Zootaxa 1532: 51–68 (2007)
www.mapress.com/zootaxa/
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ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Get an eyeful of this: a new species of giant spitting cobra from eastern and north-eastern Africa (Squamata: Serpentes: Elapidae: *Naja*)

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Abstract

We describe a new species of giant spitting cobra, *Naja ashei* sp. nov., from eastern and north-eastern Africa. The species was previously regarded as a colour phase of the black-necked spitting cobra, *N. nigricollis*. However, mtDNA sequence data show it to be more closely related to *N. mossambica* than *N. nigricollis*. The new species is diagnosable from all other African spitting cobras by the possession of a unique clade of mtDNA haplotypes and a combination of colour pattern and scalation characteristics. Its distribution includes the dry lowlands of northern and eastern Kenya, north-eastern Uganda, southern Ethiopia and southern Somalia.

Key words: *Naja ashei* sp. nov., *Naja nigricollis*, *Naja mossambica*, Serpentes, Elapidae, Africa, mitochondrial DNA, phylogeny, multivariate morphometrics

Introduction

Among venomous snakes, cobras are among those that have the highest public awareness profile. Nevertheless, our understanding of the taxonomy of the group has until recently remained woefully inadequate, particularly in terms of understanding the species limits within different well differentiated species groups. Within the genus *Naja*, the most extensively revised taxa are the Asian representatives of the genus, where successive revisions have raised the number of recognised species from one to eleven (Wüster & Thorpe, 1991; Wüster *et al.*, 1995; Wüster, 1996; Slowinski & Wüster, 2000) and the African spitting cobras, in which the number of recognised species has risen from one to five (Broadley, 1968, 1974; Roman, 1968, 1969; Wüster & Broadley, 2003). Although conventional morphological approaches have contributed considerably to the resolution of the systematics of these complexes (e.g., Broadley, 1968, 1974), more advanced approaches such as multivariate morphometrics (e.g., Wüster & Thorpe, 1989, 1992) and their combined use with mtDNA sequences (e.g., Wüster *et al.*, 1995, 1997; Slowinski & Wüster, 2000; Wüster & Broadley, 2003; Broadley & Wüster, 2004) have been especially valuable in unravelling the systematics of groups with more subtle patterns of morphological variation.

The combined use of morphological data and mtDNA adds considerable additional rigour to any attempt to diagnose and understand species limits, compared to using either marker system in isolation. Morphological differences between populations may be due either to natural selection, independent of phylogenetic affinities, or to phylogenesis (Thorpe *et al.*, 1994, 1995), and the pattern of variation alone cannot differentiate between these two hypotheses. On the other hand, the presence of multiple mtDNA haplotype clades does not necessarily indicate the presence of multiple species (e.g., Puorto *et al.*, 2001), and may even mask patterns of

Accepted by P. David: 20 Jun. 2007; published: 26 Jul. 2007

gene flow and incipient speciation (Thorpe & Richard, 2001; Ogden & Thorpe, 2002). Congruence between molecular and morphological markers indicates that the observed morphological differences are indeed a result of the populations being different evolutionary lineages, and that the mtDNA haplotype clades correspond to separate organismal lineages.

The nomenclatural history of the African spitting cobras parallels that of other taxa that fell victim to the phenomenon of the "inertial species concept" (Good, 1994): a plethora of forms was described in the 19th and early 20th century, often as full species, followed by the lumping of all African spitting cobras into the single species *N. nigricollis* Reinhardt in the 20th century, with most authors following the taxonomy of Boulenger (1896).

The first description of an African spitting cobra was by Andrew Smith, who described *Naja nigra* in 1838, but this name is preoccupied by *Naja nigra* Gray, a synonym of *N. atra* of China. Smith subsequently (1842) illustrated this snake, known as a 'spitter', under the name "*N. haje* Var. C", but Boulenger nevertheless included it in the synonymy of *N. flava* Merrem [= *N. nivea*].

The taxonomy of the African spitting cobras has fluctuated greatly since the description of the Black-necked Spitting Cobra *N. nigricollis* from Ghana by Reinhardt (1843). *Naja mossambica* was described from Tete and Sena by Peters (1854). In 1894 Günther described *N. nigricollis* var. *crawshayi* on the basis of a dry skin from Lake Mweru, while Bocage (1895) described from Angola the varieties *occidentalis, melanoleuca* [preoccupied by *N. melanoleuca* Hallowell] and *fasciata* [preoccupied by *N. fasciata* Laurenti, a synonym of *N. naja*]. Boulenger (1896), in the third volume of his *Catalogue of Snakes,* included var. *crawshayi* under the *forma typica* and listed a new variety *pallida* as well as Peters' *N. mossambica* under *N. nigricollis,* thus establishing the basis for the assumption of monospecificity for the African spitting cobras that was to become the generally accepted arrangement for more than seventy years.

Additional forms were described as varieties or subspecies of *N. nigricollis* during the first half of the 20th century. The form *katiensis* was described as a variety of *N. nigricollis* from Kati, Mali, by Angel (1922), but two specimens of typical *N. nigricollis* (MNHN 1921.611) were catalogued from the same locality. Bogert (1940) described *N. nigricollis nigricincta* from south-western Angola, Laurent (1955) described *Naja nigricollis atriceps* from Burundi and then revived *N. n. crawshayi* (Laurent 1956) and *N. n. occidentalis* (Laurent 1964) as valid subspecies, and finally Pringle (1955) described *N. n. woodi* from the western Cape Province, this being the all black species first recorded by Smith in 1838.

The splitting up of the *N. nigricollis* complex began with Broadley (1968), who reinstated *N. mossambica* as a full species, sympatric with *N. nigricollis* in eastern Zambia. He treated *pallida, katiensis, nigricincta* and *woodi* as subspecies of *N. mossambica*, while *crawshayi, occidentalis* and *atriceps* were considered synonyms of *N. nigricollis.* However, analysis of geographical variation in the spitting cobras of south-western Africa revealed sympatry between *N. mossambica* and *nigricincta,* so both the latter form and *woodi* were reinstated as subspecies of *N. nigricollis* (Broadley 1974).

Roman (1968) described *N. trilepis* from Burkina Faso, but this is a synonym of *N. katiensis* Angel. Later, the same author (Roman, 1969) confirmed widespread sympatry between *N. nigricollis* and *N. katiensis* in Burkina Faso and treated the latter as a full species.

Laurent (1973) accepted that *occidentalis* formed a genuine cline with *crawshayi,* but considered that the latter and *atriceps* should be retained as subspecies of *N. nigricollis.* Boycott & Haacke (1979) mapped the distribution of *N. n. nigricincta* and *N. n. woodi* and described variation of colour pattern of intergrade populations. Although many authors have considered *N. pallida* to be a full species (e.g., Hughes & Barry, 1969; Branch, 1979; Hughes, 1983), this was not confirmed until Wüster & Broadley (2003) analysed its phylogenetic affinities and described its sister species, *N. nubiae,* from north-east Africa.

One of the remaining puzzles in the systematics of the African spitting cobras has been the status of some eastern and north-eastern African forms. Several authors have noted the existence of two distinct colour forms of *Naja nigricollis* in eastern Africa, a smaller blackish form found in parts of Kenya as well as southern

Uganda and Tanzania, and a larger brown form found in eastern and northern Kenya, north-eastern Uganda, as well as Somalia and parts of Ethiopia (Spawls & Branch, 1995; Spawls *et al.*, 2002). However, in the absence of clear differences in scalation, the status of this form has remained unresolved. Here we use both morphological data and mtDNA sequences to investigate the affinities and status of this form. Based on congruence between morphological variation and mtDNA phylogeny, we describe the brown spitting cobra as a new species

Materials and methods

Morphology

Based on initial observation and published literature data, 14 characters relating to scalation and colour pattern were recorded from a series of specimens of spitting cobras representing different populations conventionally assigned to *Naja nigricollis*. This included both museum specimens and live specimens of the brown form kept at the Bio-Ken Snake Farm, Watamu, Kenya. The specimens examined are listed in Appendix 1.

The characters recorded from each specimen were:

1. Number of undivided subcaudal scales. In cobras, most subcaudals are divided, but some specimens have a few undivided subcaudals on the proximal portion of the tail. We recorded the absolute number rather than the proportion of subcaudals that are undivided because a high proportion of specimens have missing tail tips.

2. Mean number of postoculars on each side. The most common number is 3, but some specimens have only two postoculars on one or both sides.

3. Total number of cuneates. See Wüster (1998) for an illustration of this character.

4. Mean number of posterior temporals. We defined posterior temporals as the number of scales contacting the posterior edges of the anterior temporals (excluding supralabials and parietals).

5. Nuchals. These were defined as the number of scales contacting the lateral and posterior edges of the parietals, exclusive of the postoculars—see Broadley (1968) for an illustration.

6. Number of dorsal scale rows at 10% of the ventral scale count. The dorsal scale rows were counted in a straight line rather than a V-shaped count line.

7. Number of dorsal scale rows at midbody (i.e. midpoint of snout-vent length).

8. Number of dorsal scale rows at the level of the last ventral scale.

9. Position of the reduction from ten to eight dorsal scale rows on the tail. For this, the subcaudals were numbered from the vent, we recorded the number of the subcaudal scale opposite which the reduction had come into effect. Since many specimens have incomplete tails, we did not adjust for the total number of subcaudals.

10. Position of the reduction from eight to six dorsal scale rows on the tail, recorded as in character 9.

11. Position of the reduction from six to four dorsal scale rows on the tail, recorded as in character 9.

12. Position of the first entirely dark ventral of the main (= deepest) dark throat band.

13. Position of the last entirely dark ventral of the main dark throat band

14. Proportion of ventral scales at midbody covered in dark pigment (0 = 0–19%, 1 = 20–39%, 2 = 40–59%, 3 = 60–79%, 4 = 80–99%, 5 = 100%).

In order to visualise the pattern of variation in morphology among the two taxa included in the study, we used principal components analysis (PCA), run on the data recorded from individual specimens. One of the strengths of PCA for this type of analysis is that the method requires no *a priori* assumptions about the taxon affinities of individual specimens, and is therefore particularly suited for analyses of weakly differentiated taxa with possible areas of sympatry, where the affinities of individual specimens may be difficult to ascertain a priori. Before analysis, each character was converted to zero mean and unit standard deviation. We carried out separate PCAs for males and females as well as a PCA for both sexes together. A number of additional

specimens (Appendix 1) were examined by one of us (D.G.B.) in order to assess the variation in standard scale counts.

Molecular methods

We obtained tissue (ventral scale clippings), blood samples or shed skins from specimens of all the species of the African spitting cobra complex from eastern and north-eastern Africa (*Naja nigricollis*, *N. mossambica*, *N. pallida* and *N. nubiae*). The sampling localities are shown in Fig. 1, and the specimens listed in Appendix 2. Total DNA was extracted by standard methods (Sambrook *et al.*, 1989). Two regions of the mitochondrial DNA molecule were amplified using the polymerase chain reaction (PCR): we used primers ND4 and Leu (Arévalo *et al.*, 1994) to amplify a section of the ND4 gene and adjoining tRNAs. In the case of cytochrome *b*, we used the primers Gludg (5'-TGACTTGAARAACCAYCGTTG-3') (Palumbi, 1996) and H16064 CTTTGGTTTACAAGAACAATGCTTTA (Burbrink *et al.*, 2000). PCR reactions were set up with 10xPCR buffer, 3–3.5 mM $MgCl_2$, 0.4µM each primer, 0.8µM total dNTPs, 1 unit of *Taq* (Invitrogen, product code 10342-020) and made up to of 25µl with ultrapure water. Amplification conditions involved initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 94°C for 30s, 47°C (cyt*b*) or 60°C (ND4) for 30s, then 72°C for 2 m, followed by a final extension step of 72°C for 5m. Sequencing was carried out using the same primers by Macrogen (Seoul, S. Korea—http://dna.macrogen.com). As outgroups, we included sequences of one African non-spitting cobra, *N. nivea* (Linnaeus), and the Asian cobra *N. kaouthia* Lesson.
To test for the presence of nuclear pseudogenes (Zhang & Hewitt, 1996), we translated the DNA sequences into amino acid sequences in MEGA 2.1 (Kumar *et al.*, 2001) to check for premature stop or nonsense codons or frameshifts.

For phylogenetic analysis, we used maximum parsimony (MP) and Bayesian inference (BI) methods. MP analysis was carried out using the software PAUP* 4.0b10 (Swofford, 2002). For MP analysis, we carried out an unweighted analysis, using branch and bound searching. Internal support for different nodes was estimated using non-parametric bootstrap searching (Felsenstein, 1985), using 10000 bootstrap replicates and branch and bound searching. *Naja kaouthia* and *N. nivea* were designated as outgroups. Fixed nucleotide differences were identified using MEGA 2.1.

For phylogenetic analysis using BI, we used MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). There is increasing evidence that complex models of sequence evolution can extract additional phylogenetic signal from data, especially where saturation of base pair substitutions is commonplace (Castoe *et al.*, 2004, 2005; Castoe & Parkinson, 2006). Therefore, we used different models of sequence evolution for biologically relevant partitions of our data. In the case of protein coding mitochondrial genes, the most relevant partitions are first, second and third codon positions, which are known to display different patterns of sequence evolution. We therefore partitioned our data into six separate data partitions, namely first, second and third codon positions separately for cytochrome *b* and ND4. To identify the most appropriate models of sequence evolution for each data partition, we used MrModeltest 2.2 (Nylander, 2004), and selected the model favoured under the Akaike Information Criterion for each category in our Bayesian analysis. Since MrBayes can only use a single outgroup taxon, *N. nivea* was specified as the sole outgroup and *N. kaouthia* was excluded from all BI analyses. We ran the analysis for 4×10^6 generations using 4 simultaneous independent runs initiated with different random starting trees. Plots of lnL against generation were inspected to determine the burn-in period, and trees generated prior to the completion of burn-in were discarded, with a five-fold "safety margin".

To test whether the data reject the monophyly of the haplotypes found in the populations normally assigned to *N. nigricollis* with statistical significance, we used PAUP* to build the most parsimonious trees constrained to be consistent with that hypothesis. We then used Wilcoxon signed-ranks test (Templeton, 1983, implemented in PAUP*) to test for the significance of differences in tree length between the most parsimonious trees and the constrained tree.

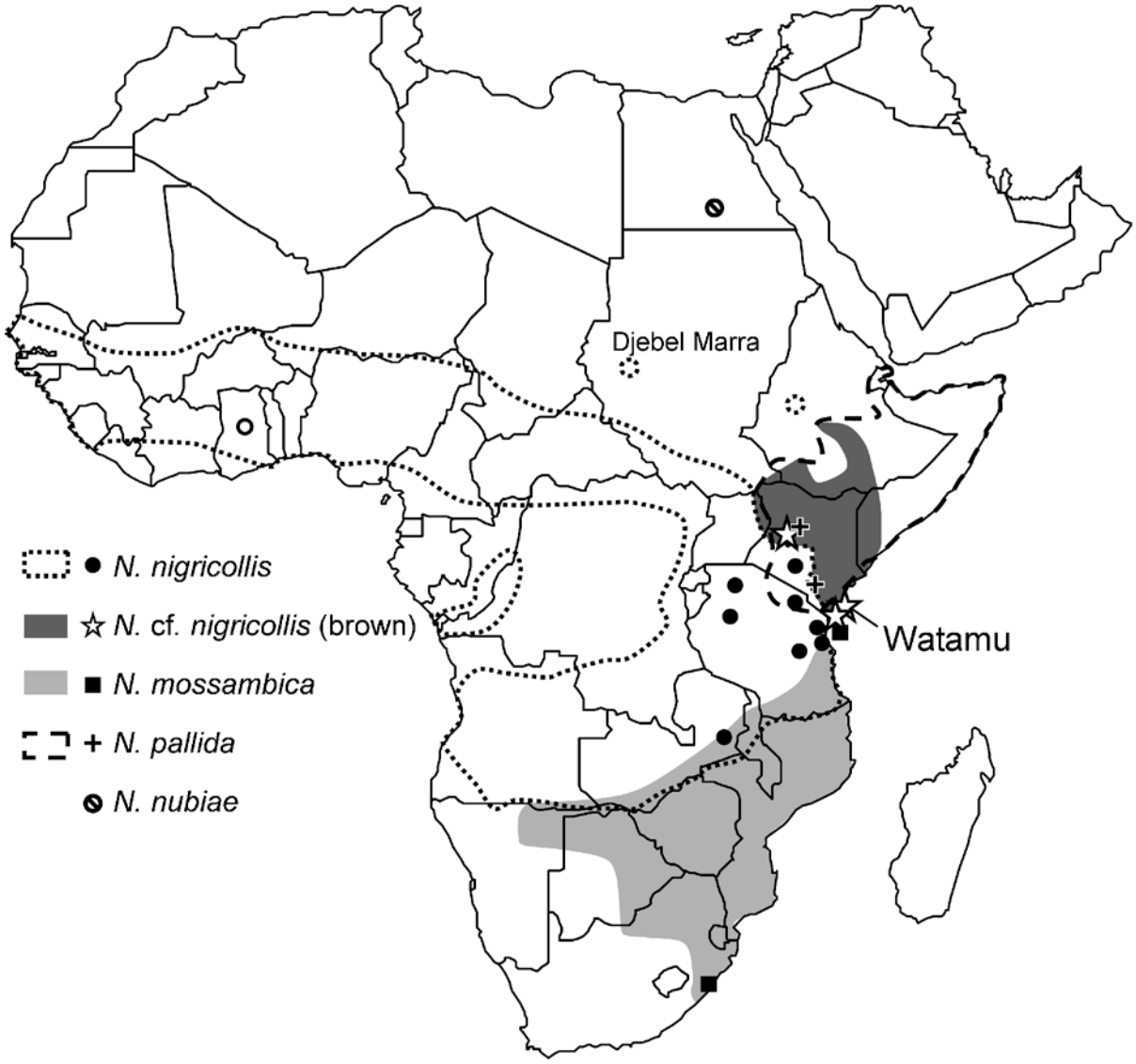

FIGURE 1. Sampling localities for molecular analysis and distribution of the spitting cobra species occurring in eastern Africa.. Hollow symbols for *N. nigricollis* in Ghana and *N. nubiae* in Egypt indicate approximate localities.

Results

Morphology

Patterns of variation visualised 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, we only present the results of the joint analysis, thus benefiting from the greater joint sample size.

The ordination plot of the individual specimens along the first two principal components of the joint PCA is shown in Fig. 2, and the PC variable loadings of the individual characters in Table 1. It can be seen that the specimens of the brown form from eastern Africa are clearly separated from other specimens of *N. nigricollis* by their high second principal component ordination scores, which are associated with high dorsal scale row counts on the neck, reduced numbers of postocular scales, a relatively posterior start to the dark throat band and a light venter. Specimens from the Djebel Marra region of Sudan did not group with the eastern African brown specimens, forming instead a somewhat distinct cluster most closely associated with black *N. nigricollis*.

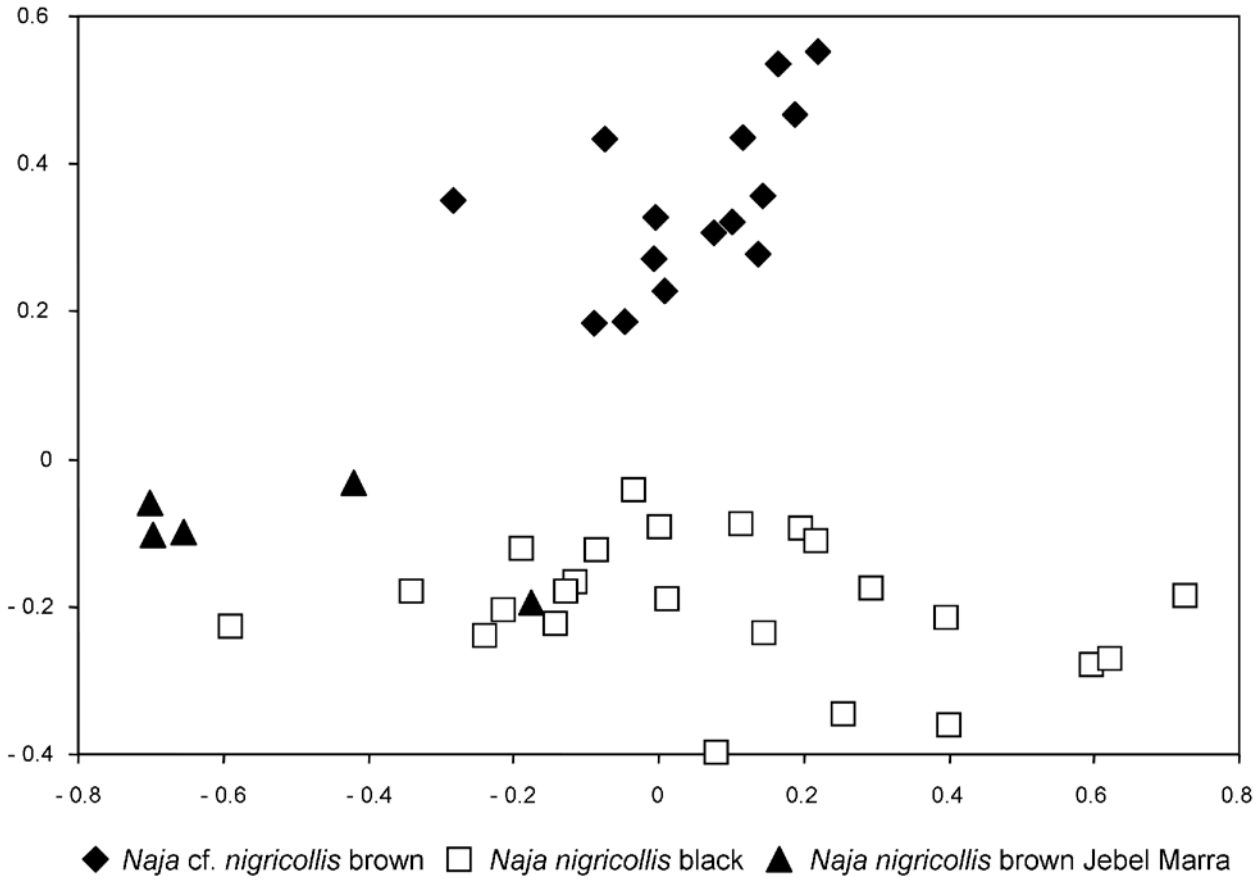

FIGURE 2. Ordination of individual specimens along the first two principal components of the PCA. The first two principal components account for 31.5 % and 22.85 % of total variance, respectively.

TABLE 1. PCA variable loadings for individual characters in PCA of specimens of both sexes.

	PC 1	PC 2
# undivided SC	0.003	-0.244
Postocs mean	-0.071	-0.37
Total no. cuneates	-0.163	-0.124
Post temp mean	-0.107	0.21
Nuchals	-0.138	-0.239
D10	-0.318	0.366
D50	-0.403	0.185
D100	-0.406	-0.05
Position of reduction from 10 to 8 scale rows on tail	-0.389	-0.069
Position of reduction from 8 to 6 scale rows on tail	-0.404	-0.131
Position of reduction from 6 to 4 scale rows on tail	-0.393	-0.15
First dark ventral of main band	-0.057	0.43
Last dark ventral of main band	0.168	0.265
Darkness of ventrals at midbody	0.096	-0.465

Molecular data

We aligned a total of 1333 base pairs, 606 for ND4 and 727 for cytochrome b. From 28 ingroup specimens, we identified 16 distinct haplotypes. The sequences were deposited with GenBank (accession numbers in Appendix 2). There were no indels, frameshifts or unexpected stop codons, leading us to conclude that our sequences represented mitochondrial DNA rather than nuclear insertions (Zhang & Hewitt, 1996). Of the 1333 b.p., 257 were variable and 216 parsimony informative across all taxa.

The one million random trees generated in PAUP* produced a g1 statistic of -0.929379, suggesting that the data contain significant phylogenetic signal ($p < 0.01$) (Hillis and Huelsenbeck, 1992)

Unweighted parsimony analysis of the sequence data yielded two equally most parsimonious trees of 543 steps (consistency index: 0.7422, retention index 0.8006). The two trees differed only in the placement of haplotypes "*N.* cf. *nigricollis* brown Watamu" and "*N.* cf. *nigricollis* brown Baringo", which exchanged places between the two trees. One of the two trees and bootstrap support for the nodes is shown in Fig. 3.

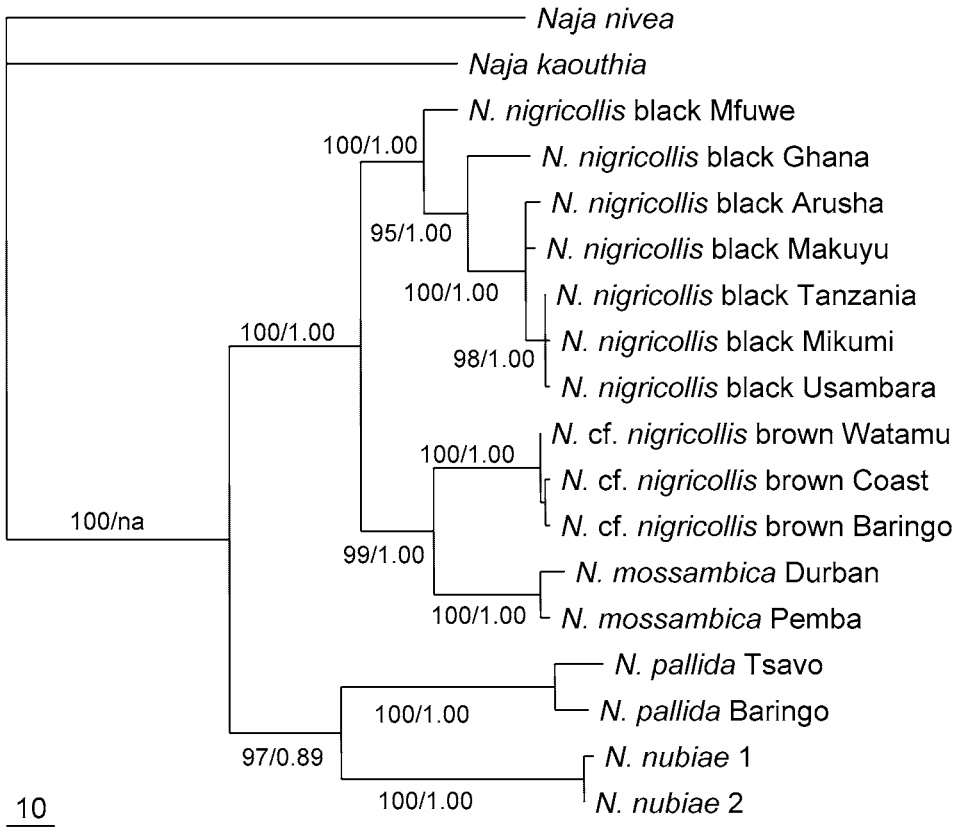

FIGURE 3. One of two equally most parsimonious trees of the combined cytochrome *b* and ND4 data. Numbers along branches indicate % bootstrap support (MP) and Bayesian posterior probability.

For Bayesian analysis, the models of sequence evolution identified as optimal by MrModeltest for the six data partitions used in this study are shown in Table 2. These were implemented for the six data partitions. Burn-in was achieved after approximately 80,000 generations, but we conservatively discarded all trees produced in the first 500,000 generations. The Bayesian tree was entirely congruent with one of the two MP trees resulting from the Bayesian analysis, except that haplotypes *N. nigricollis* Arusha and *N. nigricollis* Makuyu are shown as monophyletic in the Bayesian analysis, whereas the relationships between them and a clade of three Tanzanian haplotypes was unresolved in the MP analysis. Bayesian posterior probabilities are shown in Fig. 3.

TABLE 2. Models of sequence evolution applicable to the data partitions selected for Bayesian inference under the Akaike Information Criterion.

	ND4	cytochrome *b*
Position 1	HKY	HKY + I
Position 2	HKY	HKY
Position 3	GTR + I	HKY + G

TABLE 3. Pairwise matrix of p-distances (lower left) and associated standard errors (upper right) between the five African spitting cobra species included in this study.

	nigricollis	ashei	pallida	nubiae	mossambica
nigricollis		0.0053	0.0069	0.0072	0.0056
ashei	0.0462		0.0071	0.0080	0.0046
pallida	0.0871	0.0903		0.0072	0.0072
nubiae	0.0839	0.0926	0.0786		0.0080
mossambica	0.0489	0.0362	0.0907	0.0932	

All trees showed a clade consisting of *N. nubiae* and *N. pallida* to be the sister group of the remaining spitting cobras included in the analysis. Within the remaining spitting cobras, the haplotypes of *N. nigricollis* are polyphyletic: the eastern African brown "*N.* cf. *nigricollis*" haplotypes (samples from Watamu, Diani Beach and Lake Baringo, all in Kenya) consistently grouped as the sister taxon of *N. mossambica*, not *N. nigricollis*. This relationship was supported by strong bootstrap and Bayesian posterior probability support.

The monophyly of the haplotypes traditionally attributed to *N. nigricollis* was rejected by significant Wilcoxon signed-ranks tests (length difference = 9 steps, -Z = 2.3238–2.4962, $P < 0.05$)

Systematics

Our molecular results show that *N. nigricollis* in its traditional sense is not a monophyletic taxon, as the large brown from the eastern African coast shares a more recent common ancestor with *N. mossambica* than with *N. nigricollis*. Our morphological analyses and comparisons show that the brown spitting cobras from northern and eastern Kenya, Ethiopia and southern Somalia represent a taxon clearly distinguishable from other *N. nigricollis* in a number of features of colour pattern and scalation. Given the congruent patterns of variation in morphology and mtDNA, we consider our data to provide evidence that this form represents a separate evolutionary species from both *N. nigricollis* and *N. mossambica*. As summarised in the introduction, a number of taxa have been described within the *Naja nigricollis* complex, but none of these names appear to be applicable to this brown spitting cobra. No previously described spitting cobra taxon has its type locality within the range of the brown form (Fig. 1), and all existing names, in particular *nigricollis* (type locality: Ghana), *crawshayi* (type locality: Lake Mweru, Zambia/DRC) and *atriceps* (type locality: Burundi) are applicable to sets of populations of largely black spitting cobras that were represented in our morphological and/or molecular analyses, and were shown to be highly distinct from the brown form. Since no name appears to be available for the latter, we describe it as new:

Naja ashei sp. nov. — Ashe's spitting cobra

Holotype. National Museums of Kenya NMK S/3993, a female specimen from Watamu, Kenya (3º 21'S: 40º 01'E), coll. Royjan Taylor, maintained in captivity at Bio-Ken Snake farm until 29/09/2004 with reference

number BK 10030 (Fig. 4,5).

Paratypes (three males and two females):

BMNH 1955.1.12.4a and 4b (Kilifi, Kenya) BMNH 1963.456 (Kiboko, Kenya); BMNH 2005.1604 (Baringo, Kenya); NMZB 3349 (Ex USNM 40954) (Guaso Nyiro [=Ewaso Ng'iro], Kenya).

Diagnosis. *Naja ashei* differs from all other African spitting cobras in possessing a unique clade of mtDNA haplotypes. From the data presented here, we identified 12 fixed nucleotide differences that differentiate *N. ashei* from the other eastern African spitting *Naja*. These correspond to positions 105, 169 and 315 of the ND4 sequence of the holotype (DQ897706), and to positions 60, 108, 153, 201, 348, 381, 507, 630 and 676 of the cytochrome b sequence of the same specimen (DQ897749), the diagnostic bases at these positions being C, T, G, C, G, T, T, T, A, C, T and A, respectively.

Morphologically, *N. ashei* differs from East African *N. nigricollis* in a number of characters relating to adult colour pattern and scalation. In particular, its midbody and posterior ventral colour is predominantly light, with dark pigment encroaching mostly from the sides of the body (venter normally largely or entirely dark in *N. nigricollis*), it lacks any red, orange or pink pigment under the throat (usually pronounced in *N. nigricollis*), and the head is the same olive-brown colour as the rest of the body (often black above and below in East African *N. nigricollis*). Scalation does not provide any absolutely diagnostic characters for *N. ashei*, but mean scale counts and the range differ clearly from those of East African *N. nigricollis* (Table 4). In particular, *N. ashei* can be distinguished from most eastern African *N. nigricollis* by the combination of high ventral scale and dorsal scale row counts. Most *N. ashei* have over 195 ventrals and at least 21 and typically more scale rows around the neck, whereas most *N. nigricollis* with 195 or more ventrals have at most 21, and usually 19 or fewer scale rows around the neck, whereas higher scale row counts around the neck tend to be found in specimens with fewer ventral scales.

Naja ashei differs from the more closely related *N. mossambica* in lacking any dark edges on the labial scales and ventral scales, in having a less complex ventral banding pattern, and in having higher average ventral scale counts, but lower dorsal scale row counts. *Naja pallida* and *N. nubiae* differ in having higher midbody dorsal scale row counts (usually 25, compared to 21–23 in *N. ashei*). In addition, *N. pallida* differs from *N. ashei* in having a single, very clearly defined and clean-edged throat band (which very obviously crosses the neck except in older, darker specimens), in usually having higher ventral scale counts, and in the frequent presence of a single preocular and seven supralabials. *Naja nubiae* also has a cleaner, neater throat pattern, and two dark bands across the neck and two or three across the throat; a characteristic black tear-drop marking (consisting of dark edges to the supralabial suture below the eye) is almost invariably present; moreover, *N. nubiae* has almost consistently higher ventral scale counts, and often has seven supralabials and/or a single preocular (see Wüster & Broadley, 2003). *Naja katiensis* has consistently lower ventral and subcaudal scale counts (Table 4), a much smaller adult size, and lacks cuneate scales. Among the non-spitting cobras, *N. ashei* is most likely to be confused with *N. haje*, on account of its drab brownish coloration and large size. However, *N. haje* differs in having a single preocular, a row of suboculars separating the eyes from the supralabials, a greatly enlarged sixth supralabial, a single anterior temporal, and in lacking spitting adaptations to the fangs (Bogert, 1943), and thus being incapable of spitting venom. *Naja melanoleuca* similarly differs from *N. ashei* in having a single preocular, no suboculars, an enlarged sixth supralabial and a single anterior temporal.

Description of holotype. Body dimensions: Snout-vent length 1268 mm, tail length 239 mm, dorsal head length (snout to end of parietal suture) 33.3 mm, lateral head length (snout to posterior end of lower jaw articulation) 51.7 mm. Head width across supraoculars 19.7 mm, maximum overall width of head 39.7 mm.

Head broad, heart-shaped from above. Eye small to moderate, diameter much less than distance from mouth or from nostril.

Body scalation: 197 ventrals, 55 subcaudals, all paired except for the first, the intact tail terminates in a spine. Dorsal scale rows: 23 on neck, 21 at midbody, 15 one head length ahead of vent.

TABLE 4. Scale counts for the African species of spitting cobra.

	pallida	*nubiae*	*katiensis*	*nigricollis* (West)	*nigricollis* (Central)	*nigricollis* (South)
Scale rows on neck						
N	97	36	26	108	62	230
Range	23–30	23–27	23–27	19–23	17–23	17–23
Mean	26.32	25.28	23.61	20.26	20.25	18.64
Standard Deviation	1.59	1.32	1.1	1.24	1.23	1.46
Scale rows at midbody						
N	103	38	25	129	65	230
Range	21–27	23–27	23–25	19–23	17–21	17–21
Mean	25.34	24.6	24.52	21.49	18.77	19.57
Standard Deviation	1.15	1.03	0.82	1.05	1.14	1.19
Ventrals - males						
N	55	19	15	70	41	134
Range	192–218	207–221	165–174	185–209	177–203	175–194
Mean	204.82	212.89	169.07	198.29	191.63	184.82
Standard Deviation	5.65	3.75	2.84	4.88	4.35	4.96
Ventrals - Females						
N	46	20	11	75	21	94
Range	203–227	207–226	167–176	195–212	187–209	178–201
Mean	211.13	219	172.45	203.23	195.16	188.55
Standard Deviation	5.81	4.76	2.98	4.21	5.65	5.99
Subcaudals - Males						
N	47	18	13	57	31	132
Range	56–81	59–69	49–57	54–70	56–65	55–68
Mean	67.45	64.59	53.62	63.89	61.27	60.67
Standard Deviation	4.78	2.37	2.33	3.36	2.19	2.95
Subcaudals - Females						
N	42	18	6	62	20	91
Range	57–72	58–69	47–56	55–66	50–60	47–63
Mean	64.36	64.16	50.83	59.15	57.01	56.74
Standard Deviation	4.08	2.96	3.49	7.38	2.69	3.03

continued.

	ashei	*mossambica*	*nigricollis nigricincta*	*nigricollis woodi*
Scale rows on neck				
N	42	553	155	52
Range	21-25	21–29	21–25	21–25
Mean	22.69	24.46	22.14	22.1
Standard Deviation	1.26	1.36	1.2	1.03
Scale rows at midbody				
N	42	556	160	54
Range	20–23	19–27	21–23	21–22

Mean	21.31	23.31	21.27	21.02
Standard Deviation	0.72	1	0.67	0.14
Ventrals - males				
N	19	304	45	18
Range	192–204	170–196	190–218	213–231
Mean	197.79	186.57	205.07	224.28
Standard Deviation	3.46	4.58	7.28	3.54
Ventrals - Females				
N	12	243	39	24
Range	194–207	182–207	196–226	216–231
Mean	200.00	193.59	212.41	224.67
Standard Deviation	3.88	5.14	5.94	3.71
Subcaudals - Males				
N	19	260	41	25
Range	57–65	51–71	59–73	65–74
Mean	60.58	60.98	68.17	69.56
Standard Deviation	2.19	3.96	3.65	2.53
Subcaudals - Females				
N	11	201	34	21
Range	55–62	49–70	57–71	59–78
Mean	58.00	59.02	65.85	67.52
Standard Deviation	2.53	4.24	3.42	4.5

Dorsal scale row reduction formula: 25 5+6(2) 24 7+8(4) 23 4+5(12/13) 21 4+5/5+6(21) 19 +6(30/30) 21 4+5/5+6(122) 19 4+5/5+6(131) 17 4+5(151/154) 15 3+4(186) 14 +4(187) 15 4+5(191/194) 13 +3(195/195) 15

Caudal scale reduction formula: 11 2+3(2) 10 2+3(3/3) 8 4+5(5) 7 3+4(6) 6 2+3(16/17) 4

Head scalation: Preoculars 2/2, postoculars 2/2, supralabials 6/6, third enters eye, infralabials 8/9, first four contact anterior chin shields. On the left, infralabials 5 and 6 are homologous to the cuneate scales of Asiatic cobras (Wüster, 1998), whereas on the right hand side, infralabials five and seven are cuneates (Fig. 5). Anterior temporals 2/2, posterior temporals 5/5. Seven temporals and nuchals contact the lateral and posterior edges of the parietals. Rostral 1.5 times wider than high, visible from above. Posterior chin shields separated by two rows of smaller, elongate scales. Nasal scale entirely divided into a prenasal and a postnasal scale by the large, vertically elongate nostril. Frontal longer than wide (9.0 x 7.1 mm), slightly shorter than distance from rostral (10.3 mm), shorter than supraoculars (12.0 mm), widest along anterior edge; shape pentagonal, anterior edge straight, posterior edge ends in obtuse angle, border with supraoculars slightly concave.

Colour and pattern in life: Head uniformly brownish olive on top, paler and greyer in supralabial region and around eye. Underside of head very finely dusted with brownish grey pigment, scale bases cream, overall impression light brownish grey. Dorsal colour generally olive-brown. Neck immediately posterior to head darker than top of head or the remainder of the dorsum. Otherwise, overall appearance largely uniform. Most dorsal scales with a slightly lighter lower basal edge. Interstitial skin mostly dark grey, with indistinct lighter variegations, visible especially when exposed by inflation of the body. Dorsal scales within lighter variegations have more pronounced light bases, giving an indistinct mottled appearance. Throat and ventral pattern (Fig. 5): first seven ventrals heavily mottled with greyish brown, scale bases creamy-white, light area sharply demarcated from darker pigment. Ventrals 8–10 similarly patterned, but with a slightly darker, more saturated

brown pigment, covering 85–90% of each scale except the base near the middle of the scale. Ventrals 11–13 as ventrals 1–7. Ventrals 14–20 almost entirely covered with pigment of intermediate density, with only a few lighter flecks on some scale bases. The remainder of the ventral and subcaudal scales are creamish with isolated blotches of greyish-brown pigment. Distal lateral tips of the ventrals also covered in greyish brown pigment, which forms a continuation of the colour of the lower dorsal scale rows. There are no dark scale bases or edges on the ventral surface.

Variation for all material examined. Variation in scale counts in *N. ashei* and other African spitting cobras is given in Table 4. In addition to the characters listed there, *N. ashei* is notable for frequently having only two postocular scales, rather than three. Among the specimens included in our principal components analysis, eight out of fifteen *N. ashei* had two postoculars on at least one side, compared to one out of twenty-nine *N. nigricollis*. Variation in colour and pattern concerns especially the ventral pattern. The first ventrals may be largely light or more or less heavily suffused with dark pigment, but the transition from these to the main dark throat band normally remains distinguishable. Juveniles have a lighter dorsal ground colour, often with a faint "herring-bone" pattern, but the top and upper sides of the head and the neck are dark greyish brown (Fig. 6). The darker colour on the neck is more intense on the sides (where it merges into the dark throat band), and gradually merges into the dorsal body colour, without there being a clearly defined band.

Size. This appears to be the largest spitting cobra, at least in terms of average size. Largest male examined (NMK/O 2401—Nguni, Kitui District, Kenya) 1750 + 360 = 2110 mm; largest female (NMK, unnumbered, "Kenya") 1800 + 350 = 2150 mm. However, giant specimens are generally underrepresented in collections. Specimens measuring 2 metres are not rare along the Kenyan coast, and a number of specimens of well over 2 metres in total length have been recorded. Pitman (1974) records males with total lengths of 2743 and 2311 mm from the Baringo region of Kenya, which are almost certainly referable to *N. ashei*. However, a record specimen measuring 2819 mm (Seronera, Serengeti National Park, Tanzania—Pitman, 1974) cannot confidently be attributed to *N. ashei*, as there are no records of the species from the park, and some northern Tanzanian *N. nigricollis* also reach very large sizes (W.W., pers. obs.).

FIGURE 4. Holotype of *Naja ashei* (NMK S/3993) in life.

Etymology. We dedicate this species to the memory of the late James Ashe (1925–2004), in recognition of his contributions to East African herpetology, of the inspiration he gave to others working on the herpetofauna of this part of the world (see Spawls, 2004), of his early recognition of the distinctiveness of the species that now bears his name, and in gratitude for his support for this work.

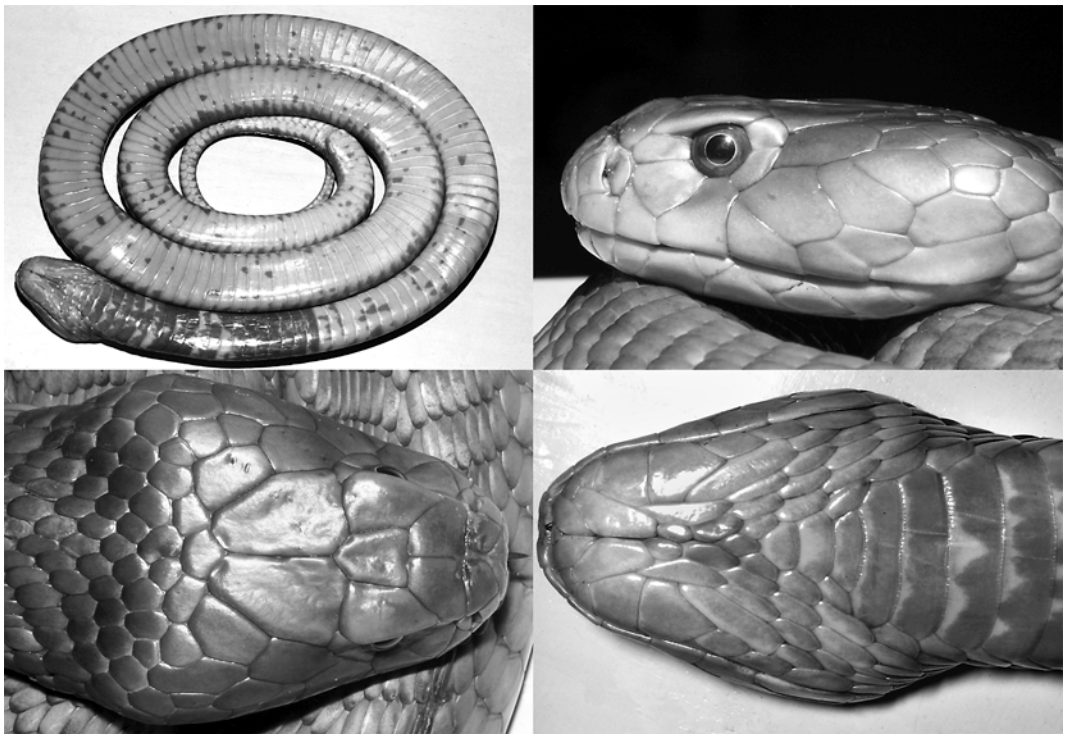

FIGURE 5. Holotype of *Naja ashei.* Note in particular the predominantly light ventral surface without black edges to the ventrals, and the presence of only two postoculars.

FIGURE 6. Juvenile specimen (total length approx 55 cm) from Watamu, Kenya, illustrating pattern and coloration (BioKen, Watamu, live collection).

Distribution. *Naja ashei* appears to be sympatric with *N. pallida* over much of its range, i.e. dry lowland regions of northern and coastal Kenya, extending south along the coast to at least Diani Beach and north into southern Somalia and south-eastern Ethiopia. It occurs in northeast Uganda at Amudat in Karamoja District (BMNH 1954.1.12.46, 1974.5145–7). It probably also occurs in the far north and/or northeast of Tanzania, but there appear to be no confirmed records. It should be looked for in the Serengeti National Park and the northernmost parts of the Tanzanian coast. The brown-headed and often very large spitting cobras from the Usambara Mountains and the central coastal region of Tanzania are referable to *N. nigricollis*, as demonstrated by our molecular analyses here. The northern and western distributional limits of *N. ashei* remain somewhat unclear. Some specimens of *N. nigricollis* from southern Sudan, northern Uganda and north-eastern Congo are also brownish above, but differ from *N. ashei* as highlighted in the diagnosis. However, the precise distributions of these forms require further investigation. The isolated population of spitting cobras assigned to *N.*

nigricollis by Wüster & Broadley (2003), from Jebel Marra, Darfur Province, Sudan, where it occurs sympatrically with *N. nubiae*, also superficially resembles *N. ashei* due to its colour pattern, but clusters with *N. nigricollis* in our multivariate analyses. Further genetic studies are required to ascertain the status of this form.

Medical relevance. As always, the discovery of a new species of venomous snake raises the question of whether existing antivenoms provide adequate protection (Wüster & McCarthy, 1996; Fry *et al.*, 2003). The question is particularly relevant as large *Naja ashei* can secrete prodigious quantities of venom. A large specimen milked at the Bio-Ken snake farm in Watamu, Kenya, produced 6.2 ml of liquid venom, weighing 7.1 g (Fig. 7). Dry weight was not recorded, but if the ratio of 34.6–41.3% solids by weight obtained by Mirtschin *et al.* (2006) from a selection of four species of *Naja* applies to *N. ashei*, then this suggests venom yields of up to 3 grams of dry venom, a record-breaking yield emphasising the potential danger of this species. Case histories have not been documented specifically for *N. ashei*, but bites by African spitting *Naja* typically result in severe necrosis (Warrell *et al.*, 1976; Tilbury, 1982), but often limited systemic symptoms.

FIGURE 7. Venom extraction from an adult specimen of *Naja ashei*, illustrating the enormous quantities of venom secreted by this species. All the venom at the bottom of the receptacle (6.2 ml) stems from this specimen.

Acknowledgements

We are deeply grateful to the numerous people who supplied us with samples, access to preserved specimens, or other logistical help, particularly Joe Beraducci (MBT, Arusha, Tanzania), Deon Naude (Meserani Snake Park, Arusha), the late James Ashe, Sanda Ashe, Royjan Taylor, Anthony Childs (Bio-Ken Snake Farm, Watamu, Kenya), Patrick Malonza and Domnick Victor Wasonga (National Museum of Kenya, Nairobi), Jonathan Leakey and Dena Crain (Lake Baringo, Kenya), Phil Berry (Mfuwe, Zambia), Craig Doria (Arusha,

Tanzania), R. David G. Theakston and Paul D. Rowley (Liverpool School of Tropical Medicine), Hans-Werner Herrmann (CRES), Colin Tilbury (University of Stellenbosch, S. Africa), Colin McCarthy (Natural History Museum, London), Natalia B. Ananjeva and Konstantin D. Milto (Russian Academy of Sciences, St. Peterburg) the late Jens B. Rasmussen (Zoological Museum, University of Copenhagen), Esther Wenman and Heather Hall (Zoological Society of London). Richard Cooper, Steven Crookes, Carlotta E. Ercolani, Catharine E. Pook and Ina Schättler contributed to the molecular work. This study was funded in part by a grant from the Leverhulme Trust to WW.

References

Angel, F. (1922) Sur une collection de reptiles et de batraciens, recueillis au Soudan français par la mission du Dr. Millet Horsin. *Bulletin du Muséum national d'Histoire naturelle de Paris* 28, 39–41.

Arévalo, E., Davis, S.K. & Sites, J.W. (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. *Systematic Biology*, 43, 387–418.

Bocage, J. V. Barboza du (1895) *Herpétologie d'Angola et du Congo.* Imprimerie Nationale, Lisbon, 203 pp.

Bogert, C.M. (1940) Herpetological results of the Vernay-Angola Expedition, with notes on African reptiles in other collections. I. Snakes, including an arrangement of African Colubridae. *Bulletin of the American Museum of Natural History*, 77, 1–107.

Bogert, C.M. (1943) Dentitional phenomena in cobras and other elapids, with notes on adaptive modification of fangs. *Bulletin of the American Museum of Natural History*, 81, 285–360.

Boulenger, G.A. (1896) *Catalogue of the Snakes in the British Museum (Natural History)* 3. British Museum (Natural History), London, 727 pp.

Boycott, R.C. & Haacke, W.D. (1979) Note on the type-locality, distribution and juvenile colouration of *Naja nigricollis woodi* (Serpentes: Elapidae) and an account of the colour- pattern variation in intergrade populations. *Annals of the Cape Provincial Museums (Natural History)*, 13, 31–38.

Branch, W. R. (1979) The venomous snakes of southern Africa Part 2. Elapidae and Hydrophidae. *Snake*, 11, 199-225.

Broadley, D.G. (1968) A review of the African cobras of the genus *Naja* (Serpentes: Elapinae). *Arnoldia*, 3, 1–14.

Broadley, D.G. (1974) A review of the cobras of the *Naja nigricollis* complex in south-western Africa. *Cimbebasia (A)*, 2,155–162.

Broadley, D.G. & Wüster W. (2004) A review of the southern African 'non-spitting' cobras (Serpentes: Elapidae: *Naja*). *African Journal of Herpetology*, 53,101–122.

Burbrink, F.T., Lawson, R. & Slowinski, J.B. (2000) Mitochondrial DNA phylogeography of the polytypic North American Rat Snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution*, 54, 2107–2118.

Castoe, T.C., Doan, T.M. & Parkinson, C.L. (2004) Data partitions and complex models in Bayesian analysis: the phylogeny of gymnophthalmid lizards. *Systematic Biology*, 53, 448–469.

Castoe, T.A. & Parkinson, C.L. (2006) Bayesian mixed models and the phylogeny of pitvipers (Viperidae: Serpentes) *Molecular Phylogenetics and Evolution*, 39, 99–110.

Castoe, T.C., Sasa, M. & Parkinson, C.L. (2005) Modeling nucleotide evolution at the mesoscale: The phylogeny of the Neotropical pitvipers of the *Porthidium* group (Viperidae: Crotalinae). *Molecular Phylogenetics and Evolution*, 37, 881–898.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791.

Fry, B.G., Winkel, K.D., Wickramaratna, J.C., Hodgson, W.C. & Wüster, W. (2003) Effectiveness of snake antivenom: species and regional venom variation and its clinical impact. *Journal of Toxicology -Toxin Reviews*, 22, 23–34.

Good, D.A. (1994) Species limits in the genus *Gerrhonotus* (Squamata: Anguidae). *Herpetological Monographs*, 7, 180–202.

Günther, A. (1894) Second report on the reptiles and batrachians transmitted by Mr. H.H. Johnston, C.B., from British Central Africa. *Proceedings of the Zoological Society of London*, 1893, 616–628.

Hillis, D.M. & Huelsenbeck, J.P. (1992) Signal, noise and reliability in phylogenetic analyses. *Journal of Heredity*, 83, 189–195.

Hughes, B. (1983) African snake faunas. *Bonner Zoologische Beiträge*, 34, 311-356.

Hughes, B. & Barry, D. H. (1969) The snakes of Ghana. *Bulletin de l'Institut fondamental d'Afrique noire*, A31, 1004-1041.

Kumar, S., Tamura, K., Jakobsen, I.B. & Nei., M. (2001) MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* ,17, 1244–1245

Laurent, R.F. (1955) Diagnoses préliminaires de quelques serpents venimeux. *Revue de Zoologie et Botanique Africaine*, 51, 127–139.
Laurent, R.F. (1956) Contribution à l'herépetologie de la région des Grands Lacs de l'Afrique centrale. I. Généralitiés. II. Chéloniens. III. Ophidiens. *Annales du Musée royal du Congo Belge, Série in 8o*, 48, 1–390.
Laurent, R.F. (1964) Reptiles et Amphibiens de l'Angola. (Troisième contribution). *Publicações Culturais da Comphania de Diamantes de Angola*, 67, 11–165.
Laurent, R.F. (1973) Le concept de sous-espèce et la variation géographique chez *Naja melanoleuca* et *Naja nigricollis*. *Revue de Zoologie et Botanique Africaine*, 87, 198–204.
Leviton, A.E., Gibbs, R.H. Jr., Heal, E. & Dawson, C.E. (1985) Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985, 802-832.
Leviton, A.E. & Gibbs, R.H. Jr. (1988). Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Supplement No. 1: Additions and corrections. Copeia 1988, 280-282.
Mirtschin, P.J., Dunstan, N., Hough, B., Hamilton, E., Klein, S., Lucas, J., Millar, D., Madaras, F.& Nias, T. (2006) Venom yields from Australian and some other species of snakes. *Ecotoxicology*, 15, 531–538
Nylander, J.A.A. (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.
Ogden, R. & Thorpe, R.S. (2002) Molecular evidence for ecological speciation in tropical habitats. *Proceedings of the National Academy of Sciences*, 99, 13612–13615.
Palumbi, S.R. (1996) Nucleic acids II: the polymerase chain reaction. *In*: Hillis, D.M., Moritz, C. & Mable, B.K. (Eds.), *Molecular Systematics. Second Edition.* Sinauer, Sunderland, Massachusetts, pp. 205–247.
Peters, W.C.H. (1854) Diagnosen neuer Batrachier, welche zusammen mit der früher (24. Juli und 17. August) gegebenen Übersicht der Schlangen und Eidechsen mitgetheilt werden. *Bericht über zur Bekanntmachung geeignete Verhandlungen der Königlichen preussischen Akademie der Wissenschaften zu Berlin*, 1854, 614–628.
Pitman, C.R.S. (1974). *A Guide to the Snakes of Uganda.* Revised Edition. Wheldon & Wesley, Codicote, 290 pp.
Pringle, J.A. (1955) A new subspecies of the spitting cobra *Naja nigricollis* Reinhardt from the Cape Province. *Annals of the Natal Museum*, 13, 253–254.
Puorto, G., Salomão, M.G., Theakston, R.D.G., Thorpe, R.S., Warrell, D.A. & Wüster, W. (2001) Combining mitochondrial DNA sequences and morphological data to infer species boundaries: phylogeography of lanceheaded pitvipers in the Brazilian Atlantic forest, and the status of *Bothrops pradoi* (Squamata: Serpentes: Viperidae). *Journal of Evolutionary Biology*, 14, 527–538
Reinhardt, J.T. (1843) Beskrivelse af nogle nye Slangearter. *Det Kongelige Danske Videnskabernes Selskabs Naturvidenskabelige og Mathematiske Afhandlinger*, 10, 233–277.
Roman, B. (1968) Une nouvelle espèce de *Naja* en Haute-Volta. *Carrefour Africain* 299: 273–274, 286, 310.
Roman, B. (1969) Les *Naja* de Haute-Volta. *Revue de Zoologie et Botanique Africaine*, 82, 1–14.
Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
Sambrook, F., Fritsch, E.F. & Maniatis, T (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
Slowinski, J.B. & Wüster, W. (2000) A new cobra (Elapidae: *Naja*) from Myanmar (Burma). *Herpetologica*, 56, 257–270.
Smith, A. (1838) Contributions to South African Zoology. *Magazine of Natural History*, 2, 30–33, 92–94.
Smith, A. (1842) *Illustrations of the Zoology of South Africa—Reptilia*. London: Smith. Elder & Co., Pls. i–ixxviii. Part 16 (Plate x).
Spawls, S. (2004) James Ashe: 1925–2004. African Journal of Herpetology, 53, 203–204.
Spawls, S. & Branch, B. (1995) The Dangerous Snakes of Africa. Blandford, London, UK, 192 pp..
Spawls S., Howell K., Drewes R. & Ashe J. (2002) A field guide to the reptiles of East Africa. Academic Press, London, 543 pp.
Swofford, D.L. (2002) PAUP*—Phylogenetic Analysis Using Parsimony (*and Other Methods). Beta version 4.0b10. Sinauer, Sunderland.
Templeton, A.R. (1983) Phylogenetic inference from restriction endonuclease cleavage sites maps with particular reference to the evolution of humans and the apes. *Evolution*, 37, 221–244.
Thorpe, R.S. & Richard, M. (2001) Evidence that ultraviolet markings are associated with patterns of molecular gene flow. *Proceedings of the National Academy of Sciences*, 98, 3929–3934.
Thorpe, R.S., Brown, R.P., Day, M., Malhotra, A., McGregor, D.P. & Wüster, W. (1994) Testing ecological and phylogenetic hypotheses in microevolutionary studies: an overview. *In*: Eggleton, P. & Vane-Wright, R. (Eds.), *Phylogenetics and Ecology.* Academic Press, London, pp. 189–206.
Thorpe, R.S., Malhotra, A. Black, H., Daltry, J.C. & Wüster, W. (1995) Relating geographic pattern to phylogenetic process. *Philosophical Transactions of the Royal Society of London, B.*, 349, 61–68.

Tilbury, C.R. (1982) Observations on the bite of the Mozambique spitting cobra (*Naja mossambica mossambica*). *South African Medical Journal*, 61, 308–313.
Warrell, D.A., B.M. Greenwood, N. McD. Davidson, L.D. Ormerod & C.R.M. Prentice (1976) Necrosis, haemorrhage and complement depletion following bites by the spitting cobra (*Naja nigricollis*). *Quarterly Journal of Medicine, New Series*, 45, 1–22.
Wüster, W. (1996) Taxonomic changes and toxinology: systematic revisions of the Asiatic cobras (*Naja naja* species complex). *Toxicon*, 34, 399–406.
Wüster, W. (1998) The cobras of the genus *Naja* in India. *Hamadryad*, 23, 15–32.
Wüster, W. & Broadley, D.G. (2003) A new species of spitting cobra from north-eastern Africa (Serpentes: Elapidae: Naja). *Journal of Zoology, London*, 259, 345–359.
Wüster, W. & McCarthy, C.J. (1996) Venomous snake systematics: implications for snakebite treatment and toxinology. In C. Bon & M. Goyffon (Eds.), *Envenomings and their Treatments*. Fondation Mérieux, Lyon, pp. 13–23.
Wüster, W. & Thorpe, R.S. (1989) Population affinities of the Asiatic cobra (*Naja naja*) species complex in south-east Asia: reliability and random resampling. *Biological Journal of the Linnean Society*, 36, 391–409.
Wüster, W. & Thorpe, R.S. (1991) Asiatic cobras: systematics and snakebite. *Experientia*, 47, 205–209.
Wüster, W. & Thorpe, R.S. (1992) Asiatic cobras: population systematics of the *Naja naja* species complex (Serpentes: Elapidae) in India and Central Asia. *Herpetologica*, 48, 69–85.
Wüster, W., Thorpe, R.S., Cox, M.J., Jintakune, P., Nabhitabhata, J. (1995) Population systematics of the snake genus *Naja* (Reptilia: Serpentes: Elapidae) in Indochina: multivariate morphometrics and comparative mitochondrial DNA sequencing (cytochrome oxidase I). *Journal of Evolutionary Biology*, 8, 493–510.
Wüster, W., Warrell, D.A., Cox, M.J. Jintakune, P., Nabhitabhata, J. (1997) Redescription of *Naja siamensis* Laurenti, 1768 (Serpentes: Elapidae), a widely overlooked spitting cobra from Southeast Asia: geographic variation, medical importance and designation of a neotype. *Journal of Zoology, London*, 243, 771–788.
Zhang, D.-X. & Hewitt, G.M. (1996) Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution*, 11, 247–251.

Appendix 1.

Specimens examined for morphological analyses and range of variation. Institutional acronyms follow Leviton *et al.* (1985), and Leviton & Gibbs (1988). Additional acronyms: AAU/H: Addis Ababa University Museum; MA/A: Awash National Park.

Naja ashei. Included in PCA: KENYA: Watamu: NMK S/3993, BioKen live collection 10510, 10564, 10655, 10656, 10657, 10658, unregistered juvenile; Kiboko: BMNH 1963.456; Kilifi: BMNH 1955.1.12.4a–b; West of Isiolo: NMK 0/3065; Baringo: BMNH 2005.1604. ETHIOPIA: Arba Minch, Gemu Gofa: ZFMK 15883; Awash National Park: BMNH 1973.3271. Additional material used to compile variation in scale counts: KENYA: AMNH 73368; Isiolo: CAS 123159, NMK O/3221; Mombasa: MCZ 18234, NMK O/1484; Kataungi: ZMB 17471; Nguni, Kitui District: NMK O/2401; Kiboko Ranger Station: NMK O/2956; Gede Forest Station: NMK O/3166; Marich Pass, West Pokot Dist.: CAS 154409; Wei Wei River, 5 km N. Sigor, West Pokot: LACM 63380, 63391; Craig Farm: LACM 63389; Hills west of Mt. Kenya: MCZ 7988; Guaso Nyiro/Uaso Nyiro River: NMZB 3349, MCZ 9044; Lake Turkana: BMNH 95.12.31.25; Buna, Wajir Dist.: CAS 130093. ETHIOPIA: 25 km NE Afdem: AAU H 416; Arba Minch: AAU H 636; Awash National Park: BMNH 1973.3273-4, MA A 047-8; Owaramulka: BMNH 1916.6.24.14; Kalam, Omo River: USNM 218621. SOMALIA: Mareri, Lower Juba River: CAS 153434-5.

Naja nigricollis (material included in PCA only): KENYA: Darinyiro Ranch, Laikipia, Kenya: NMK S/3119; Ol'Manyatta Estate, Subukia: BMNH 1932.9.8.5. TANZANIA: Liwale: BMNH 1959.1.7.30; Tunduru: BMNH 1952.1.9.97–98a,b; Bombani, near Amani, Usambara Mountains: J. Beraducci, personal collection, 2 specimens. UGANDA: Eastern Province: BMNH 1933.9.8.38; Kaimja, Lake Edward, Tor: BMNH 1959.1.7.26–27; Mokia, SE Ruwenzori, Uganda: BMNH 1907.4.30.11; Bussu: BMNH 1911.7.8.17–18; Serere Teso: BMNH 1959.1.7.25, 1959.1.7.35; ETHIOPIA: 10 km W Mabel, Blue Nile Gorge: BMNH 1973.3184. RWANDA: Mpanga Ranch: ZFMK 57283. DRC: Rwindi, Kivu: ZFMK 50004 (Paratype, *Naja nigricollis atriceps* Laurent). RWANDA: Butare: ZIL 19379. GUINEA BISSAU: Bubaque, Bijagos Archipelago: ZFMK 58330; SENEGAL: ZFMK 17579–80. LIBERIA: Voinjama: NHRM, uncatalogued specimen.

Naja cf. *nigricollis*: SUDAN: Djebel Marra: ZFMK 39878, 39879, 39882, 39883, 39884.

Appendix 2

Samples, haplotypes and GenBank accession numbers of sequences used. GenBank accession numbers with asterisks were not generated by the authors. Institutional acronyms follow Leviton *et al*. (1985), and Leviton & Gibbs (1988); JB = Joe Beraducci, live collection; LSTM = Liverpool School of Tropical Medicine, live collection, WW = Wolfgang Wüster

Taxon	Locality	Voucher(s) / sample(s)	Haplotype label (Fig. 3)	Genbank accession number (ND4, cyt*b*)
Naja nigricollis	Ghana	LSTM, live coll. / WW842	*N. nigricollis* Ghana	**DQ897695, DQ897738**
Naja nigricollis	Arusha, Tanzania	WW296	*N. nigricollis* Arusha	**DQ897698, DQ897741**
Naja nigricollis	Kigosi Camp, Tanzania	WW297	*N. nigricollis* Tanzania	**DQ897699, DQ897742**
Naja nigricollis	Mbwewe, Tanzania	JB, live coll. / WW1404, WW1405	*N. nigricollis* Tanzania	**DQ897699, DQ897742**
Naja nigricollis	Bombani, Amani, Tanzania	JB, live coll. / WW1403, WW1406, WW1407	*N. nigricollis* Usambara	**DQ897703, DQ897746**
Naja nigricollis	Bulyanhulu, Tanzania	NMZB 15423 / WW1614	*N. nigricollis* Tanzania	**DQ897699, DQ897742**
Naja nigricollis	Makuyu, Kenya	Bio-Ken live coll. BK10221, BK 10246 / WW1271, WW1272	*N. nigricollis* Makuyu	**DQ897700, DQ897743**
Naja nigricollis	Mfuwe, Chipata District, Zambia	NMZB 16970, NMZB 17353 / WW1198, WW1393	*N. nigricollis* Mfuwe	**DQ897701, DQ897744**
Naja nigricollis	Mikumi NP, Tanzania	WW1415	*N. nigricollis* Mikumi	**DQ897702, DQ897745**
Naja ashei	Watamu, Kenya—HOLOTYPE	NMK S-3993 / WW1430	*N.* cf. *nigricollis* brown Watamu	**DQ897706, DQ897749**
Naja ashei	Watamu, Kenya	Bio-Ken live coll. BK10022, BK10177 / WW1267, WW1268	*N.* cf. *nigricollis* brown Coast	**DQ897704, DQ897747**
Naja ashei	Diani, Kenya	Bio-Ken live coll. BK10248 / WW1270	*N.* cf. *nigricollis* brown Coast	**DQ897704, DQ897747**
Naja ashei	Baringo, Kenya	BMNH 2005.1604	*N.* cf. *nigricollis* brown Baringo	**DQ897705, DQ897748**
Naja pallida	Tsavo East N.P., Kenya	Bio-Ken live coll. BK-10054 / WW1273	*N. pallida* Tsavo	**DQ897715, DQ897758**
Naja pallida	Lake Baringo, Kenya	Bio-Ken live coll. BK10659 / WW1431	*N. pallida* Baringo	**DQ897717, DQ897760**
Naja nubiae	Unknown	ZSL live coll. / WW836	*N. nubiae*1	**DQ897718, DQ897761**
Naja nubiae	Unknown	ZSL live coll. / WW837	*N. nubiae*2	**DQ897719, DQ897762**
Naja mossambica	Durban, KwaZulu-Natal, South Africa	WW590, gift H.-W. Herrmann	*N. mossambica* Durban	**DQ897720, DQ897763**
Naja mossambica	Pemba Island, Tanzania	KMH26380, KMH26381 / WW1391, WW1392	*N. mossambica* Pemba	**DQ897727, DQ897770**
Naja nivea			*Naja nivea*	***AY058983, *AF217827**
Naja kaouthia		CAS 206602	*Naja kaouthia*	***AY058982, *AF217835**