the other hand, it might be produced by non-lymphoid cells of the pineal. It is conceivable that the substance associated with the modified photoreceptor cell may have an immuno-endocrine role.

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Asiatic cobras: Systematics and snakebite

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Summary. The population affinities of the Asiatic cobras of the genus *Naja* are investigated, using multivariate analysis of a range of morphological characters. This complex, which was formerly thought to be monospecific, consists of at least eight full species. In some cases, species whose bites require different antivenoms occur sympatrically. The new understanding of the systematics of the Asiatic cobra complex calls for a reappraisal of cobra antivenom use in Asia, and for more research into venom composition.
Key words. *Naja*; cobra; Serpentes; systematics; snakebite; antivenom.

The Asiatic cobras of the genus *Naja* are of considerable medical, social and economic importance in many parts of Asia, killing thousands of people every year[1]. The complex is usually thought to consist of a single species, *Naja naja*, with ten generally recognized subspecies[2-4] (fig. 1). Many of these are poorly defined and heterogeneous, and the population systematics of the group have been a source of confusion for many years. Our study has already shown that some of the conventional subspecies are in fact full species[5-7].

The poor understanding of the population systematics of these snakes is regrettable in view of their medical importance: as has been demonstrated in the carpet viper (*Echis carinatus*) species complex, closely related and morphologically similar species can have venoms with different antigenic qualities. Consequently, the antivenom against one species may not neutralize the venom of another[8], which can lead to greatly increased fatality rates[9,10]. The aim of this paper is to summarize the population affinities of the Asiatic cobra complex, and to relate these findings to the literature on the treatment of bite victims and to venom research.

This study is based on the multivariate analysis of morphological characters recorded from approximately 700 preserved specimens loaned from 29 museums in Europe, the United States and India[11]. 66 morphological characters, listed in table 1, relating to scalation, colour pattern, dentition, internal anatomy and body proportions were recorded from each specimen. Specimens were grouped into operational taxonomic units (OTUs) on the basis of collecting gaps and potential physiographic barriers. The homogeneity of the OTUs was confirmed by principal components analysis. Canonical variate analysis (CVA), one of the most widely used techniques in the analysis of population affinities[12-15], was used for the investigation of the population systematics of the group.

The four CVAs, presented in figure 2, show that the Asiatic cobra populations comprise eight distinct taxa, which we regard as separate species:

- the Indian spectacled cobra, *Naja naja*, from India and neighbouring areas;
- the Central Asian cobra, *Naja oxiana*, ranging from the Caspian Sea to northern India;
- the monocellate cobra, *Naja kaouthia*, which occurs from northern India to Malaysia, Vietnam and the Andaman Islands;
- the Chinese/Indochinese spitting cobra, *Naja atra*, which occurs from eastern China to Thailand. The affinities of the Indochinese populations here included in this species are still unclear, and under further investigation. More than one species may be involved;
- the equatorial spitting cobra, *Naja sumatrana*, from the Malayan Peninsula, equatorial Indonesia and Palawan;

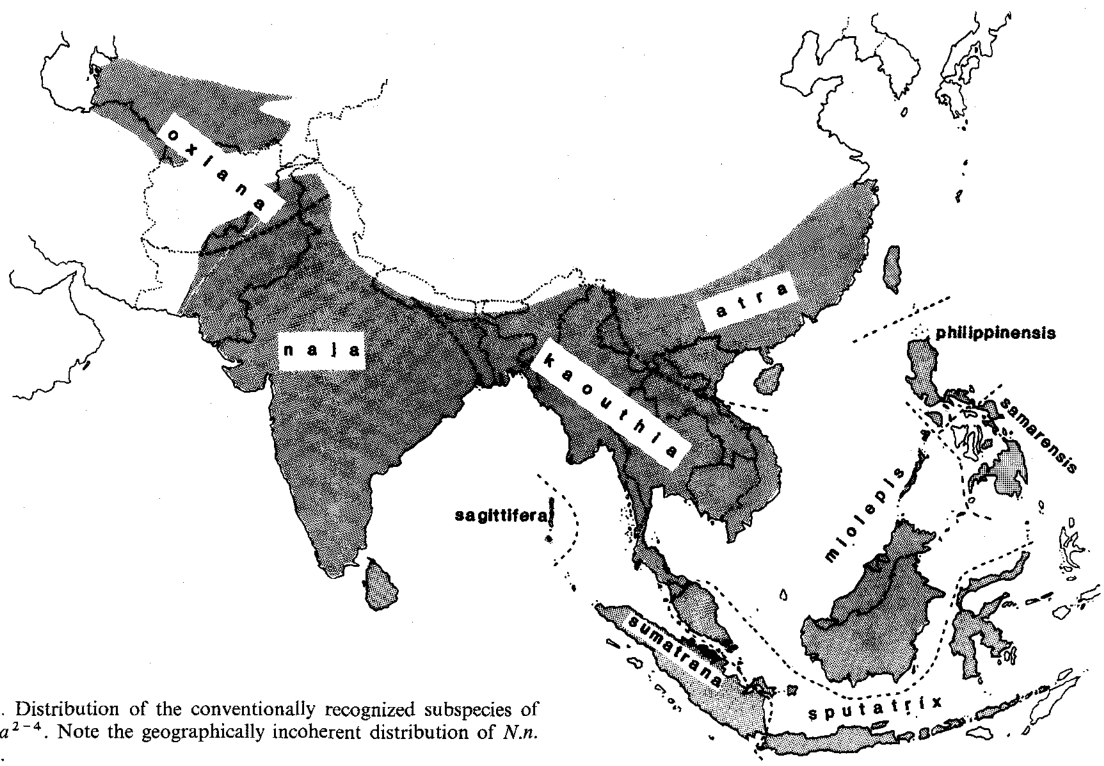

Figure 1. Distribution of the conventionally recognized subspecies of *Naja naja*[2–4]. Note the geographically incoherent distribution of *N.n. sputatrix*.

Table 1. List of characters recorded from the preserved *Naja* material. Detailed descriptions of characters and recording methods have been published elsewhere[6, 11].

1	No. ventral scales	35	No. pterygoid teeth
2	No. subcaudal scales	36	No. dentary teeth
3	% of subcaudals undivided	37	%VS position of last ventral involved in formation of light throat area
4	No. cuneates	38	No. lateral throat spots
5	No. posterior temporals	39	No. solid teeth on maxilla
6	No. temporals and nuchals in contact with parietals	40	%VS length of longest cervical rib
7	No. scale rows at 10th ventral	41	%DS width of hood mark
8	No. scale rows at 20% VS length	42	%VS position of anterior edge of hood mark
9	No. scale rows at 40% VS length	43	%VS position of posterior edge of hood mark
10	No. scale rows at 60% VS length	44	%DS encroachment of light throat colour onto sides of neck
11	No. scale rows at 80% VS length	45	%VS position of anterior edge of largest pair of lateral throat spots
12	No. scale rows at level of last ventral	46	%VS length of largest pair of lateral throat spots
13	%CS tail segments with two scale rows	47	%DS encroachment of largest pair of throat spots onto sides of neck
14	%CS position of reduction from 6 to 4 scale rows on tail	48	%VS width of first ventral band
15	%CS position of reduction from 8 to 6 scale rows on tail	49	Length of frontal scale
16	%CS position of reduction from 10 to 8 scale rows on tail	50	Width of frontal scale
17	%VS position of anterior thyroid edge	51	Distance between anterior edge of frontal scale and posterior tip of rostral scale
18	%VS position of posterior heart tip	52	Length of supraocular scales
19	%VS position of systemic junction	53	Length of suture between prefrontal scales
20	%VS position of anterior liver tip	54	Length of interparietal suture
21	%VS position of posterior liver tip	55	Length of parietal scales
22	%VS position of anterior pancreas tip	56	Length of fang
23	%VS position of cystic duct-intestine junction	57	Length of fang discharge orifice
24	%VS length of cystic duct	58	Distance from tip of fang to proximal end of discharge orifice
25	%VS position of anterior tip of right testis	59	Snout-vent length
26	%VS position of posterior tip of right testis	60	Tail length
27	%VS position of anterior tip of left testis	61	Maximum width of head across supraoculars
28	%VS position of posterior tip of left testis	62	Distance from snout tip to posterior end of interparietal suture
29	%VS position of anterior tip of right kidney	63	Distance from snout tip to posterior end of lower jaw bone
30	%VS position of posterior tip of right kidney	64	Head depth across middle of supraoculars
31	%VS position of anterior tip of left kidney	65	Head depth from lower edge of supralabials to top surface of supraoculars
32	%VS position of posterior tip of left kidney	66	Width of widest ventral scale
33	No. renal arteries		
34	No. palatine teeth		

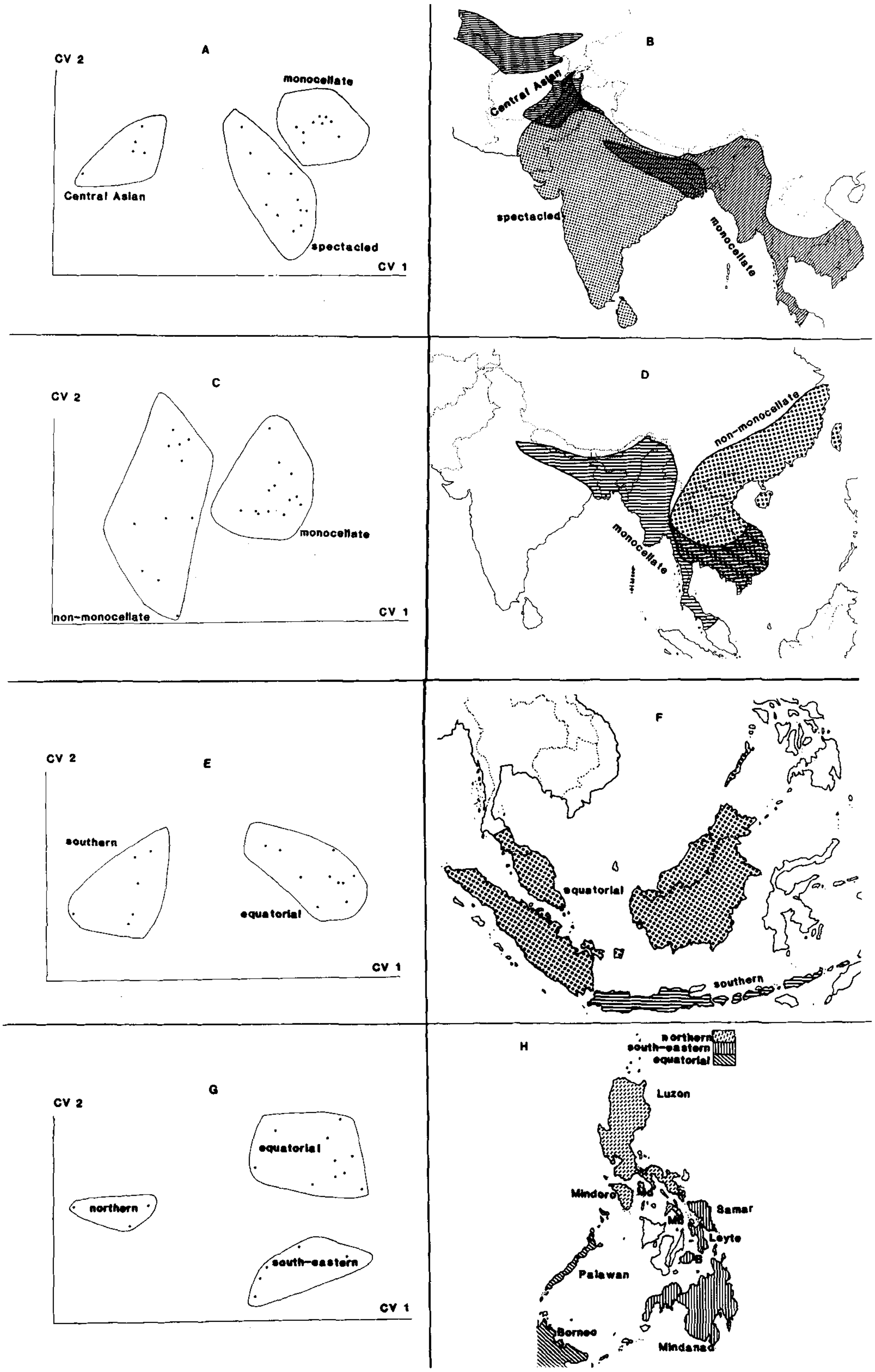

Figure 2. Determination of Asiatic cobra taxa through the use of CVA. For each CVA, the ordination of the specimens along the first two canonical variates is shown. Points correspond to OTU means, lines indicate the scatter of individual specimens. *A* CVA 1: Ordination of cobra populations from India, Central Asia and Indochina. Three distinct taxa are evident: a Central Asian taxon, a taxon with a spectacle-shaped hood mark, and a taxon with a monocellate mark. *B* Distribution of the Central Asian, spectacled and monocellate taxa revealed by CVA 1. The three taxa are partially sympatric, and therefore separate species. *C* CVA 2: Ordination of cobra populations from China and Indochina. Two distinct taxa are evident: a taxon with a monocellate hood mark, and a taxon with a variable, but non-monocellate mark. *D* Distribution of the two cobra taxa from China and Indochina, revealed by CVA 2. They occur sympatrically, and are therefore different species. *E* CVA 3: Ordination of cobra populations from the Malayan Peninsula and Indonesia. Two distinct taxa are evident: an equatorial taxon, and a southern Indonesian taxon. *F* Distribution of the two Malayan and Indonesian cobra taxa revealed by CVA 3. Because of consistent and considerable morphological differences, we consider them to be separate species. *G* CVA 4: Ordination of the cobra populations of the Philippines and equatorial Indonesia. Three distinct taxa are evident: the equatorial cobra, a southeastern taxon, and a northern taxon. *H* Distribution of the three Philippine cobra taxa revealed by CVA 4. The three forms are considered separate species, since they constitute phenetically cohesive and highly distinct taxa. (Md: Marinduque; Mb: Masbate; Bh: Bohol).

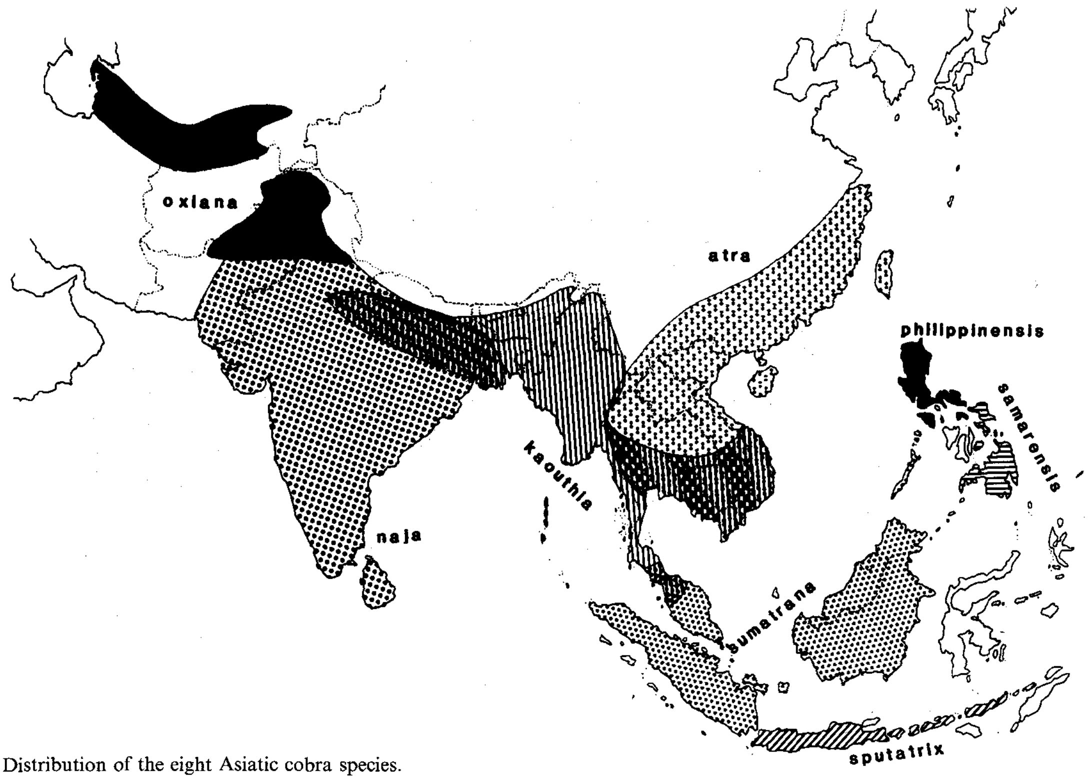

Figure 3. Distribution of the eight Asiatic cobra species.

- the southern Indonesian spitting cobra, *Naja sputatrix*, from Java and the Lesser Sunda Islands;
- the southeastern Philippine spitting cobra, *Naja samarensis*, from Mindanao, Samar, Leyte, Bohol and Camiguin;
- the northern Philippine cobra, *Naja philippinensis*, from Luzon, Mindoro, Masbate, and Marinduque.

Their distribution is shown in figure 3. The extensive areas of sympatry between several pairs of species are clearly evident.

Cobras are an important cause of snakebite morbidity and mortality in much of tropical Asia[16-18]. Cobra bites in humans result in a variety of symptoms, the most important being potentially fatal neurotoxic effects, and local tissue damage, which can be crippling. The relative incidence of these symptoms varies enormously between, and, to a lesser extent, within the newly recognized Asiatic cobra species[19-21] (table 2).

These enormous differences between these species indicate major differences in the composition of their venoms. Antigenic differences between these venoms are therefore likely, so that an antivenom against one species may be ineffective against the venom of another.

Such problems have been demonstrated in some cases: Indian antivenom (manufactured on the basis of *Naja naja* venom) shows poor neutralizing ability against the venom of *N. philippinensis*[22], and antivenom against cobras from southern Malaysia (*N. sumatrana*) is ineffective against the venom of *N. kaouthia*[16]. Chinese *N. atra* venom appears to be poorly neutralized by most commercially available cobra antivenoms[23].

These problems illustrate the need for a sound understanding of the population systematics of venomous snakes. *N. sumatrana* and *N. kaouthia*, which are known to require different antivenoms, occur sympatrically in parts of the Malayan Peninsula[9] (fig. 3). *N. kaouthia*, and the Indochinese spitting cobras here referred to *N. atra*, occur sympatrically in much of Indochina. The reported differences in venom composition between *N. kaouthia* and the Chinese populations of *N. atra*[23] sug-

Table 2. Geographic variation in the relative incidence of the symptoms of Asiatic cobra bites

	% cases with neurotoxic symptoms	% cases with necrosis	Ratio neurotoxicity / necrosis
Luzon[21] (n = 39) (*N. philippinensis*)	97%	7%	14:1
C. Thailand[20] (n = 24) (*N. kaouthia*)	54%	30%	1.8:1
N. Malaysia[19] (n = 47) (*N. kaouthia* and *N. sumatrana*)	12%	44%	1:3.7

The nomenclature has been modified from the original references, and altered to the one proposed in this paper. Many patients experienced neither necrosis nor neurotoxicity, symptoms being absent or limited to local swelling and/or pain. In the study from northern Malaysia[19], two patients were bitten by *Naja sumatrana*, the remainder probably all by *Naja kaouthia*.

gest that there may be problems with regard to antivenom efficacy in Indochina. In both cases, no polyvalent antivenom against the venoms of both species involved is available, since it had not hitherto been realized that these forms are separate, sympatric species.

Problems may also occur in countries with several non-sympatric species of cobra, since the antivenom manufacturers generally produce antivenom only on the basis of the venom of snakes from their immediate vicinity. In the Philippines, only antivenom against *N. philippinensis* is produced, even though two other species (*N. samarensis* and *N. sumatrana*) occur in the country, and in Indonesia, antivenom is only manufactured on Java against the venom of *N. sputatrix*, even though *N. sumatrana* is widespread in the equatorial parts of the country. Differences in venom composition, and antivenom cross-reactivity, between these species have not been researched in depth. This should now be done as a matter of urgency. Although venoms are likely to vary more between species than within them, problems may also be caused by intraspecific differences in the antigenic properties of venoms: different geographic populations of the same species may have very different venoms, resulting in different symptoms (eg. *N. kaouthia* venom appears to be more neurotoxic and less necrotic in Thailand than in northwestern Malaysia – see table 2), and possibly in antivenom incompatibility. Manufacturers of antivenoms against widespread species should use venoms from specimens from all parts of the species' range to cover as much antigenic variability as possible.

The interpretation of past research into venom differences is difficult, due to the hitherto insufficient understanding of the systematics of the group. Since many of the conventional subspecies of *Naja naja* did not correspond to the actual species, it is often impossible to ascertain which species an experimental venom was taken from: for instance, venom labelled simply "*Naja naja sputatrix*", without locality indication, may come from the Malayan populations which were assigned to that taxon, but actually belong to *N. sumatrana*, or from the Javan populations, which are *N. sputatrix*. Without knowledge of the locality of origin of the snakes, there is no way of establishing which species is involved.

More work is needed on the systematics of many groups of dangerously venomous snakes. Researchers participating in snakebite and venom studies need to bear the importance of sound taxonomy in mind, and should ensure that the snakes they are dealing with are correctly identified. If possible, specimens should be deposited in a museum, so that their identity can later be confirmed.

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