A new species of spitting cobra (*Naja*) from north-eastern Africa (Serpentes: Elapidae)

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Abstract

A new species of spitting cobra *Naja nubiae* sp. nov. is described from north-eastern Africa. The distinctiveness of the new species is confirmed by multivariate analysis of pattern and scalation data. Phylogenetic analysis of mitochondrial DNA sequences reveals the new species to be the sister taxon of *N. pallida*, but with considerable levels of sequence divergence relative to that species. The populations concerned had previously been assigned to *N. pallida*. The new species differs from *N. pallida* principally in having more than one dark band across the neck and under the throat, as well as a pair of spots under the throat. It occupies a disjunct range across Egypt, the Sudan, Chad, Niger and Eritrea, where it seems to occupy primarily relatively mesic habitats. *Naja mossambica* is more closely related to *N. nigricollis* than to *N. pallida* and the new species. A key to the African species of *Naja* is presented.

Key words: *Naja*, taxonomy, phylogeny, multivariate morphometrics, Africa, *Naja nubiae* sp. nov.

INTRODUCTION

From the point of view of popular awareness, cobras are among the best-known snakes in the world. Their characteristic defensive posture and their venomous nature have captured the human imagination for millennia, and made them cultural icons and objects of veneration in many parts of the world. The ability of some species to spit venom at an adversary (Bogert, 1943; Wüster & Thorpe, 1992*a*; Rasmussen, Young & Krimm, 1995) has further added to their notoriety (e.g. Larson, 1990).

Despite the high profile of these snakes, their systematics have remained surprisingly poorly known until recently. In-depth analyses of several species groups have revealed the existence of unrecognized species previously lumped under a single name, as part of the phenomenon referred to as the 'inertial species concept' by Good (1994) (for further examples and discussion see Zamudio & Greene, 1997; Wüster, Yrausquin & Mijares-Urrutia, 2001). In what could be termed the 'Afro-Asian *Naja* diversity race', the number of recognized African *Naja* species has risen from four to eight since 1968 (Broadley, 1995; Spawls & Branch, 1995), and that of Asiatic species from one to 11 since 1989 (Wüster, 1996; Slowinski & Wüster, 2000).

Among the African *Naja*, the spitting cobras have been most extensively revised. Until 1968, all populations of the group were regarded as part of a single species, *Naja nigricollis*. Broadley (1968, 1974) split the complex into two species, *Naja nigricollis* and *N. mossambica*, the latter including the taxa *katiensis*, *mossambica* and *pallida* as subspecies (Broadley, 1974). Roman (1969) demonstrated widespread sympatry between *N. nigricollis* and *N. katiensis* and treated the latter as a species separate from *N. mossambica*. Hughes & Barry (1969), Branch (1979) and Hughes (1983) regarded the taxon *pallida* as a full species, separate from *N. mossambica*. Although they provided no concrete evidence for this interpretation, it was followed by many (e.g. Golay, 1985; Broadley, Rage & Toriba, 1993; Spawls & Branch, 1995), but not all (e.g. Meirte, 1992), subsequent workers. Haagner, Branch & Haagner (2000) considered the two southern subspecies of *N. nigricollis*, *N. n. nigricincta* and *N. n. woodi*, to be subspecies of a separate species, *N. nigricincta*, but without providing new data. The status of these forms, as well as the subspecies of *Naja haje*, are the subject of ongoing studies by the authors, and will be published elsewhere. For the purposes of this paper, the taxa *nigricincta* and *woodi* will be discussed as part of *N. nigricollis*, unless specifically excluded.

The only one of these species recorded from the north-eastern parts of Africa is *Naja pallida*, which has been reported from Egypt south to north-eastern Tanzania, and east to Somalia (Broadley, 1968; Spawls & Branch, 1995).

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Table 1. Characters tested for significance of variation between populations and used for multivariate analyses. Characters showing no significant between-locality variation were not used in multivariate analyses. %VS, % ventral scale count

	Significance of between-locality variation	Eigenvector coefficient for PC 1	Eigenvector coefficient for PC 2
Ventrals	0.0039	−0.263	0.185
No. of preoculars (mean of both sides)	0.0040	0.021	−0.678
No. of supralabials (mean of both sides)	0.0001	−0.165	0.614
Cuneates (mean of both sides)	NS		
Posterior temporals (sum of both sides)	NS		
Nuchal scales	NS		
Dorsal rows at 20% VS	NS		
Dorsal rows at 40% VS	NS		
Dorsal rows at 60% VS	NS		
Dorsal rows at 80% VS	0.0051	0.229	−0.073
Dorsal rows at 100% VS	NS		
Position of reduction from 8 to 6 caudal scale rows	NS		
No. of lateral spots under throat	< 0.0001	−0.391	0.052
No. of bands under throat	< 0.0001	−0.354	−0.013
No. of bands across neck	< 0.0001	−0.376	0.004
%VS position of first ventral in main dark throat band	< 0.0001	−0.370	−0.152
%VS width of main dark throat band	< 0.0001	0.311	0.273
Presence/absence of dark tips to ventrals	< 0.0001	−0.288	−0.141
Presence/absence of dark bases to dorsal scales	< 0.0001	−0.338	−0.050

Previous workers have noted colour pattern variation in this species, specimens from the Nile Valley (Egypt, northern Sudan) being olive, with black interstitial skin (Eiselt, 1962; Osman & El Sir, 1988; Spawls & Branch, 1995). However, there have been no systematic studies addressing the status of these northern populations.

An investigation of the systematics of the *N. pallida* complex was prompted by difficulties experienced by the first author in reconciling the external appearance of three live spitting cobras kept at London Zoo, corresponding to the description of northern *N. pallida*, with the notion that they are conspecific with the typical, southern form of the species. The analysis of a large series of museum specimens led to the conclusion that the northern populations hitherto assigned to *Naja pallida* represent a separate, undescribed species of African spitting cobra.

Multivariate morphometrics and analysis of mitochondrial DNA sequences were then used to confirm the status of these northern populations *vis-à-vis* typical *Naja pallida*. Multivariate morphometrics can portray the pattern of variation in several characters simultaneously, and thus provide a comparison of the generalized phenotypes of different populations. This methodology has proved to be an important tool in visualizing patterns of variation and revealing the distinct nature of cryptic species in a variety of snake groups (e.g. Wüster & Thorpe, 1991, 1992b; Wüster, Otsuka et al., 1992; Wüster, Thorpe et al., 1996; Lenk & Wüster, 1999; Wüster, Yrausquin et al., 2001). The analysis of mtDNA sequences has similarly proved to be a useful tool in assessing the phylogenetic history of populations, and thus revealing lineages with a long history of independent evolution (e.g. Wüster & Thorpe, 1994; Zamudio & Greene, 1997; Slowinski & Wüster, 2000), while remaining largely unperturbed by the confounding effects of geographical differences in natural selection pressures (Thorpe et al., 1995).

MATERIALS AND METHODS

Multivariate morphometrics

Based on initial observation and published literature data, 19 characters relating to scalation and colour pattern (Table 1) were recorded from a series of specimens of spitting cobras assignable to the new species and *N. pallida*. The live specimens of the new species from London Zoo, from which DNA samples were obtained, were also included in the morphological analysis. The specimens examined are listed in the Appendix.

To standardize the position of colour pattern characters or scale row counts along the body, the ventral scales were numbered from the head according to the method of Dowling (1951). The position of each character was noted as the number of the ventral opposite which it was situated. This was then converted to % ventral scale count (%VS) to compensate for differences in the number of ventral scales among different individuals. Since a high proportion of specimens had missing or partially regenerated tail tips, subcaudal scale counts were not included, nor was it possible to standardize the position of the reduction from 8 to 6 caudal scale rows by expressing it as a percentage of the total subcaudal scale count.

To assess which characters show significant variation between localities, specimens were grouped into 5 samples according to region of origin and sampling gaps: (1) specimens of the new species from Egypt and

Table 2. Species and specimens used for mtDNA analysis

Species	GenBank accession no.	Locality and voucher (if any)	Sample size
Naja nivea	AF 217827	Unknown, no voucher (Slowinski & Keogh, 2000)	1
Naja kaouthia	AF 217835	Burma: Ayeyarwady Div.; CAS 206602	1
Naja nubiae	AF 399751–2	Unknown, London Zoo, live collection nos. C1591–1592, to be vouchered in BMNH after death[a,b]	2
Naja pallida	AF 399748	Unknown, London Zoo, live collection	2
Naja pallida	AF 399749	Kenya, LATOXAN, live collection nos. 90007–90008; to be vouchered in MNHN	1
Naja pallida	AF 399750	Tanzania, Reptiles du Monde, live collection	1
Naja mossambica	AF 399747	South Africa: KwaZulu Natal, Durban, gift from H. W. Herrmann	1
Naja nigricollis	AF 399746	Ghana, Liverpool School of Tropical Medicine, live collection	1
Naja nigricollis	AF 399745	Lara, Kaélé, Cameroon, LATOXAN, live collection nos. 9735002–003; to be vouchered in MNHN[a]	2

[a] Photographs available from WW.
[b] Morphological data available from WW.

northern Sudan; (2) specimens of the new species from Chad; (3) 3 specimens of the new species of unknown origin from London Zoo, which furnished the DNA used in this study; (4) specimens of *N. pallida* from Kenya and adjoining south-western Ethiopia and southern Somalia; (5) specimens *N. pallida* from northern Somalia, Djibouti and eastern Ethiopia. Each character in the resulting database was subjected to an analysis of variance (ANOVA), using BMDP 1V (Dixon, 1991). Characters showing no significant between-locality variation were not considered further (see Table 1).

To visualize the pattern of variation in morphology among the 2 taxa included in the study, principal components analysis (PCA) was run on the data recorded from individual specimens. Before analysis, each character was converted to zero mean and unit standard deviation. Only characters found to display significant between-locality variation were used. Separate PCAs were carried out for males and females as well as a PCA for both sexes together.

Molecular methods

Blood samples or clippings of ventral scales were obtained from specimens of several African spitting cobra species (Table 2). DNA was purified from the samples using standard protocols (Sambrook, Frisch & Maniatis, 1989) of RNase and proteinase k digestion, phenol, phenol-chloroform and chloroform centrifugation and ethanol precipitation, or by using various commercial kits. Outgroup sequences of the African non-spitting cobra *Naja nivea* and the Asiatic *N. kaouthia* were obtained from Slowinski & Keogh (2000).

A 758 bp fragment of the cytochrome *b* gene was amplified using the polymerase chain reaction (PCR), using primers 5′-TCA AAC ATC TCA ACC TGA TGA AA-3′ (L-strand; modified from Kocher *et al.*, 1989) and 5′-GGC AAA TAG GAA GTA TCA TTC TG-3′ (H-strand, modified version of primer MVZ 16; Moritz, Schneider & Wake, 1992). The 5′ ends of these primers correspond to positions 14977 and 15735 of the total mtDNA sequence of *Dinodon semicarinatus* (Kumazawa *et al.*, 1998). See Pook, Wüster & Thorpe (2000) for details of PCR conditions.

Sequences were obtained using an ABI 377 Automated Sequencer, following manufacturer's protocols. The different sequencing operations used pooled products of several PCR reactions, and several specimens were sequenced for several of the species, thus minimizing the potential impact of PCR amplification errors.

Sequence analysis

All sequence and phylogenetic analyses were carried out using PAUP* 4.0b8 (Swofford, 2001), unless stated otherwise.

All sequences were checked for insertions, deletions or the presence of stop codons. Any of these would have indicated that the sequences represented nuclear insertions of the mitochondrial genes (Zhang & Hewitt, 1996). The sequence data were assayed for the presence of a significant phylogenetic signal by means of the g1 tree skewness statistic (Hillis & Huelsenbeck, 1992), calculated from all 2 027 025 possible trees.

Average genetic distances between species were calculated using the program Phyltest (Kumar, 1996), using the Kimura 2-parameter distance to reduce the effect of partial saturation of some types of base pair substitution.

Phylogenetic analysis

Maximum parsimony (MP) searches, using *Naja nivea* and *N. kaouthia* as outgroups, were carried out using the exhaustive search algorithm of PAUP*. No differential weighting of different codon positions or transversions *vs* transitions was used. Bootstrap analysis was implemented using 1000 pseudoreplicates and branch-and-bound searching. Branch support (Bremer, 1994) was found by re-running the parsimony analysis while retaining successively longer trees and calculating their consensus. The branch support for each node is the difference in the number of steps between the most parsimonious tree and the shortest tree in which the node is broken up.

Maximum likelihood (ML) analyses require an appropriate model of sequence evolution. For this, the program Modeltest 3.0 (Posada & Crandall, 1998) was used. This generates a Jukes–Cantor distance-based neighbour-joining (NJ) tree, and calculates likelihood scores as well as the different parameters of sequence evolution for 56 different models. The model giving the highest likelihood score was then selected, and the parameters fixed as estimated by Modeltest 3.0 in a heuristic ML search. For this, a NJ tree was used as a starting tree, and tree bisection-reconnection (TBR) branch swapping used. The parameters from the resulting ML tree were then re-estimated, and a further heuristic search run using these new parameter settings. This was repeated until the analysis retained the same tree at each iteration. ML bootstrap analysis was carried out using 100 pseudoreplicates, and the same settings as the original analysis.

DESCRIPTION OF NEW SPECIES

The results of our morphological and molecular analyses support our initial impression that the north-eastern spitting cobras represent a hitherto undescribed species. Detailed discussion of the results will follow the description of the new species, which we hereby name *Naja nubiae* sp. nov., the Nubian spitting cobra.

Naja nubiae sp. nov.

Vipera (Naia) haje (not Linnaeus) Geoffroy St. Hilaire, 1827: 157, pl. vii, fig. 3. (part).

Naja nigricollis (not Reinhardt) Anderson, 1896: 109; 1898: 322, pl. XLV; Andersson, 1904: 5; Werner, 1908: 1883; Boulenger, 1915: 656 (part); Werner, 1919: 507; 1927: 77; Scortecci, 1928: 306; 1930: 204; Zavattari, 1930: 177; Flower, 1933: 826; Corkill, 1935: 25 (part); Villiers, 1950*a*: 121; 1950*b*: 341 (part); Saleh, 1997: 173.

Naia nigricollis var. *mossambica* (not Peters) Boulenger, 1896*b*: 379 (part, Assouan).

Naja nigricollis nigricollis (not Reinhardt) Loveridge, 1945: 3; Marx, 1968: 40.

Naja nigricollis pallida (not Boulenger) Eiselt, 1962: 294.

Naja mossambica pallida (not Boulenger) Broadley, 1968: 11 (part); Harding & Welch, 1980: 12 (part); Meirte, 1992: 58 (part).

Naja pallida (not Boulenger) Branch, 1979: 215 (part); Hughes, 1983: 332, 352 (part); Largen & Rasmussen, 1993: 382 (part); Largen, 1997: 93; Spawls & Branch, 1995: 76 (part); Golay, 1985: 47 (part); Osman & El Sir, 1988: 77; Broadley *et al.*, 1993: 190 (part); David & Ineich, 1999: 165 (part).

Naja katiensis (not Angel) David & Ineich, 1999: 166 (Aïr massif).
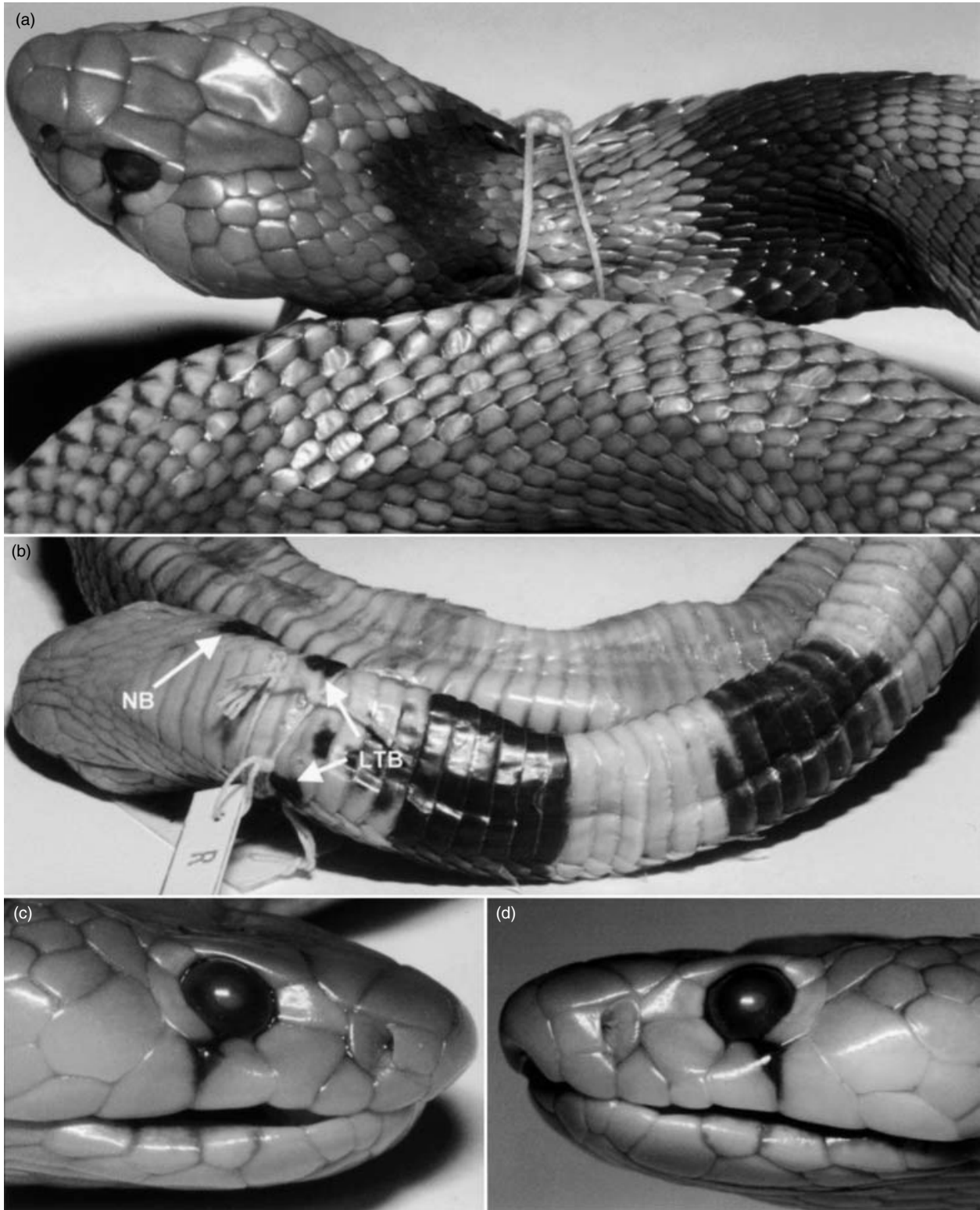
Holotype

Peabody Museum, Yale University, specimen YPM R 3916, a male from Kom Ombo (= Kawn Umbû), Aswan Governorate, Egypt (24°28′ N, 32°57′ E) (Fig. 1).

We designate as paratypes those vouchered specimens that were included in the morphometric analyses (for locality details, see Appendix 1): BMNH 97.10.28.615-616, 1911.7.15.11, 1959.1.5.28; MHNG 1328.43-44; MNHN 7694, 3961, 7695a & b, 8787-8788; YPM R 5210; ZFMK 32391, 39880-81; NHRM 1588.

Diagnosis

The new species can be differentiated from *Naja pallida*, with which it was until now confused, through its throat and neck pattern: *N. pallida* normally has a single, broad dark band across the throat; this encircles the body and crosses the neck. There is no other pattern on the body or throat, except in a small minority (*c*. 5%) of specimens, which have a more or less obvious second dark band across the throat, but not across the neck. In *N. nubiae*, there are two bands across the neck: a nape band situated immediately behind the parietal scales, and the main band further back. The nape band may or may not cross the throat. The second, main neck band invariably crosses the throat, usually in a more posterior position than the throat band of *N. pallida* (Fig. 2). A further dark ventral band is present in *N. nubiae*, but may be faint in older specimens. There is a distinct light throat area before the main throat band, and practically all specimens feature a small dark spot on each side of the throat, at the junction of the ventrals and the dorsals (Fig. 1). This resembles the lateral throat spots seen in most Asiatic species. Additionally the bases of the scales are strongly blackened in *N. nubiae*, but not in *N. pallida*. Most *N. nubiae* have seven supralabials, even when two preoculars are present, whereas in most *N. pallida*, specimens with two preoculars have only six supralabials (see below).

Naja mossambica is very similar to *N. nubiae* in overall appearance and coloration, However, the dark throat pattern is more irregular, and the throat bands do not cross the neck of the snake. Also, *N. mossambica* has lower ventral scale counts (177–205; Broadley, 1983)

Fig. 1. Holotype of *Naja nubiae* (YPM R 3916). (a) Head and neck region in dorsal view. Note dark nape band immediately behind head and the second, main neck band. (b) Throat region. NB, nape band; LTB, lateral throat blotches. (c), (d) Supralabial and preocular configuration on both sides of the holotype. Note large third supralabial contacting eye and nasal in (c), compared with the same scale split into a small supralabial and a large lower preocular in (d).

than *Naja nubiae* (207–226), and never has more than six supralabials. In *N. mossambica*, most supralabials and head scales are more or less distinctly edged with black, whereas this is only the case for the 'tear drop' marking on the posterior edge of the fourth supralabial, below the eye, in *N. nubiae* and *N. pallida*.
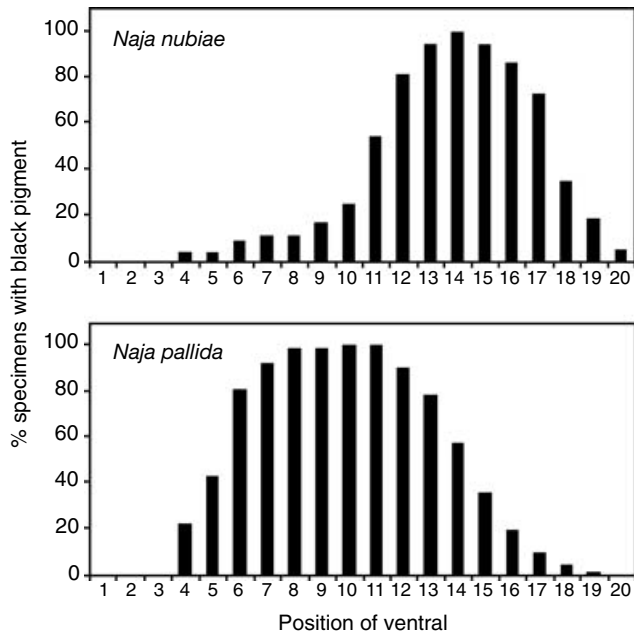
Fig. 2. Black throat banding in the *Naja pallida* complex, showing the percentage of specimens in which each ventral scute is largely black due to involvement in the main throat band. Note that in *N. nubiae* the first throat band almost invariably covers at least V 13–15, whereas in *N. pallida*, the first band is placed further forward, centred on V 8–11.

Naja katiensis is easily distinguished by the absence of a small fifth supralabial (cuneate) and its much lower scale counts for ventrals (165–186) and subcaudals (47–55; Broadley, 1968).

Naja nigricollis differs in dorsal coloration (mostly dark, or patterned or variegated), in having a broad dark band across the throat, and a mostly dark venter. Additionally, this species also usually has 23 or fewer dorsal scale rows at midbody, *vs* the usual 25 in *Naja nubiae*. The southern taxon *woodi* is uniformly black as an adult, and grey with a black head as a juvenile, and the taxon *nigricincta* is boldly barred in black and white or red.

In *Naja haje* and *N. annulifera*, the supralabials are separated from the eyes by a series of subocular scales, and these cobras do not spit. The other non-spitting species of African cobra (*N. nivea*, *N. melanoleuca*) differ in having an enlarged sixth supralabial, which contacts the postoculars, a single anterior temporal, versus two or three in spitting cobras, and 21 or fewer dorsal scale rows (*vs* 25 in *N. nubiae*).

Description of holotype (Fig. 1)

Body dimensions. SVL 654 mm, tail length 134 mm, head length 30.1 mm, total length 788 mm. Body scalation: 218 ventrals (count slightly ambiguous due to extensive scarring of the ventral surface between ventrals 150 and 165), 63 divided subcaudals, anal single. Dorsal scale rows: 27 on neck, 25 at midbody (50% VS length),

17 at vent. Dorsal scale rows fuse and split irregularly along the body, as observed in some other *Naja* (pers. obs.) and *Walterinnesia* (Marx, 1953), therefore a dorsal scale row reduction formula is not provided. Caudal scale row reduction formula: 14 2 + 3(2) 12 2 + 3(3) 10 3 + 4(5) 8 3 + 4(14/15) 6 2 + 3(36/41) 4.

Head scalation: supralabials 7/7; on right hand side, third supralabial large and high, contacts orbit and nasal scale, on left hand side, third supralabial very small and low, fourth contacts orbit on both sides. Infralabials 9/10, first four contact anterior chin shields. Fifth to tenth infralabials small, fifth, sixth and seventh homologous to cuneate scale of Asiatic cobras. Anterior chin shields much wider but shorter than posterior chin shields. Posterior chin shields broadly separated by two rows of intervening sublingual scales. Nasal almost completely divided by large, vertically oval nostril. 1/2 preoculars, all contact nasal scale but not the internasals. Lower preocular on left hand side homologous to upper part of third supralabial on right hand side. 2/2 postoculars, lower larger than upper, showing faint crease indicative of subdivision. Rostral wider than high, barely visible from above. Prefrontals larger than internasals. Frontal longer than wide (5.7 × 4.0 mm), slightly shorter than distance from rostral (6.0 mm), shorter than supraoculars (6.3 mm); anterior edge straight, posterior edge ends in obtuse angle, border with supraoculars slightly convex. Anterior temporals 2/2, posterior temporals 5/5, small and poorly differentiated. Ten temporals and nuchals contact the lateral and posterior edges of the parietals.

Coloration and pattern in preservative. Head pattern: top of head brownish-beige, lighter and somewhat yellowish on sides and, and greyish-cream under head. A conspicuous, well-defined 'tear drop' marking under the eye, consisting of black pigment on the upper posterior edge of the fourth supralabial, the upper anterior edge of the fifth supralabial, and the lower edge of the lower postocular. The black pigment also extends along the orbit onto the edge of the third supralabial (right) or the lower preocular (left). Additional dark markings on the head are very inconspicuous and poorly defined, and include darker mottling on supralabials 5–7, and along the outer and posterior edges of the parietals. Body pattern: dorsal surface brownish grey, basal portion of each dorsal scale black, interstitial skin between scales black. Ventral side slightly lighter, with a yellowish tinge in the posterior third of the body as well as the subcaudal surface. Very faint, inconspicuous darker speckling along the middle third of the ventrals. A conspicuous nape band, in the shape of a forward-pointing chevron, crosses the nape behind the head, the anterior edge separated from the parietals by two dorsal scales, and extends laterally to reach the second dorsal scale row between the first and fourth ventrals. Outer tips of ventrals 6–7 and the lower two-thirds of the adjoining lowest dorsal scale row black, giving rise to a pair of eyespots when seen from below. A well-defined black ring extends across ventrals 11–16, and across the neck. The posterior edge of the band is indented by *c*. the width of 1.5 dorsal scales. Ventrals 7–10 have medial black spots of increasing width. A further dark band of

Table 3. Variation in scalation characters displayed in *Naja nubiae* and *Naja pallida*. Exceptional values (< 10%) are shown in parentheses

	Naja nubiae	*Naja pallida*
Ventrals	207–226	192–227
Subcaudals	58–72	56–81
Dorsals around neck	23–29	21–29
Dorsals at midbody	23–27	21–27
Dorsals one head length anterior to vent	15–17 (19)	(15) 17–19
Preoculars	1–2	1–2
Postoculars	(2) 3	(2) 3 (4)
Supralabials	7 (6–8)	6–7 (8–9)
Infralabials	8–9 (10)	9 (8–10)
Nuchals (Broadley, 1968)	(10) 11–13 (14–15)	(9–10) 11–14 (15)

lesser intensity covers ventrals 24–29, as well as the right halves of ventrals 23 and 30. The continuation of this band across the neck is barely perceptible on the interstitial skin.

Variation

Scalation. Midbody scale rows 23–27 (mean = 24.54, $n = 35$), ventrals in males 207–221 (mean = 213.13, $n = 15$), in females 209–226 (mean = 218.72, $n = 18$); anal entire; subcaudals in males 56–69 (mean = 64.13, $n = 15$), in females 61–69 (mean = 64.0, $n = 17$). Preoculars 1 or 2; postoculars 3 (rarely 2; the holotype is unusual in this respect); temporals $2 + 4$, $2 + 5$, rarely $3 + 4$ or $3 + 5$ (except in the Ennedi population); cuneates 2 or 3; nuchals bordering parietals 10–15; supralabials 6–8 (usually 7), the fourth, or third and fourth (rarely third only) entering orbit; infralabials 9 (rarely 7, 8 or 10), the first 4 (rarely 3) in contact with the anterior sublinguals. Variation in a number of scalation characters in *Naja nubiae* compared with *Naja pallida* is shown in Table 3.

The arrangement of the preoculars and supralabials is of particular interest. In African spitting cobras, three possible configurations exist (Fig. 3). Configuration 1 consists of a single preocular, and a large third supralabial, which contacts both the eye and nasal scale. This is the configuration found in non-spitting African *Naja* and all Asiatic *Naja*, as well as other related genera, such as *Boulengerina*, *Pseudohaje*, *Hemachatus* and even *Elapsoidea*, and is therefore likely to represent the primitive condition. Normally, these snakes have seven supralabials. In configuration 2, the third supralabial has been squeezed out from contact with the mouth, and now forms a second, lower preocular, which contacts both the orbit and the nasal. This arrangement is found in many African spitting cobras, such as *Naja nigricollis*, *N. mossambica*, and *N. katiensis*, and these snakes normally have six supralabials. In configuration 3, the third supralabial is split into two, a small lower part, which contacts the edge of the mouth, and a larger upper part,
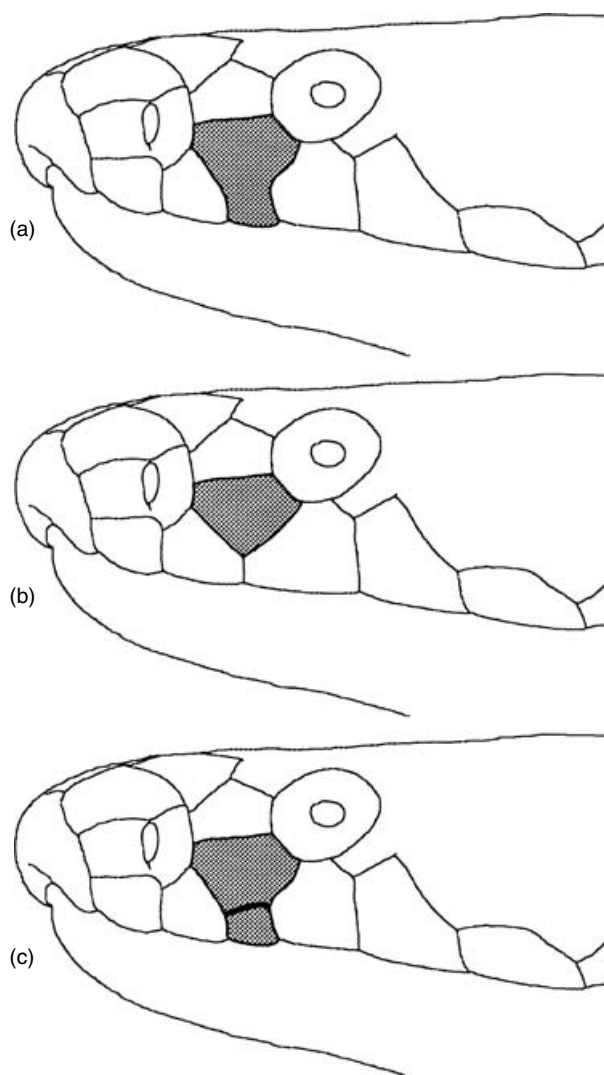
Fig. 3. Variation in the configuration of the preocular and supralabial scalation among African cobras. In each drawing, the scale homologous with the third supralabial of non-spitting cobras is shaded. (a) Configuration 1, found in non-spitting cobras, Asiatic cobras, and some *N. nubiae* and *N. pallida*: third supralabial contacts eye and nasal. (b) Configuration 2, found in some African spitting cobras: third supralabial no longer contacts edge of mouth, two preoculars present. (c) Configuration 3, found in *N. nubiae* and some *N. pallida*: the third supralabial has become split into a small supralabial and a large lower preocular.

which now forms a second, lower preocular, and contacts both the orbit and the nasal scale. This configuration normally results in a total of seven supralabials.

Naja nubiae and *N. pallida* differ considerably in the frequency in which these different configurations are found. Whereas *N. nubiae* tends to have either configuration 1 or 3, *N. pallida* most often has either 1 or 2; most *N. pallida* with two preoculars thus have six supralabials (55 out of 77 examined by DGB), whereas most *N. nubiae* with two preoculars have seven (17 out of 20 specimens examined by DGB). However, it should be

Fig. 4. Live male specimen of *Naja nubiae*; London Zoo, captive collection. Note the characteristic tear-drop marking under the eye.

noted that specimens displaying different arrangements on the two sides of the head are not uncommon in either species, as exemplified by the holotype of *N. nubiae*.

The occurrence of two preocular scales in African spitting *Naja* is unique among elapids (except for *Dendroaspis*, which have three preoculars of unclear homology), and must be regarded as a morphological synapomorphy of the African spitting *Naja*. Since *N. pallida* and *N. nubiae* are polymorphic in this respect, the fixation or near-fixation of this trait in *N. katiensis*, *N. nigricollis* and *N. mossambica* may be regarded as a morphological synapomorphy for those three species.

Size. Like *N. pallida*, *N. nubiae* is a relatively small cobra. Largest male (MNHN 76956, Egypt) *c*. 1200 + 235 = 1435 mm; largest female (MSNM 1790, Cheren, Eritrea) 1250 + 230 = 1480 mm (Scortecci, 1939*a*).

Colour in life. Living *N. nubiae* exhibit warmer, slightly more reddish tones than preserved specimens, without, however, differing grossly. A live specimen is shown in Fig. 4, and a colour photo was published by Saleh (1997).

Etymology

The species is named after the region of Nubia, home of the first black African civilization, which occupied the Nile Valley between Aswan and Khartoum, in present-day southern Egypt and northern Sudan. Most museum specimens of this species originate from this region. We suggest the common name 'Nubian spitting cobra' for this species.

Synonymy and comparative data for *Naja pallida*

Naja pallida Boulenger, 1896

Naia nigricollis (not Reinhardt) Stejneger, 1893: 734; Boulenger, 1895: 168; 1896*a*: 216; 1897: 279; Meek, 1897: 179; Sternfeld, 1908: 241; Boulenger, 1915: 656 (part); Loveridge, 1929: 36 (part); Parker, 1932: 364; Loveridge, 1929: 36 (part); Scortecci, 1934: 34, fig. 15; 1939*a*: 194, fig. 107–109; 1939*b*: 286; 1940: 18; 1943: 287.

Naia nigricollis var. *pallida* Boulenger, 1896*b*: 379. Type locality: inland of Berbera, British Somaliland [= Somalia] (restricted by Bogert, 1940); 1896*c*: 13; 1896*d*: 21; 1898: 721; 1909: 311; Lönnberg & Andersson, 1913: 5; Calabresi, 1918: 124; 1927: 33.

Naja nigricollis nigricollis (not Reinhardt) Loveridge, 1936: 272; Bogert, 1940: 88.

Naja nigricollis pallida Bogert, 1942: 5; Loveridge, 1947: 58; Parker, 1949: 90; Battersby, 1954: 248; 1955: 150; Loveridge, 1957: 292; Balletto 1968: 268.

Naja mossambica pallida Broadley, 1968: 11 (part); Lanza, 1972: 180; Spawls, 1978: 11; Harding & Welch, 1980: 12 (part); Meirte, 1992: 58 (part).

Naja mossambica (not Peters), Hoevers & Johnsen, 1982: 199; Lanza, 1983: 229.

Naja pallida Branch, 1979: 215 (part); Hughes, 1983: 320, 352 (part); Golay, 1985: 47(part); Broadley *et al.*, 1993: 190 (part). Lanza, 1990: 422; Largen & Rasmussen, 1993: 382 (part); Spawls & Branch, 1995: 76 (part); David & Ineich, 1999: 165 (part).

Variation. Midbody scale rows 21–27 (mean = 25.29, $n = 104$); ventrals in males 192–218 (mean = 204.83, $n = 55$), in females 199–227 (mean = 210.91, $n = 48$); anal entire; subcaudals in males 56–81 (mean = 67.47, $n = 48$), in females 55–72 (mean = 64.43, $n = 43$). Preoculars 1 or 2; postoculars 3 (rarely 2 or 4); temporals usually 2 + 4 or 2 + 5, rarely 2 + 3, 2 + 6, 3 + 4, 3 + 5 or 3 + 6; supralabials 6 or 7 (very rarely 8 or 9), the third, fourth or third and fourth entering orbit; infralabials 9 (rarely 8 or 10), the first four (rarely three) in contact with the anterior sublinguals; cuneates usually 2 or 3, rarely 1, 4 or 5; nuchals bordering parietals 11–15 (rarely 9 or 10).

Size. Largest male (NMK 1489, Olorgesaille, Kenya) 1300 + 235 = 1535 mm; largest female (NMK 2770, Kibwezi, Kenya) 1170 + 240 = 1410 mm.

RESULTS OF ANALYSES

Multivariate morphometrics

The ANOVA showed that 11 out of the 19 characters recorded display significant between-locality variation (Table 1). Subsequent analyses were restricted to these characters. Patterns of variation visualized by separate PCAs of male and female specimens were identical to that shown by the analysis of both sexes together. Since sexual dimorphism did not affect the results, only the results of the joint analysis are presented.

The ordination plot of the individual specimens along the first two principal components of the joint PCA is shown in Fig. 5. The separation between the eastern, typical *N. pallida* and *N. nubiae* is clearly evident, irrespective of the sex of the specimens. The eigenvector loadings of different characters is shown in Table 1. It
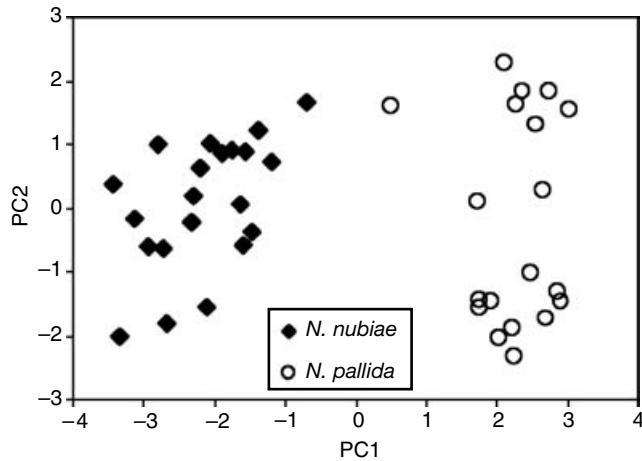
Fig. 5. Ordination of first and second principal component scores of the specimens of *Naja pallida* and *N. nubiae* included in this study. The first two principal components summarize 48.8% and 17.1% of the total variance, respectively.

Fig. 6. One of two equally most parsimonious maximum parsimony trees derived from an exhaustive analysis of the unweighted sequence data. Figures above nodes indicate branch support (Bremer, 1994), figures below indicate % MP bootstrap support/% ML bootstrap support.

can be seen that the separation of specimens along the first principal component is due primarily to characters relating to colour pattern, whereas separation along the second principal component is primarily governed by the configuration of preocular and supralabial scales.

Sequence analysis

We aligned 717 bp of cytochrome *b* sequence for all taxa. GenBank accession numbers can be found in Table 2. No stop codons, insertions or deletions were found. Two hundred nucleotide positions were variable, and 115 were parsimony-informative. The g1 tree skewness distribution statistic was –0.920787, which allows us to reject the null hypothesis that the data contain no significant phylogenetic signal ($P < 0.01$; Hillis & Huelsenbeck, 1992).

Unadjusted pairwise sequence divergences within the ingroup ranged from 0.14% (between two *N. nubiae* haplotypes) to 10.9% (between Tanzanian *N. pallida* and Ghanaian *N. nigricollis*). The average Kimura two-parameter distance, determined using the software PHYLTEST (Kumar, 1996), between *N. nubiae* and *N. pallida* was 8.75% ± 1.2%, compared to 6.3% ± 1.0% between *N. mossambica* and *N. nigricollis*.

Phylogenetic analysis

The unweighted MP analysis resulted in two equally most parsimonious trees (EMPTs) of 286 steps (c.i. = 0.7937; h.i. = 0.2063; r.i. = 0.7592), which differ only in that one tree resolves the relationships among the three *N. pallida* haplotypes whereas the other represents them as a trichotomy (Fig. 6). The haplotypes of *Naja nubiae* formed the sister clade of the *N. pallida* haplotypes,

and the *nubiae* + *pallida* clade constituted the sister clade of a group consisting of *N. mossambica* and *N. nigricollis*. Bootstrap support for all nodes was high (> 80%). Reanalysis with transversions weighted twice or 10-fold relative to transitions resulted in no change of tree topology (not shown).

The model of sequence evolution selected as most appropriate for the present sequences was the HKY85 model with a gamma shape parameter of 0.2056. A ML search using the parameters identified by Modeltest resulted in a single most likely tree (-lnL = 2221.5378). Further re-estimation of sequence evolution parameters resulted in no further change of parameters or tree topology. The ML tree was topologically identical to the consensus of the two EMPTs identified in the MP search (not shown). Bootstrap support values for the different nodes are shown in Fig. 6.

DISCUSSION

The results of the morphological and molecular analyses clearly demonstrate the highly distinct nature of *Naja nubiae* from typical *N. pallida*. The pattern differences between the two forms are qualitative and categorical, features present in one form being replaced with different features in the other. Similarly, in the DNA sequence analysis, the sequence divergence of > 8.7% between *N. nubiae* and *N. pallida* is more consistent with that usually found between closely related but clearly distinct
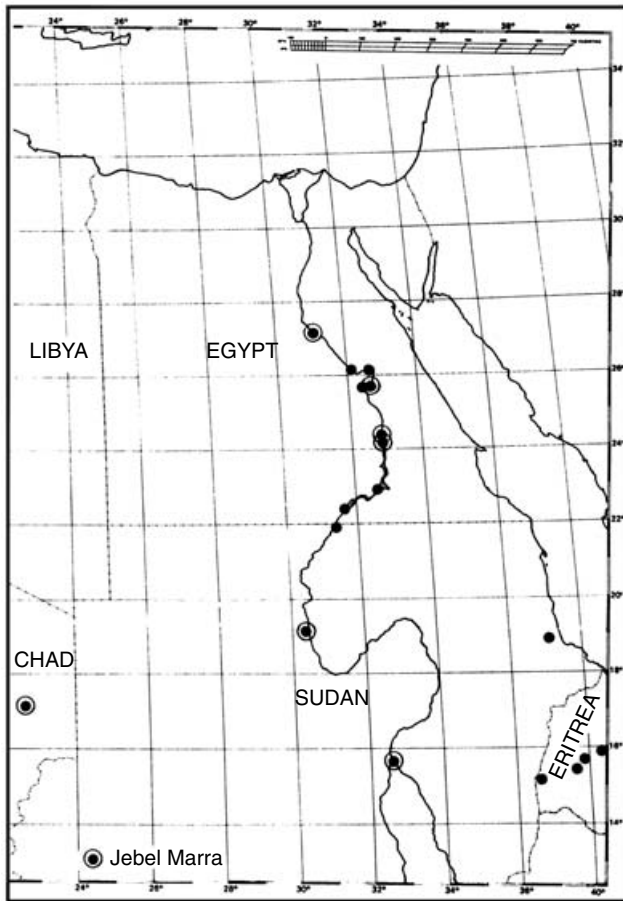
Fig. 7. Locality records for *Naja nubiae* in Egypt, Chad, Sudan and adjacent northern Eritrea. Localities of specimens used for PCAs are encircled.

Fig. 8. Locality records for *Naja nubiae* and *N. pallida* in Eritrea, Ethiopia, Djibouti, Somalia, Kenya and Tanzania. Localities of specimens used for PCAs are encircled.

species of squamates rather than at the intraspecific level (e.g. Zamudio & Greene, 1997; Avise, Walker & Johns, 1998). Moreover, it is considerably greater than the distance of 6.3% between our sequences of *N. mossambica* and *N. nigricollis*, two partly sympatric, and thus un questionably distinct, species.

These results leave little doubt that the spitting cobra of north-eastern Africa is on a separate evolutionary traject-ory from its nearest relative, *N. pallida*, and therefore constitutes a distinct evolutionary species. The initial separation of *N. pallida* from *N. mossambica* (Hughes & Barry, 1969; Branch, 1979; Hughes, 1983) was based on little concrete evidence, but was nevertheless widely accepted. Our mtDNA analyses confirm the status of *N. pallida* and *N. mossambica* as separate species: *N. mossambica* is more closely related to *N. nigricollis*, with which it is sympatric in parts of its range (Broadley, 1968), than to *N. pallida* and *N. nubiae*.

Distribution and natural history notes

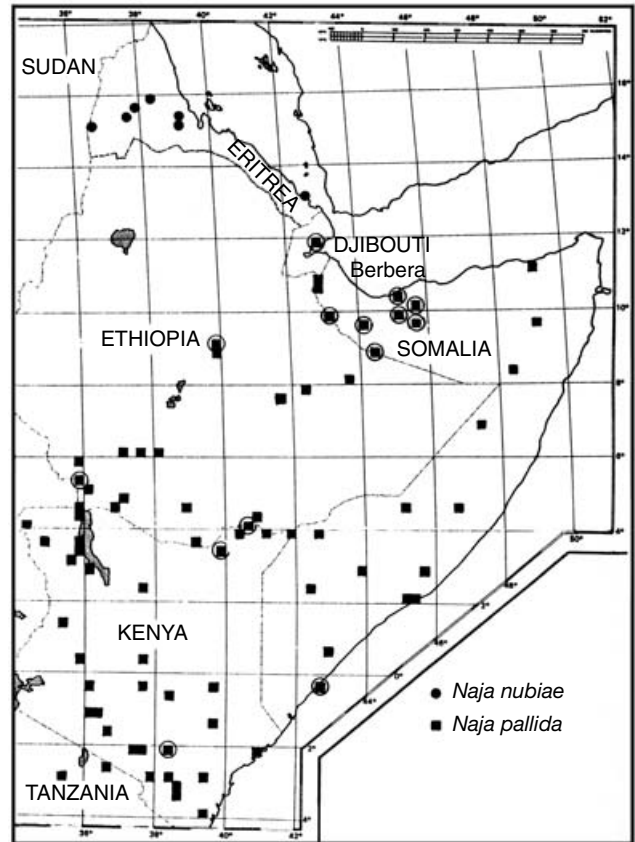*Naja nubiae* has a wide but apparently patchy distribution in north-eastern Africa, particularly in the south-eastern part of the Saharan region. The majority of available museum material originates from Upper Egypt, south of Asyut (Marx, 1968). In addition, the species has been documented from the Nile Valley of the northern Sudan (south to Khartoum, Osman & El Sir, 1988). An apparently isolated population occurs at altitudes of *c*. 1800–2000 m on Jebel Marra, Darfur Province, Sudan, where it is sympatric with *Naja nigricollis*. This locality represents a considerable northerly range extension for *N. nigricollis* (specimens ZFMK 39878–79 and 39882–84) when compared with published distributions maps (e.g. Spawls & Branch, 1995). Three specimens of *N. nubiae* are known from the Ennedi Massif in north-eastern Chad, and two from the Aïr Massif 1500 km to the west in north-western Niger. *Naja nubiae* may yet be found in other isolated mesic habitats, such as oases and hill areas, in the Sahara. Additionally, there are records from the Red Sea coast of Sudan (Erkowit) and Eritrea (Assab), as well as from the mountains of northern Eritrea (Largen, 1997). Available records from north-eastern Africa are mapped in Figs 7 & 8.

Although most of the documented range of *N. nubiae* is arid, the species seems to favour mesic microhabitats within its broader range. Along the Nile valley, Hegner (1994) reports that *N. nubiae* lives in low vegetation along the banks of the river Nile, and reports it to be a good

swimmer. Eiselt (1962) and Joger (1984) surmised that the flooding of Lake Nasser following the construction of the Aswan Dam would have resulted in the extinction of populations between the dam and Wadi Halfa, at the upper end of the Lake. However, Hegner (1994) noted the continued presence of the species at Abu Simbel. It may therefore be expected that populations continue to exist along the shores of Lake Nasser. The present range of the species seems to consist of relict populations remaining from a broader geographic range, dating back to times of more mesic climates in the present-day Sahara desert.

Spawls & Branch (1995) mentioned (under *N. pallida*) specimens of a black cobra with yellow bands on the neck, from the Aïr Massif, Niger. Their translation from the French (Villiers, 1950*b*) is somewhat misleading, and these snakes are here assigned to *Naja nubiae*.

Medical importance

There are no records of bites by this species, although they must occur from time to time. A case of snake venom ophthalmia in a zookeeper resulted in pain, palpebral oedema, blepharospasm and reddening of the eye. The condition resolved within a few days, without further complications (pers. obs.).

Key to the African species of *Naja*

In view of the numerous changes in the systematics of the African species of *Naja*, which have seen the number of recognized species rise from four to nine since 1968, it seems useful at this stage to provide a key to the species of this genus. Previous keys (e.g. Golay, 1985; Meirte, 1992) are taxonomically outdated, sometimes use suboptimal characteristics, or do not take into account intraspecific variation in important characters, such as the configuration of the preoculars in *N. pallida* and *N. nubiae*. In the key of Golay (1985), any African spitting cobra with a single preocular would key out as *Naja naja*, and a *N. nubiae* with two preoculars would key out either as *N. mossambica* (for very low ventral scale counts), or fail to key out because of conflict between coloration and ventral scale counts. In the key of Meirte (1992), *N. nubiae* would key out as *N. mossambica*, within which that author included the taxa *katiensis*, *mossambica* and *pallida* as subspecies. However, it would not be clearly assignable to any of these three taxa.

1. Eye separated from supralabials by a series of subocular scales   2
   Eye in contact with supralabials   3

2. Rostral scale not enlarged, snout rounded; usually 21 scale rows   *Naja haje*
   at midbody; ventrals usually more than 200
   Rostral scale enlarged, snout pointed; usually 17–19 scale rows   *Naja annulifera*
   at midbody; ventrals usually less than 200

3. Sixth supralabial high, enlarged, contacts postocular scales; one   4
   anterior temporal shield; fangs not adapted for spitting
   Sixth supralabial scale low, not enlarged, does not contact postocular scales; two   5
   or more anterior temporal shields; fangs adapted for spitting

4. Usually 21 dorsal scale rows at midbody; rostral as broad as high;   *Naja nivea*
   posterior chin shields widely separated
   Usually 19 dorsal scale rows at midbody; rostral broader than high;   *Naja melanoleuca*
   posterior chin shields in contact or narrowly separated

5. Fifth infralabial large, in contact with chin shields; ventrals 164–186;   *Naja katiensis*
   subcaudals 47–56
   Fifth infralabial very small (cuneate), well separated from chin shields;   6
   ventrals usually 180 or more; subcaudals usually 55 or more

6. A single, regular band around the throat and/or neck, otherwise   *Naja pallida*
   more or less uniform reddish or brown
   More than one band around throat and/or neck, or irregular throat pattern,   7
   or other body pattern, or largely dark or black

7. Body uniformly olive or greyish, throat light with dark pattern, part of   8
   anterior throat area not covered in dark pigment
   Pattern variable, often entirely black or very dark, usually a very broad   *Naja nigricollis*
   dark band across the throat, including the first few ventrals

8. Throat pattern consisting of somewhat irregular bands that do not cross   *Naja mossambica*
   the neck; many head scales with dark edges; southern and eastern Africa
   Throat pattern consisting of 1–3 regular bands, some of which cross the neck;   *Naja nubiae*
   only border between supralabials below eye with dark edges
   ('teardrop marking'); north-eastern Africa

Acknowledgements

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APPENDIX

Material examined by the authors or for which data was supplied by B. Hughes, plus literature records. *, Specimens used for PCAs. Collection acronyms follow Leviton *et al.* (1985), with the following additions: KMH, University of Dar es Salaam, Tanzania; MWC, Mweka Wildlife College, Tanzania.

Naja nubiae: EGYPT. No precise locality MNHN 7694*, 3961*, 7695a* & b*; NMW 16870; SMF 20611; ZMB 2810; Abu Simbel (Hegner, 1994); Aswân (Anderson, 1896, 1898; Boulenger, 1896b) BMNH 97.10.28.615*; Asyût YPM/R, 5210*; Atrun Island (Aswân) (Werner, 1927; Eiselt, 1962) NMW 16865; Balyana ZFMK 50236 (not seen); 10 km E of Idfu (Aswân) UMMZ 177828; Kawn Umbû YPM 3 3916*; Luxor [= Al Uqsur] NMBA 14389, 14424; ZFMK 50232–5; 24 km north of Luxor FMNH 153051; Qena FMNH 75227–8; Seiyala (Eiselt, 1962) NMW 17001; Thebes IRSNB 10891; MHNG 1328.43*–44*; NMBA K. 1176, 1214, 1836; Wadi Nasim (Qena) USNM 134987. SUDAN. Dongola BMNH 97.10.28.616*; Erkowit ZFMK 32391*, 38416; Khartoum (Andersson, 1904; Werner, 1908, 1919; Flower, 1933) BMNH 1911.7.15.11*; NHRM 1588*; 'Nubia' ZMB 2806; Wadi Halfa (Eiselt, 1962) NMW 17002; White Nile NMBA 2224. Darfur: Jebel Marra: Suni on east slope at 1800 m ZFMK 39880*–81*. ERITREA (Largen, 1997). Assab MHNG –; Cheren (Scortecci, 1939a) MSNM 1790; Ghinda MZUT R. 3396 (not seen); Gura (Loveridge, 1945) ANSP 25207; Tessenei (Scortecci, 1930) MSNM 801 (not seen). CHAD. Archei, Ennedi BMNH 1959.1.5.28*; Oued Basso, Ennedi MNHN 8787*–8*. NIGER. Irabellaben, Bagzane Mts, Aïr (Villiers, 1950a,b) IFAN 47-4-41 & 42. NO DATA. London Zoo – live collection, 3 unnumbered specimens*; NMW 19069 ('Arabar, Cartago'); USNM 94444.

Naja pallida: DJIBOUTI. Tadjoura District BMNH 1971.1478*. SOMALIA. Afgoi MZUF 24206, 24208; Bardera MSNG 40805; Belet Uen (Scortecci, 1939*b*); Inland of Berbera BMNH 95.6.14.28 (lectotype)*; Bihen BMNH 1931.7.20.404; Borama District (Parker, 1949) BMNH 1949.2.2.86*; Burao BMNH 1949.1.3.48*; Deragodleh (Meek, 1897); Galgala Oasis MZUF 6041; Gardo MZUF 6707; Giohar (Scortecci, 1939*b*); Goolis Mts BMNH 98.5.21.30*; Guban (Parker, 1949) BMNH 1949.2.2.89; Hargeisa BMNH 1940.2.8.1*; 1959.1.3.63; Harradigit (Parker, 1949) BMNH 1949.2.2.90; Haud (Parker, 1949) BMNH 1949.2.2.87*; Kismayu (Lönnberg & Andersson, 1913) NHRM 2327*; Lugh BMNH 98.1.28.17; MSNG 29240a & b, 29243; Mareri, Juba River (Hoevers & Johnson, 1982) CAS 153430–3; 153450; 'Somaliland' BMNH 1954.1.12.90–1; MSNG 29023; NMW 19075; Wagga, Goolis Mts BMNH 1905.11.7.55*–56*. ETHIOPIA. 'Abyssinien' NMW 19071, 19074; Arba Minch ZFMK 15882, 15884 (sympatric with *N. nigricollis)*; Awash National Park MA-A 032; Badditu Region MSNG 28832a & b; Deghabur (Parker, 1949) BMNH 1954.1.12.90; El Dire (Scortecci, 1943); Jabolo River ZMB 27452; 30 km N of Kalam, Omo valley MNHN 1973–368*; Kefa, west of Omo River BMNH 1900.11.29.4*; Lake Stephanie ANSP 4719; Mount Fantelle (Lanza, 1972) MZUF 12337; Moyale MZUF 620 (not seen); Mui, Omo National Park AAU-H. 2112; Okutha (?) NMW 19073 (2); Tedetchamalka MNHN 05–225*. KENYA, (Spawls, 1978) No locality NMW –; ZMB 21286, 22454; Amalem Irrigation Scheme (Nakuru) NMK 2615; 40 km south-east of Bura AMNH 88630; Dandu (Battersby, 1955) BMNH 1954.1.2.89*, 90*, 91; Eliye Springs NMK 2474; Ewaso Nyiro (Isiolo) NMK 2403; Garissa (photo, S. Spawls); Isiolo CAS 123159; NMK 1192; Ithumba BMNH 1963.56*; Kakuma CAS 13178–81; NMK 2843; Kalakol River Mouth, Lake Turkana BMNH 1932.5.2.105*; Kargi CAS 122983; Kauriro NMZB-UM 5546; Kiboko Range Station NMK 2955a & b; NMZB 11800–1; Kibwezi MCZ 40742; NMK 2592, 2770; Kililama ZMB 28468; Kisiwani NMB 7376; Lali, Galana River LACM 35352; Lake Hannington UMMZ 132047; Lake Turkana NMK 1562; Lodwar; NMK 2367, 2457, Loiyangalani LACM 60739; Lokichokio NMK 3157; Lokitaung CAS 131254; Lothagam Hill MCZ 89796; Manyani (Voi) NMK 435–6; Marigat NMK 3120; Mount Suswa NMK 2799; Murri (Battersby, 1954) BMNH 1952.1.9.9*; 100 km E of Nairobi ZFMK 29760–1; Nakuru FMNH 203995, 204100; Ngomeni NMK 2397, 2400; Ngura, (Kitui) NMK 2667; Olorgesaillie NMK 1172, 1489; Samburu NMK 1173; Sigor NMK 1967; Tana River (Stejneger, 1893; Loveridge, 1929) USNM 20090; Tsavo (Bogert, 1940) AMNH 51821; 'Uganda [Railway]' BMNH 1909.10.30.32; Voi (Bogert, 1942) AMNH 61628; BMNH 1963.55; CAS 122366; NMK 1467. TANZANIA. Iboya School, Mkomezi Game Reserve MWC –; Longido CM 332; NMK 1592; Longido West camp (Loveridge, 1947) MCZ 18237; Mweka MWC –; Olduvai Gorge KMH 2583; NMZB 7207; ZSM –.