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# Phylogeography and systematic revision of the Egyptian cobra (Serpentes: Elapidae: *Naja haje*) species complex, with the description of a new species from West Africa

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## Abstract

We use a combination of phylogenetic analysis of mtDNA sequences and multivariate morphometrics to investigate the phylogeography and systematics of the Egyptian cobra (*Naja haje*) species complex. Phylogenetic analysis of mitochondrial haplotypes reveals a highly distinct clade of haplotypes from the Sudano–Sahelian savanna belt of West Africa, and that the haplotypes of *Naja haje arabica* form the sister group of North and East African *N. h. haje*. Multivariate morphometrics confirm the distinctness of the Arabian populations, which are consequently recognised as a full species, *Naja arabica* Scortecci. The Sudano-Sahelian populations are also found to represent a morphologically distinct taxon, and thus a separate species, which we describe as *Naja senegalensis* sp. nov. The new species differs from all other members of the *N. haje* complex by a combination of colour pattern and scalation characteristics (especially higher numbers of scale rows around the neck), and the possession of a unique clade of mtDNA haplotypes. The distribution of the new species includes savanna areas of West Africa, from Senegal to western Niger and Nigeria.

**Key words**: mitochondrial mtDNA, *Naja senegalensis* sp. nov., *Naja haje*, *Naja anchietae*, *Naja annulifera*, *Naja arabica*, phylogeny, snakes, West Africa

## Introduction

Recent decades have seen a tremendous surge in the use of molecular markers both for inferring phylogenetic interrelationships of groups of organisms and for taxonomic revisions. Mitochondrial DNA (mtDNA) sequences have dominated this field (Avise, 2000). Phylogeographic studies, relating evolutionary relationships among haplotypes to their geographic distribution, have allowed the reconstruction of the biogeographical history of species complexes, and, especially when performed across multiple co-distributed species, the reconstruction of the history of entire biotas (e.g., Rull, 2008).

In systematics, important applications of mtDNA have included phylogenetic reconstruction in general, but particularly species delimitation (Wiens & Penkrot, 2002). However, for the latter, mtDNA sequences do not represent an adequate source of evidence unless supported by additional markers. Multiple distinct haplotype clades can co-exist in a single gene pool, and phylogeographic patterns can remain in place despite extensive continued gene flow across mitochondrial haplotype distribution boundaries (Thorpe & Richard, 2001; Ogden & Thorpe, 2002). Consequently, the presence of multiple haplotype clades need not indicate the presence of more than one species (Puorto *et al.*, 2001). Additional evidence is therefore required to test whether mitochondrial haplotype clades represent independently evolving organismal lineages (= species), or

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whether they co-exist within a single gene pool. Such evidence can come from unlinked nuclear genetic markers or from morphology: correspondence between mitochondrial haplotype clades and morphologically distinct sets of populations indicates that the morphologically distinct populations do indeed represent historical lineages rather than the products of ecogenesis (Thorpe *et al.*, 1991), and simultaneously that mtDNA haplotype clades do in fact denote independently evolving organismal lineages rather than a relict of matrilineal history within a wider gene pool. In addition, a combination of molecular markers and morphological data collected from both genetically characterised individuals and museum specimens accumulated over decades or centuries can maximise the information content of systematic revisions by alleviating the logistical difficulties of sampling rare, widespread, or otherwise hard-to-sample taxa for molecular analyses (Wüster *et al.*, 1995; Malhotra & Thorpe, 2004).

Resolving the systematics of groups of venomous snakes is of particular relevance due to the medical importance of some species, as well as the extensive research interest of their venoms. Given the ubiquity of variation in venom composition in snakes (Chippaux *et al.*, 1991) and its potential effects on the efficacy of antivenoms (Harrison *et al.*, 2003), correct species identification is an essential, yet frequently neglected, underpinning for the reproducibility of research results and the treatment of bite victims (Fry *et al.*, 2003). However, despite the medical importance of these animals, our knowledge of the systematics of many groups of venomous snakes remains very incomplete: a number of recent phylogenetic studies have revealed previously unsuspected patterns of genetic diversity (e.g., Kuch *et al.*, 2005a; Wüster *et al.*, 2005; Skinner *et al.*, 2005), and new species are being discovered regularly even in high-profile groups of snakes (Kuch *et al.*, 2005b; Doughty *et al.*, 2007; Campbell & Flores-Villela, 2008). In addition to contributing to the resolution of the systematics of medically important snakes, phylogenetic studies also provide a framework for the elucidation of the patterns and causes of the evolution of venom composition, even when the phylogenetic pattern itself does not predict venom composition (e.g., Daltry *et al.*, 1996; Thorpe *et al.*, 2007; Barlow *et al.*, 2009).

One group of medically important venomous snakes that has received considerable systematic attention in recent years is the genus *Naja* (cobras). Morphological methods alone have contributed to the resolution of systematic problems in these snakes (e.g., Broadley, 1968, 1995; Wüster & Thorpe, 1992a). However, the joint use of morphological and molecular markers has allowed further resolution of species limits in many complex groups, and contributed to the discovery of new taxa, as well as the synonymy of the genera *Boulengerina* and *Paranaja* with *Naja* (e.g., Slowinski & Wüster, 2000; Wüster & Broadley, 2003, 2007; Broadley & Wüster, 2004; Wüster *et al.*, 2007). As a result, the number of recognised species of *Naja* has risen from four or five African and one Asian species (Bogert, 1943; Klemmer, 1963) to 15 African and 11 Asian species (Broadley, 1968, 1995; Wüster, 1996; Slowinski & Wüster, 2000; Wüster *et al.*, 2007), which were grouped into four subgenera by Wallach *et al.* (2009).

Recent fieldwork by two of the present authors (JFT, LC) has focussed attention on the systematics of cobras in West Africa. Five species of *Naja* are currently known from West Africa: *N. nigricollis* Reinhardt, 1843, *N. katiensis* Angel, 1922, *N. nubiae* Wüster & Broadley, 2003, *N. haje* (Linnaeus, 1758) and *N. melanoleuca* Hallowell, 1857 (Villiers & Condamin, 2005; Chippaux, 2006; Trape & Mané, 2006). The first three are spitting cobras, characterised by the possession of fangs with modified discharge orifices that allow them to project venom over a considerable distance (Bogert, 1943; Wüster & Thorpe, 1992b; Westhoff *et al.*, 2005). All African spitting cobras were long considered part of a single, highly variable species, *N. nigricollis*, but a number of revisions over the last 40 years has shown this to consist of seven different species (Broadley 1968, 1974; Wüster *et al.*, 2007).

In contrast to the spitting cobras, the different groups of non-spitting cobras have received much less taxonomic attention, with the exception of the southern African taxa of the *Naja haje* complex (Broadley, 1995; Broadley & Wüster, 2004). Within the African non-spitting cobras, the *N. haje* (Linnaeus 1758; type locality: Egypt) complex is characterised by the possession of a row of subocular scales separating the eye from the supralabials, which distinguishes it from all other cobras. Together with *N. nivea*, which lacks this distinguishing feature, the *N. haje* complex was assigned to the subgenus *Uraeus* Wagler, 1830 by Wallach *et*

*al*. (2009). Historically, the distribution of *N. haje* was thought to extend from Morocco to Egypt along the northern edge of the Sahara, along the Nile valley, and then in the savannas of tropical Africa from Sudan to Senegal and South Africa, excluding the Guinean and Congo rainforests and the humid savannas of parts of western, central and eastern Africa, and with an isolated population in the south-western Arabian Peninsula. Several subspecies have been recognised: *N. h. legionis* Valverde, 1989, in Morocco and the Western Sahara, *N. h. arabica* Scortecci, 1932, in the Arabian Peninsula, *N. h. annulifera* Peters, 1854, in eastern parts of southern Africa, and *N. h. anchietae* Bocage, 1879, from western parts of southern Africa. Based on morphological character analysis, Broadley (1995) raised *N. annulifera* to species level, with *N. a. anchietae* as a subspecies, thus including all the southern populations of *N. haje* sensu lato. This was later confirmed through mtDNA sequence analysis by Broadley & Wüster (2004), who also found evidence to recognise *N. anchietae* as a separate species from *N. annulifera*.

From 1988 to 2006, collections of snakes carried out by one of the authors (JFT) in various West African countries allowed the accumulation of approximately one hundred specimens which, based on scalation data, were initially assigned to *Naja haje* (Trape & Mané, 2000, 2004). In this collection, most of the material (primarily from Senegal and Mali) differed considerably from several specimens of a population from Niger, which was referable to the nominate form. As a result, the populations from Senegal and Mali were considered as a separate subspecies by Trape & Mané (2006), but not formally described or named.

The collection of additional material from Niger by two of us (LC and JFT) demonstrated that both forms occur in sympatry in parts of that country. Independently of these field results, author WW noted considerable mtDNA sequence differentiation of a Malian sample from Kenyan, Moroccan and Egyptian material, leading to the suspicion of an additional species being present. These observations prompted the present study, in which we examine both molecular genetic and morphological variation across the entire *Naja haje* species complex, with the aim of revising the systematics of the group and reconstructing its biogeographical history. The latter is of particular interest due to the paucity of phylogeographic studies of widespread African reptiles (but see Wüster *et al.*, 2007), and also due to the disjunct distribution of the complex, including as it does the isolated populations from the south-western Arabian Peninsula (Gasperetti, 1988), a pattern paralleled by a number of other species of African affinities (Arnold, 1980), and a central African disjunction.

## Materials and methods

**Specimens examined and morphological characters.** The material examined consisted of specimens from major museums worldwide. In the case of West African material, the specimens examined are mostly included in the collection accumulated by JFT between 1990 and 2007 (63 specimens from Senegal, 32 from Mali, two from Niger). Most of this latter material is preserved at the Centre de Recherche IRD-UCAD de Hann in Dakar, Senegal (acronym: IRD), but a minority, in particular the types of the new species described in this paper, were deposited in the Muséum national d'Histoire naturelle de Paris (MNHN) or the Institut Royal des Sciences Naturelles de Bruxelles (IRSNB). The specimens from Benin and Burkina Faso, from the private collection of LC, are currently preserved in Niamey but will soon be deposited in the MNHN. Other specimens examined as part of this study are preserved in the Centre National de la Recherche in Ouagadougou, Burkina Faso, although most of these were not sufficiently well preserved to be included in our analyses. Museum acronyms follow Leviton *et al*. (1985), additions are given in Appendix 1.

The following morphological characters were recorded wherever possible: total length, tail length, ratio total length: tail length, number of dorsal scale rows around neck (NSR—counted one head length behind the head), number of dorsal scale rows at midbody (MSR), number of dorsal scale rows at vent (PSR—counted one head length ahead of vent), number of ventrals (V—the first ventral being defined as the fist scale wider than long behind the gulars), number of subcaudals (SC—excluding terminal spine), number of supralabials, infralabials, preoculars, suboculars, postoculars, anterior temporals, posterior temporals, cuneates (CUN—small additional scales inserted between the infralabials and the edge of the mouth), nuchals (NUC—temporal

and occipital scales bordering the lateral and posterior edges of the parietals—Broadley, 1968).

For statistical analysis, specimens were grouped into geographical Operational Taxonomic Units (OTUs) based on collecting gaps and obvious morphological discrepancies. The OTUs used in this study (with sample sizes) are listed in Table 1. We initially used 2-way analysis of variance (ANOVA) to test the following morphological characters for significant among-OTU differences and sexual dimorphism: NSR, MSR, PSR, V, SC, CUN, NUC. Because several characters showed significant sexual dimorphism, we carried out all further analyses separately for males and females. We used 1-way ANOVA to test for significant among-OTU differences in each sex. Patterns of morphological variation were analysed by means of canonical variates analysis (CVA), a standard approach to the analysis of geographical variation (Thorpe, 1980). To increase resolution within the *N. haje* complex north of the Equator, the 1-way ANOVAs and CVAs were repeated under exclusion of specimens of *N. annulifera* and *N. anchietae*. In each CVA, only characters found to show significant among-OTU differences for that dataset were used.

**TABLE 1.** Operational Taxonomic Units (OTUs) and their sample sizes for analysis of geographic variation in the *Naja haje* complex.

| OTU | Locality | *N* (Males) | *N* (Females) |
|---|---|---|---|
| 1 | Egypt, Libya (*haje*) | 20 | 14 |
| 2 | Northern Ethiopia (*haje*) | 2 | 0 |
| 3 | Southern Ethiopia (*haje*) | 3 | 1 |
| 4 | Kenya and Tanzania (*haje*) | 9 | 6 |
| 5 | Southern Sudan and Uganda (*haje*) | 4 | 5 |
| 6 | Central Sudan (*haje*) | 2 | 1 |
| 7 | Northern Nigeria (*haje*) | 2 | 3 |
| 8 | Holotype, *Naja haje* var. *viridis* Peters | 1 | 0 |
| 9 | Timbuktu, Mali (*haje*) | 0 | 1 |
| 10 | Senegal (*N.* cf. *haje*) | 18 | 14 |
| 11 | South-western Saudi Arabia (*arabica*) | 6 | 3 |
| 12 | Dhofar, Oman (*arabica*) | 1 | 1 |
| 13 | Morocco (*legionis*) | 1 | 1 |
| 14 | Tunisia (*haje*) | 5 | 4 |
| 15 | *N. anchietae* | 73 | 53 |
| 16 | *N. annulifera* | 45 | 108 |
| 17 | Mali (*N.* cf. *haje*) | 7 | 3 |
| 18 | Niger (*haje*) | 2 | 2 |

**Molecular phylogenetics**

We obtained tissue (ventral scale clippings or blood samples) from specimens of the *Naja haje* complex from various parts of its known distribution. Total DNA was extracted using the GenElute™ Mammalian Genomic Miniprep kit (Sigma-Aldrich). Sample and sequence details are given in Table 2.

A ~ 900 base pair fragment of the mitochondrial NADH dehydrogenase subunit 4 (ND4) gene and the adjoining tRNA-His and tRNA-Leu genes was amplified using the primers ND4 (Arévalo *et al.*, 1994) and HIS12763v (5'–TTC TAT CAC TTG GAT TTG CAC CA–3', Sylvain Ursenbacher, pers. comm.), and an 1100 b.p. section of cytochrome *b* was amplified using primers GludG (Palumbi, 1996) and H16064 (Burbrink *et al.*, 2000).

**TABLE 2.** Samples and GenBank accession numbers for specimens included in the molecular analysis.

| Taxon | Sample/voucher reference number | Locality | GenBank accession numbers (ND4, cytb) |
|---|---|---|---|
| *Naja kaouthia* | CAS 206602 | Myanmar | AY058982, AF217835 |
| *Naja nivea* | | | AY058983, AF217827 |
| *Naja haje legionis* | WW1057 / Liverpool School of Tropical Medicine, live collection | Morocco | GQ387062, GQ387091 |
| *Naja haje haje* | WW 893 / Liverpool School of Tropical Medicine, live collection | Egypt | GQ387063, GQ387092 |
| *Naja haje haje* | WW 1077 / Latoxan live coll. N. ha. ha. 98110004 | Egypt | GQ387064, GQ387093 |
| *Naja haje haje* | WW 1078 / Latoxan live coll. N. ha. ha. 98140015 | Egypt | GQ387065, GQ387094 |
| *Naja haje haje* | WW1262 / Bio-ken live coll. BK-10043 | Athi River, Kenya | GQ387066, GQ387095 |
| *Naja haje haje* | WW1263 / Bio-ken live coll. BK10197 | Naivasha, Kenya | GQ387067, GQ387096 |
| *Naja haje haje* | WW 1651 | Zinder, Niger | GQ387068, GQ387097 |
| *Naja haje haje* | WW 1652 | Zinder, Niger | GQ387069, GQ387098 |
| *Naja haje haje* | WW 1653 | Zinder, Niger | GQ387070, GQ387099 |
| *Naja haje haje* | WW1659 / Liverpool School of Tropical Medicine, live collection | northern Nigeria | GQ387071, GQ387100 |
| *Naja haje haje* | WW1660 / Liverpool School of Tropical Medicine, live collection | northern Nigeria | GQ387072, GQ387101 |
| *Naja haje haje* | WW1661 / Liverpool School of Tropical Medicine, live collection | northern Nigeria | GQ387073, GQ387102 |
| *Naja haje arabica* | WW 1677 / Breeding Centre for Endangered Arabian Wildlife, Sharjah | Taif, Saudi Arabia | GQ387074, GQ387103 |
| *Naja haje arabica* | WW 1678 / Breeding Centre for Endangered Arabian Wildlife, Sharjah | Taif, Saudi Arabia | GQ387075, GQ387104 |
| *Naja haje arabica* | WW 1679 / Breeding Centre for Endangered Arabian Wildlife, Sharjah | Taif, Saudi Arabia | GQ387076, GQ387105 |
| *Naja haje arabica* | WW 1681 / Breeding Centre for Endangered Arabian Wildlife, Sharjah | Taif, Saudi Arabia | GQ387077, GQ387106 |
| *Naja haje arabica* | WW 1682 / Breeding Centre for Endangered Arabian Wildlife, Sharjah | Taif, Saudi Arabia | GQ387078, GQ387107 |
| *Naja haje arabica* | WW2035 / LK JEM 657 | Zinjubar, Yemen | GQ387079, GQ387108 |

continued next page.

**TABLE 2.** (continued)

| Taxon | Sample/voucher reference number | Locality | GenBank accession numbers (ND4, cytb) |
|---|---|---|---|
| *Naja senegalensis* | WW2203 / MNHN 2008.0074 | Dielmo, Sine Saloum, Senegal | GQ387080, GQ387109 |
| *Naja senegalensis* | WW1079 / Latoxan live coll. N. ha. ha. 99080019 | Bandiagara, Mali | GQ387081, GQ387110 |
| *Naja senegalensis* | WW1542 / IRD-TR 1273 | Niakoni, Mali | GQ387082, GQ387111 |
| *Naja senegalensis* | WW2018 / IRD TR 103 M | Doussoudiana, Mali | GQ387083, GQ387112 |
| *Naja senegalensis* | WW2039 / LC 6536 | Nienie, Benin | GQ387084, GQ387113 |
| *Naja anchietae* | WW591 / H.-W. Herrmann, pers. coll. | Okahandja, Namibia | GQ387085, GQ387114 |
| *Naja anchietae* | WW289 | Silumbi, Namibia | GQ387086, GQ387115 |
| *Naja anchietae* | WW1892 / Chobe Snake Park, live coll. | Kazungula, Botswana | GQ387087, GQ387116 |
| *Naja anchietae* | WW1893 / Chobe Snake Park, live coll. | Kazungula, Botswana | GQ387088, GQ387117 |
| *Naja annulifera* | WW193 | Phalaborwa, Limpopo Province, S. Africa | GQ387089, GQ387118 |
| *Naja annulifera* | WW 881 / NMZB 16066 | Bulawayo, Zimbabwe | GQ387090, GQ387119 |

The PCR protocol involved 20µl reactions that were carried out using 18µl of 1.1X ReddyMix™ PCR Mastermix (Abgene™, catalogue no. AB-0575-LD/A), consisting of 1.25 units of Thermoprime Plus DNA polymerase, 75mM Tris-HCL (pH 8.8 at 25ºC), 20mM $(NH_4)_2SO_4$, 1.5mM of $MgCl_2$, 0.01% (v/v) Tween® 20, 0.2mM of each dNTP and a precipitant red dye for electrophoresis. Primers were added to a final concentration of 0.4µM and approximately 4ng of template DNA.

The amplification protocol comprised denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturing for 45 seconds at 92°C, followed by 1 minute of annealing at 50–55°C, one and a half minutes extension at 72° C. The reaction ended with one longer extension phase at 72° C for 5 minutes. Single strand sequencing was carried out by Macrogen (Seoul, S. Korea—http://dna.macrogen.com), using the primers ND4 and GludG. 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.

We tested for the presence of a significant phylogenetic signal by examining the skewness of the distribution of the lengths of $10^6$ random trees (Hillis & Huelsenbeck, 1992). For phylogenetic analysis, we used maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods. MP analysis was carried out using the software PAUP* 4.0b10 (Swofford, 2002). For MP analysis, we performed an equal weighting, heuristic search involving 10,000 random addition sequence replicates. Internal support for different nodes was estimated using non-parametric bootstrapping (Felsenstein, 1985), using 10,000 replicates and 5 random addition sequence replicates each. We also estimated Bremer support (Bremer, 1994) by repeating the heuristic searches while retaining successively longer trees, and determining the minimum lengths of suboptimal trees not including clades present in the optimal trees. Trees were rooted using outgroup sequences from the Asiatic *Naja kaouthia* as a far outgroup and the African *N. nivea* as a near outgroup and

likely sister taxon of the *N. haje-annulifera* complex (Wüster *et al.*, 2007).

For ML analysis, we first used MrModeltest 2.2 (Nylander, 2004) to estimate the best model of sequence evolution across the entire sequence database and selected the model favoured under the Akaike Information Criterion (AIC). We then used the software PAUP* 4.0b10 (Swofford, 2002) to infer the most likely tree, using heuristic searching, TBR branch swapping, and ten random addition sequence replicates to guard against the possibility of multiple islands of optimal trees.

For phylogenetic analysis using Bayesian Inference (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). 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 model 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. kaouthia* was specified as the outgroup for all BI analyses. We ran the analysis for $5 \times 10^6$ generations using four simultaneous independent runs initiated with different random starting trees, and sampling every 500 generations. Plots of lnL against generation time were inspected to determine the burn-in period, and trees generated prior to the completion of burn-in were discarded. As a safety margin, we discarded the first $10^6$ generations.

## Results

### Morphological analysis

All seven characters initially included in the analysis showed significant among-OTU differences in both sexes. After exclusion of *N. anchietae* and *N. annulifera*, characters MSR and PSR did not differ significantly among the remaining OTUs in males, whereas characters PSR and CU did not differ significantly in females.

Canonical variates analysis (CVA) of all *N. haje* complex populations (Fig. 1) confirms the distinctness of *N. anchietae* and *N. annulifera* from each other and from the rest of the *N. haje* complex through their separation along the first canonical variate. Moreover, the Arabian Peninsula populations (*N. haje arabica*) are shown to be somewhat distinct from all other populations of the complex along the second canonical variate. The CVAs run under exclusion of the southern African populations (Fig. 2) confirm the distinctness of the Arabian populations and show that the West African savanna populations of the complex are clearly separated from the remainder of the *N. haje* complex along the first canonical variate, especially in the analysis of female specimens. Canonical variate loadings for the different characters are given in Table 3.

### Molecular phylogeography

We aligned 684 b.p. of sequence of the mitochondrial cytochrome *b* gene and 654 b.p. of the NADH dehydrogenase 4 gene for 28 ingroup samples, revealing 19 ingroup haplotypes. Translation of the sequences revealed no unexpected indels, frameshifts or stop codons. Of the 1338 base pairs, 310 were variable and 171 parsimony-informative. Levels of pairwise sequence divergence (p-distance) in the ingroup ranged from 0 to 0.0699. The analysis of $10^6$ random trees resulted in a skewness statistic of $-g1=0.800125$, rejecting the null hypotheses of there being no phylogenetic signal in the data ($p < 0.01$; Hillis & Huelsenbeck, 1992).

Equal weighting parsimony analysis yielded 18 equally most parsimonious trees of 453 steps distributed across a single tree island (c.i. = 0.6302, excluding uninformative characters). Maximum likelihood and Bayesian inference analyses revealed phylogenetic trees in broad agreement with the MP consensus tree, except where noted below; the BI tree is shown in Fig. 3.

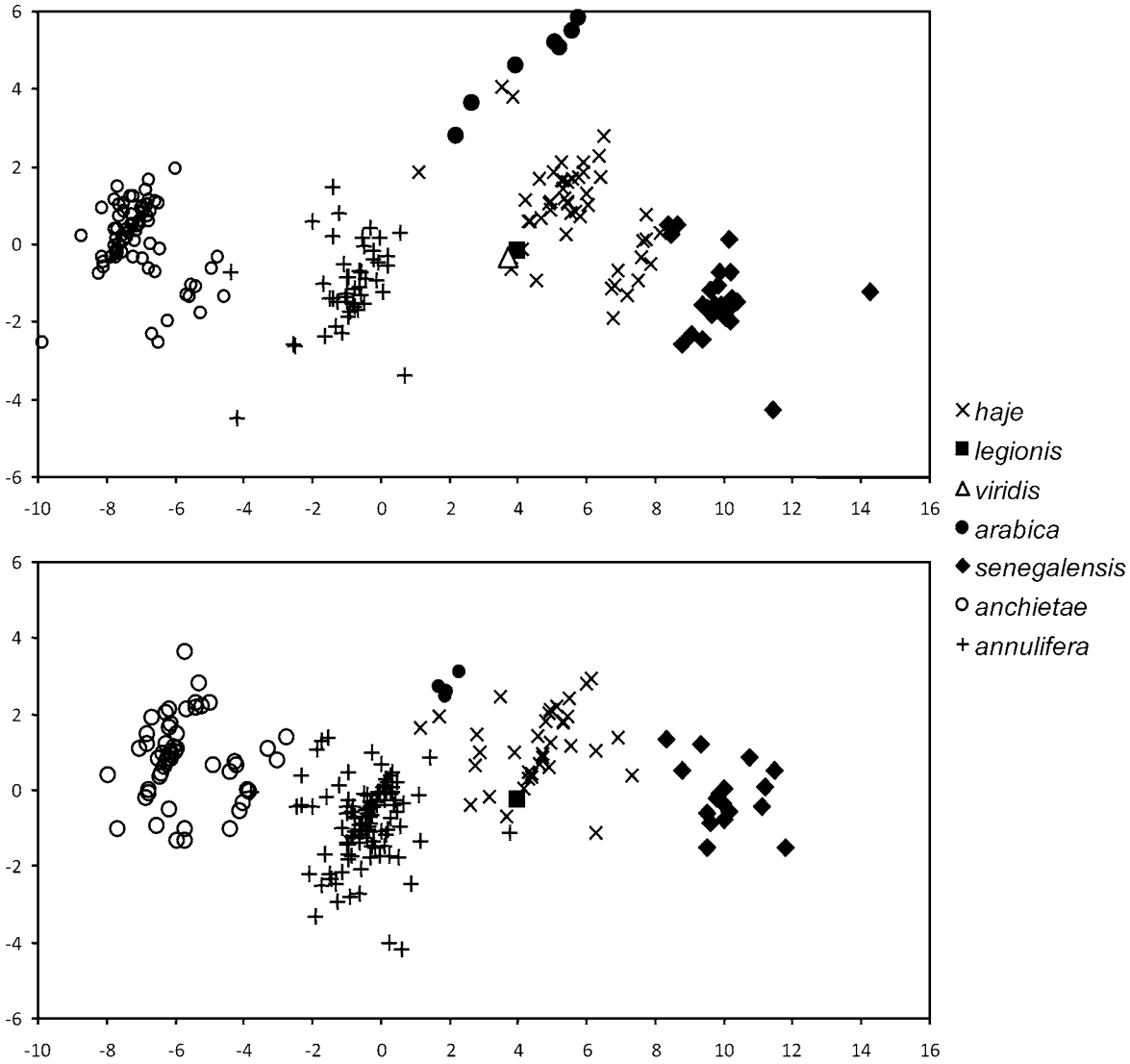


**FIGURE 1.** Ordination of the male (top) and female (bottom) specimens of the *Naja haje* complex along the first canonical variates of CVAs of seven morphological characters (neck, midbody and posterior dorsal scale rows; ventrals; subcaudals, nuchals, cuneates). Note the distinctness of *N. anchietae* and *N. annulifera* along the first canonical axis, of the distinctness of the West African savanna form (*N. senegalensis*) along the same axis, and of the Arabian populations along the second canonical axis, especially in males.

The trees revealed the following relationships: (1) The haplotypes of the *N. haje* complex fall into three main clades: the southern African *N. annulifera* + *anchietae* clade, a clade of haplotypes from northern, eastern and central western Africa and the Arabian Peninsula, and a clade consisting of haplotypes from the Sudano–Sahelian savanna of West Africa. The MP tree places the latter as the sister group of all other populations of the *N. haje* complex, whereas in the ML and BI trees, the *N. annulifera* + *anchietae* clade is the sister group of the rest of the *N. haje* complex, in all cases without significant bootstrap or Bayesian posterior probability support. (2) Within the North and East African and Arabian clade, all trees recover a northern African clade consisting of samples of *N. h. haje* from Egypt, central Niger and northern Nigeria, and *N. h. legionis* from Morocco, with little well-supported structure within it. Within this haplotype clade, the p-distance between the *N. h. legionis* haplotype and the Egyptian and W. African haplotypes ranges from 0.005–0.007. (3) Two specimens of *N. h. haje* from Kenya are the sister group to the North African clade (mean p-distance from N. African clade: 0.013). (4) Specimens from the Arabian Peninsula form a monophyletic group that constitutes the sister taxon of the North and Central West African + Kenyan clade (mean p-distance

0.029). The haplotype from Yemen differs from the Saudi (Taif) haplotypes by p-distances of 0.011–0.012. (5) Specimens of *N. anchietae* and *N. annulifera* form each other's sister group (mean p-distance 0.042). Most of these relationships are supported with strong bootstrap and Bayesian posterior probability support, except the relative positions of the three main haplotype clades. P-distances between clades are shown in Table 4.

**TABLE 3.** Canonical Variates Analyses: % of total variance represented by the first and second canonical variates of each analysis, and the standardised canonical discriminant function coefficients of each variable. Dashes indicate variables not included in the analyses because of lack of significant among-OTU variation.

| | All OTUs | | | | Excl. *N. annulifera* and *N. anchietae* | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | | Females | | Males | | Females | |
| | CV1 | CV2 | CV1 | CV2 | CV1 | CV2 | CV1 | CV2 |
| % of total variance | 91.7 | 3.9 | 69.5 | 16.5 | 90.8 | 4.4 | 80.1 | 12.3 |
| NSR | 0.564 | -0.753 | 0.865 | -0.139 | 0.717 | -0.517 | 0.910 | -0.091 |
| MSR | 0.584 | 0.388 | - | - | 0.348 | 0.042 | -0.04 | -0.079 |
| PSR | 0.044 | 0.173 | - | - | -0.060 | 0.102 | - | - |
| V | 0.480 | 0.489 | 0.423 | 0.544 | 0.587 | 0.855 | 0.496 | 0.342 |
| SC | 0.193 | 0.346 | -0.234 | 0.639 | -0.127 | -0.130 | -0.145 | 0.848 |
| NU | 0.086 | -0.180 | 0.254 | 0.15 | -0.037 | -0.322 | 0.195 | 0.016 |
| CU | -0.086 | 0.277 | -0.013 | 0.625 | -0.228 | 0.274 | - | - |

**TABLE 4.** P-distances between clades of the *Naja haje* complex

| | N. Africa & Niger +Nigeria | Kenya | Arabian Peninsula | Sudano-Sahelian savanna | *N. anchietae* | *N. annulifera* |
|---|---|---|---|---|---|---|
| N. Africa & Niger +Nigeria | — | — | 0.029 | 0.049 | 0.053 | |
| Kenya | 0.013 | — | | | | |
| Arabian Peninsula | 0.029 | 0.030 | — | | | |
| Sudano-Sahelian savanna | 0.047 | 0.050 | 0.051 | — | 0.067 | |
| *N. anchietae* | 0.050 | 0.053 | 0.057 | 0.069 | — | |
| *N. annulifera* | 0.051 | 0.052 | 0.059 | 0.063 | 0.042 | — |

## Taxonomy and discussion

Both morphological and molecular results demonstrate the distinctness of *Naja anchietae* and *N. annulifera* from each other and the rest of the *N. haje* complex, thus confirming their status as separate species (Broadley, 1995; Broadley & Wüster, 2004). Cobras from the Arabian Peninsula have not previously been included in

molecular or morphological analyses. Our molecular phylogeny shows *N. haje arabica* to constitute a distinct clade, forming the sister group of all *N. haje* populations from northern and eastern Africa. Morphologically, the populations from the Arabian Peninsula constitute a clearly distinct cluster, differing primarily through their higher subcaudal scale count and a strong tendency towards lower neck and midbody scale row counts. Arnold (1980) suggested the possibility of clinal variation from south-eastern Arabia to the north-west and on to Africa, and questioned the distinctness of the Arabian form. However, although Arnold's data do suggest that the eastern populations are most differentiated, our molecular data clearly identify the Arabian cobras as a distinct lineage. Given the morphological distinctness of the Arabian populations, and their unique mitochondrial haplotypes, we consider these cobras to represent a separate species, *Naja arabica* Scortecci, 1932.

**TABLE 5.** Meristic characters of the species of the *Naja haje* complex from northern Africa and Arabia. Numbers after the colon indicate the sample size for that particular count. Ranges in brackets for *Naja arabica* are from the larger sample of Arnold (1980), for which information on the sex of the specimens was not available.

| | *Naja senegalensis* | | *Naja haje* | | *Naja arabica* | |
|---|---|---|---|---|---|---|
| | M | F | M | F | M | F |
| Ventrals | 205–219 | 219–225 | 191–216 | 202–222 | 202–218 | 208–221 |
| | | | | | (204–226) | |
| Subcaudals | 59–66 | 56–64 | 53–67 | 53–68 | 65–73 | 70–73 |
| | | | | | (62–80) | |
| Dorsal scale rows around neck | 23: 3<br>25: 19<br>27: 2 | 23: 1<br>24: 2<br>25: 12<br>26: 1<br>27: 1 | 19: 1<br>20: 1<br>21: 35<br>23: 13 | 19: 3<br>20: 3<br>21: 27<br>22: 1<br>23: 3 | 19: 8 | 19: 5 |
| | | | | | (19–21) | |
| Dorsal scale rows at midbody | 21: 28<br>23: 1 | 21: 16<br>23: 3 | 19: 4<br>20: 2<br>21: 43<br>23: 1 | 19: 6<br>20: 3<br>21: 27 | 19: 3<br>21: 6 | 19: 3<br>21: 2 |
| Nuchals | 6: 2<br>7: 13<br>8: 8<br>9: 1 | 6: 1<br>7: 11<br>8: 2<br>9: 3 | 5: 8<br>6: 10<br>7: 33 | 5: 4<br>6: 2<br>7: 30 | 6: 1<br>7: 9 | 6: 5 |

Our molecular analysis confirms the preliminary results of Broadley & Wüster (2004) that the Moroccan populations of *N. haje*, described as a separate subspecies, *N. h. legionis* by Valverde (1989), are nested among the Egyptian and West African haplotypes of the nominate form, with minimal divergence. Our morphological analyses also do not provide any evidence that this form is distinct from other populations of *N. h. haje*. Given the extreme variability in the colour and pattern of this species, we do not consider the differences highlighted by Valverde (1989) to justify recognition of this form as a separate subspecies, and consider *Naja haje legionis* Valverde a synonym of *Naja haje* (Linnaeus).

Our molecular and morphological results show that the specimens of the *Naja haje* complex from the Sudano–Sahelian savannas of West Africa represent a separate taxon distinct in morphology and mtDNA. Morphological analysis gives no indication of intergrades or hybrids between the West African savanna form and *Naja haje* sensu stricto (Fig. 2); indeed, in males, West African *N. haje* s. str. are among the most distinct from the savanna form. Consequently, we regard this savanna form as a separate species from all other populations of the *N. haje* complex. Peters (1873) described a specimen of the *Naja haje* complex (ZMB

2820), purportedly from West Africa, as *Naja haje* var. *viridis*. However, the low ventral scale (191) and neck scale row (21) counts preclude this specimen from belonging to the Sudano–Sahelian savanna species revealed by our data (see below), and the specimen clusters with other *N. haje* sensu stricto in our morphometric analyses (Fig. 2). No existing names appear to be available for the West African savanna species, thus we describe it here as new:

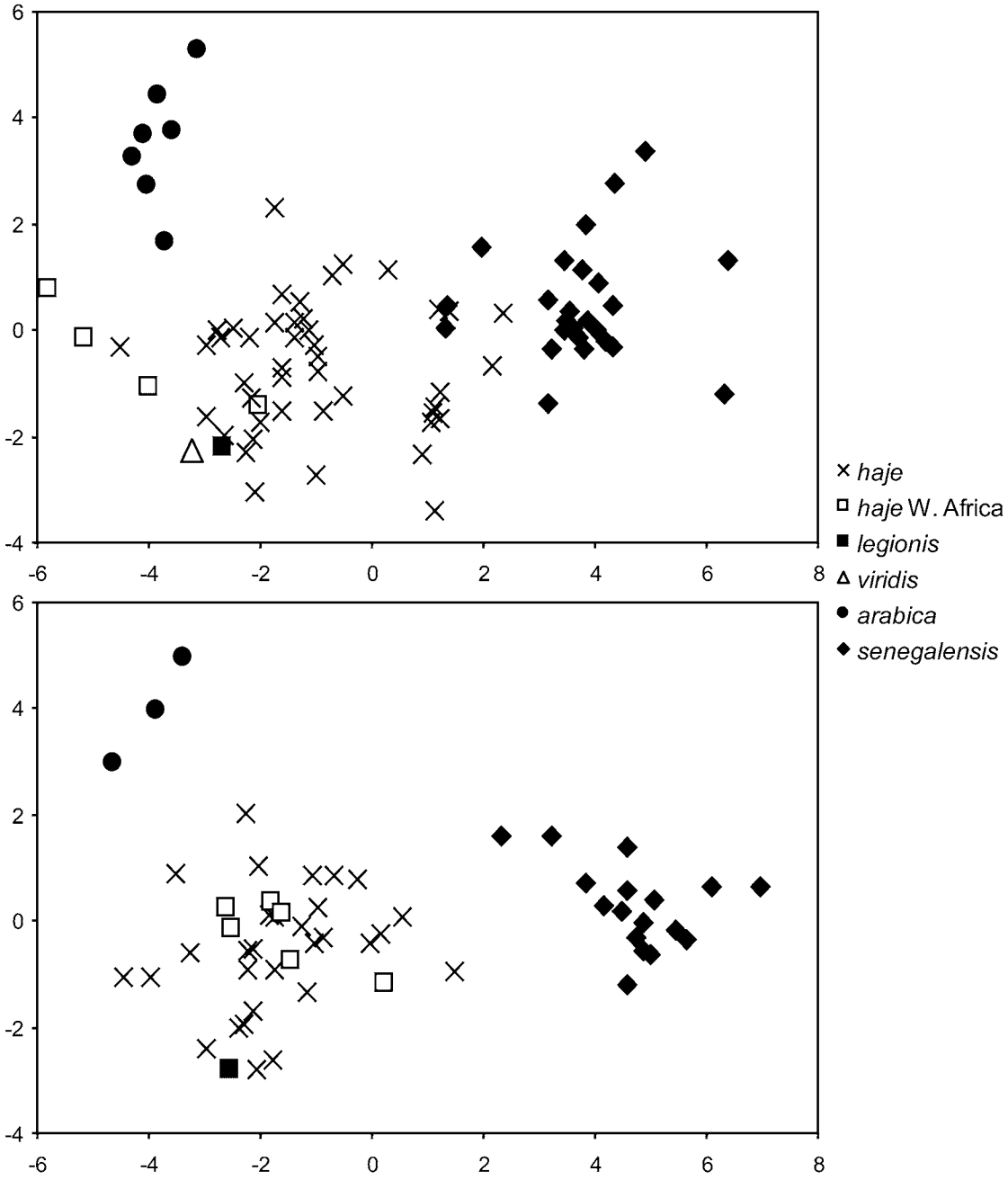


**FIGURE 2.** Ordination of male (top) and female (bottom) specimens of the *Naja haje* complex, excluding *N. anchietae* and *N. annulifera* based on canonical analysis of five morphological characters (see text for details) of. Note the distinctness of the Arabian populations and of the West African savanna form (*N. senegalensis*). Note also that, although there is some overlap between *N. haje* sensu stricto and *N. senegalensis* in males (top), this does not involve West African specimens of *N. haje* s. str. from near the contact zone with *N.* cf. *haje*. The ostensibly West African type of *N. haje* var. *viridis* Peters groups with *N. haje* s. str., not with *N. senegalensis.*

***Naja senegalensis* sp. nov. Trape, Chirio and Wüster**
Figs. 4–8

**Holotype:** MNHN 2008.0074 (previously IRD S-8549), collected in September 2008 near Dielmo (13°43'N, 16°25'W) by Mr. Babacar N'Dao, veterinary agent at Keur Lahim Fatim, who sent it to the first author (Fig. 4–6).

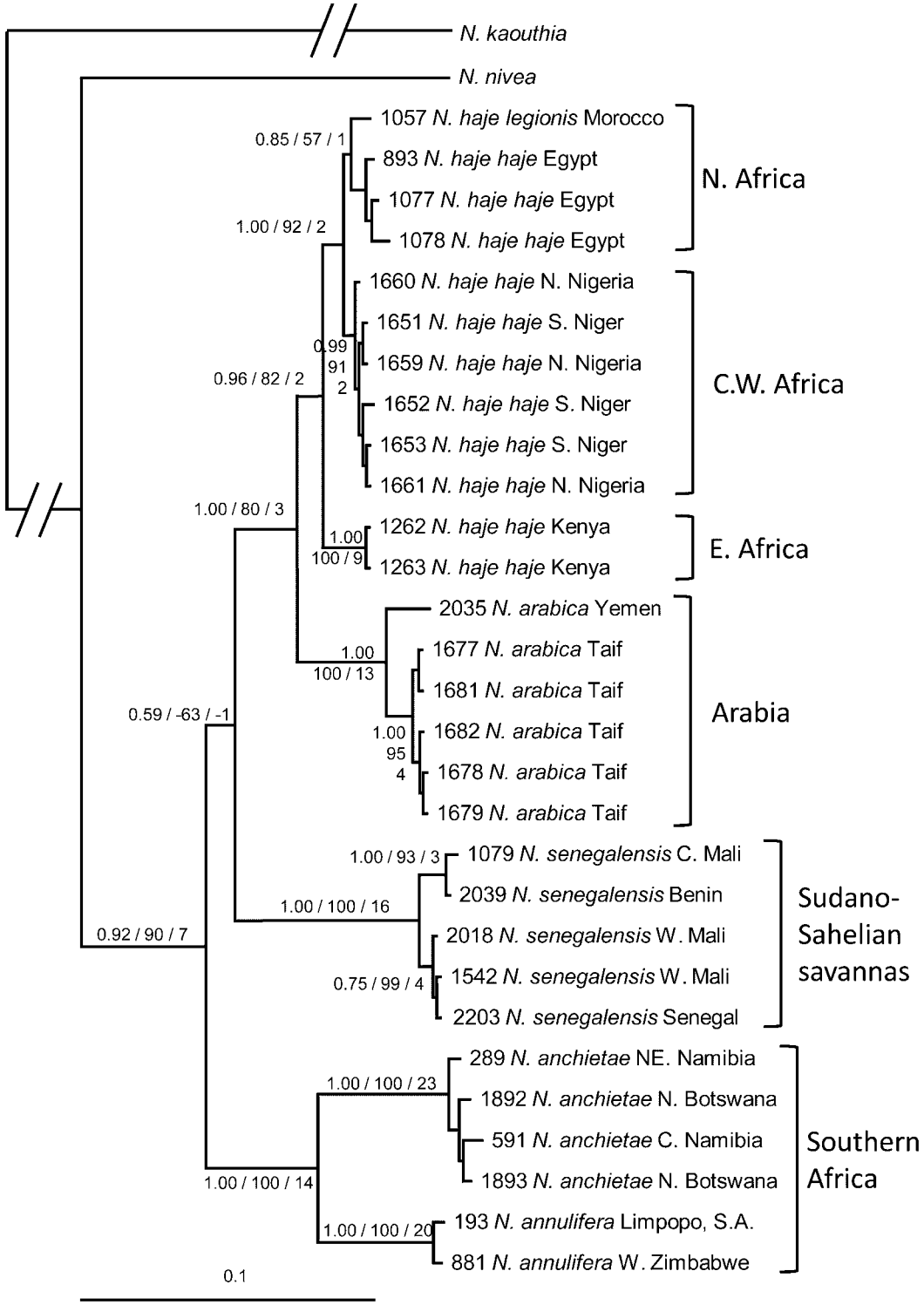


**FIGURE 3.** Bayesian inference tree of the *Naja haje* complex. Support values indicate Bayesian posterior probabilities, MP bootstrap support and Bremer (1994) support. Negative support values indicate that the node is contradicted in the MP analysis, and the support for the alternative hypothesis.

**Paratypes:** 31 specimens, all from Sénégal: MNHN 2008.0075 (previously IRD S-409), MNHN 2008.0076 (previously IRD S-1027) MNHN 2008.0077 (previously IRD S-1028), MNHN 2008.0078 (previously IRD S-1578), MNHN 2008.0079 (previously IRD S-1589): Keur Lahine Fatim (13°44'N, 16°23'N), Sine Saloum; MNHN 2008.0080 (previously IRD S-443), MNHN 2008.0081 (previously IRD S-2302), MNHN 2008.0082 (previously IRD S-2306): Keur Bakar Mané (13°37'N, 16°17'W), Sine Saloum; MNHN 2008.0083 (previously IRD S-762), MNHN 2008.0084 (previously IRD S-5283): Keur Seny Gueye (13°36'N, 16°19'W), Sine Saloum; MNHN 2008.0085 (previously IRD S-855), MNHN 2008.0086 (previously IRD S-858), MNHN 2008.0087 (previously IRD S-1640): Keur Gadji (13°38'N, 16°19'W), Sine Saloum; MNHN 2008.0088 (previously IRD S-1283): Keur Santhiou (13°39'N, 16°21'W), Sine Saloum; IRSNB 2654 (previously IRD S-605), IRSNB 2655 (previously IRD S-1429), IRSNB 2656 (previously IRD S-1435), IRD S-1439, IRD S-1440, IRD S-1442, IRD S-1461, IRD S-3429, IRD S-3430, IRD S-5692: Dielmo (13°43'N, 16°25'N), Sine Saloum; IRD S-2113: Landieni (12°33'N, 12°22'W), eastern Senegal; IRD S-5344: Saroudia (12°32'N, 11°35'W), eastern Senegal; IRD S-5427: Sambarabougou (13°06'N, 11°51'W), eastern Senegal; IRD S-5849, IRD S-5854: Guénoto (13°33'N, 13°50'W), eastern Senegal; IRD S-6204: Keur Lamine Diamé (13°37'N, 16°16'W), Sine Saloum; IRD S-6461: Touba Baria (13°38'N, 16°14'W), Sine Saloum.

**Other specimens examined.** 86 specimens: **Senegal** (46 specimens): IRD S-343, IRD S-3431: Senegal; IRD S-462: Keur Ayip Kâ (13°39'N, 16°19'W), Sine Saloum; IRD S-604, IRD S-606, IRD S-664: Keur Bakar Mané (13°37'N, 16°17'W), Sine Saloum; IRD S-856 , IRD S-1634: Keur Gadji (13°38'N, 16°19'W), Sine Saloum; IRD S-1279, S-1280, S-1281, S-1292, S-1293: Keur Santhiou (13°39'N, 16°21'W), Sine Saloum; S-1411, S-1472, S-1482: Dielmo (13°43'N, 16°25'W), Sine Saloum; IRD S-1588: Keur Lahim Fatim (13°44'N, 16°23'N), Sine Saloum; IRD S-3849: Badiara (13°13'N, 14°12'W), Haute Casamance; IRD S-3952: Goundaga (12°51'N, 14°05'W), Haute Casamance; IRD S-4806: Tialé (15°14'N, 16°49'W), Cayor; IRD S-5085: Oubadji (12°40'N, 13°03'W), Sénégal oriental; IRD S-5307: Saroudia (12°32'N, 11°35'W), Sénégal oriental; IRD S-5795, IRD S-6090: Keur Momat Souna (13°38'N, 16°17'W), Sine Saloum; IRD S-5851, IRD S-5853: Guénoto (13°33'N, 13°50'W), eastern Senegal; IRD S-5862: Médina Djikoye (13°37'N, 16°18'W), Sine Saloum; IRD S-6239: Touba Baria (13°38'N, 16°14'W), Sine Saloum; IRD S-6656: Takoudialla (12°50'N, 14°04'N), Haute Casamance; IRD S-6680: Ségoto (13°18'N, 11°49'W), Sénégal oriental; IRD S-7200: Touba Ndiaye (15°09'N, 16°52'W), Cayor; IFAN 55-4-13: Cambérène (14°45'N, 17°25'W); IFAN 52-11-90, IFAN 53-11-143: Dakar (14°42'N, 17°27'W); IFAN 47-1-10, IFAN 47-1-15, IFAN 50-9-149, IFAN 51-12-53, IFAN 52-3-23, IFAN 52-7-47, IFAN 56-5-50: Hann (14°43'N, 17°26'W), IFAN 82-1-2: Keur Massar (14°47'N, 17°18'W); IFAN 52-1-8: Malika (14°47'N, 17°20'W); IFAN 53-3-20: Ouakam (14°43'N, 17°29'W); IFAN 44-1-3: Popenguine (14°33'N, 17°07'W). **Mali** (32 specimens): IRD 2353-M, IRD 2354-M: Ballabougou (12°52'N, 06°52'W); IRD 238-M: Bangaya (13°14'N, 10°43'W); IRD 1179-M, IRD 1181-M: Djinagué (12°59'N, 09°52'W); IRD 103-M: Doussoudiana (11°09'N, 07°48'W); IRD 2368-M: Koundian (13°10'N, 10°40'W); IRD 805-M: Laminina (11°12'N, 07°47'W); IRD 1977-M, 2003-M, 2017-M: Mamoroubougou (11°13'N, 05°28'W); IRD 1796-M, IRD 3419-M: Npiébougou (11°59'N, 08°00'W); IRD 2352-M: Sadjouroubougou (12°35'N, 07°44'W); IRD 1591-M: Sare-Soma (14°45'N, 03°55'W); IRD 878-M, 957-M: Sebekourani (12°12'N, 08°42'W); IRD 184-M, IRD 2102-M, IRD 2109-M, IRD 2118-M, IRD 2145-M, IRD 2186-M, IRD 3606-M, IRD 3617-M, IRD 3618-M, IRD 3683-M: Titiéna (11°27'N, 06°33'W); IRD 156-M, IRD 581-M, IRD 2349-M, IRD 2350-M, IRD 2351-M (13°09'N, 07°57'W). **Niger** (2 specimens): IRD 201-N: Karosofua (13°37'N, 06°37'E); IRD 1504-N: Téla (12°08'N, 03°28'E). **Burkina Faso** (2 specimen): LC 6531: Kondio (11°37'N, 02°01'E); IFAN 48-2-9: Dano près Diébougou (11°09'N, 03°04'W); USNM 237088 8km S of Dana (NW 1202C1). **Bénin** (1 spécimen): LC 7109: Niénié (11°22'N, 02°12'E). **Guinée** (1 specimen): IFAN 52-6-34: Niandan-Banie (approximatively 10°20'N, 09°50'W). **Nigeria** (1 specimen) : CM 92607 Shagunu, west bank of Kainji Lake (10º20'N, 04º28'E).

**Diagnosis.** *Naja senegalensis* resembles all other members of the *N. haje* complex and differs from all other *Naja* in having a row of subocular scales separating the orbit from the supralabial scales. *Naja senegalensis* can be distinguished from other species of the *N. haje* complex through a combination of scale

counts and the coloration of juveniles and adults. Comparative scale counts are given in Table 3. *Naja senegalensis* is distinguishable from *N. haje* through its higher neck scale row count: *N. senegalensis* normally has 25 dorsal scale rows around the neck, although some specimens have 23 or 27. By contrast, W. African *N. haje* have fewer neck scale rows (19–21 in five specimens from Niger, 21–23 in three specimens from Nigeria, 21 in one specimen from Tombouctou, Mali). In other parts of Africa, the majority of specimens also have 21 or fewer scale rows around the neck (Table 3). A cobra specimen from Djibouti, with 27 scale rows around the neck and 23 at midbody, tentatively assigned to the *N. haje* complex by Ineich (2001), appears to be a spitting cobra. Other scalation characters do not distinguish *N. senegalensis* from *N. haje*, although the new species tends to occupy the upper end of the spectrum of ventral scale counts in the complex (Table 3).

Another diagnostic feature of *N. senegalensis* is the juvenile pattern: the great majority of juveniles and subadult specimens have a highly contrasting white blotch on the neck, within the dark collar encircling the neck (Fig. 7). This pale patch is present in 37 out of 39 small and medium-sized specimens, but barely discernible or absent in almost all larger adults. However, one of us (LC) recently photographed a large captive adult (approximately 200 cm total length) from the area of Bamako, Mali, that retained a very conspicuous, heart-shaped nuchal mark (Fig. 8). We have never observed this patch in *N. haje*, and therefore consider its presence to be a diagnostic character for *N. senegalensis*.

*Naja senegalensis* differs strongly in coloration from sympatric or parapatric West African *N. haje*: adults of *N. senegalensis* are almost invariably uniformly dark brown dorsally, whereas juveniles are greyish dorsally and yellowish ventrally, with a dark collar (Fig. 7) around the neck and usually a white neck blotch. Small adult specimens tend to be dark brown with paler speckles and their ventral side is yellowish. Small adult *N. senegalensis* from W National Park, in the Niger–Burkina Faso–Benin border region, have a brown dorsal coloration with small reddish dots of one scale each. The entire head, and in particular the supralabial region, are normally uniformly dark brown. In Niger and Nigeria, where both *N. senegalensis* and *N. haje* are found (the former in the Sudan savanna, the latter in the Sahelian zone, but with possible areas of sympatry), both adults and juveniles of *N. haje* display quite different colours: the body of adults is yellow to dark brown dorsally (often mostly yellow, especially in Niger), often with scattered individual dark scales, but the venter is at least partly cream-coloured, or with contrasting light and dark bands or blotches. The sides of the head, and in particular the supralabial region, normally display contrasting areas of pale and dark pigmentation, and, most noticeably, a dark spot under the eye, reminiscent of the "teardrop" marking present in *N. nubiae* (Wüster & Broadley, 2003), and a more or less distinct dark greyish neck band, approximately ten scales wide. Juveniles lack the pale neck patch present in young *N. senegalensis*. An adult *N. haje* from Niger is illustrated in Trape & Mané (2006, p. 195).

Elsewhere in Africa, *N. haje* shows great variation in colour pattern, but differs consistently from that shown in *N. senegalensis* as follows:

- The Moroccan/Western Saharan population extends into the northern parts of Western Sahara, and specimens have been illustrated by Bons & Geniez (1996, p. 251), Geniez *et al.* (2004, p. 169, 171) and Dobiey & Vogel (2007, p. 67). Adults are usually uniform black except for a yellowish gular area. Some may be dark brown above and grey below.

- *N. haje* extends across northern Algeria south of the Atlas Mountains (Schleich *et al.*, 1996), but no voucher specimens have been examined. LC did not encounter the species during two years that he spent in the region; local people knew it, but reported that it was very rare. Renker (1966) reported encountering a uniformly "sandy brown" specimen at Ghardaia and both black and brown specimens at Bir Ghellalia, Msila Province

- Nine Tunisian specimens showed great variation, but most are yellowish or mottled brown, with head and neck blackish, the venter may be dark mesially or suffused with brown. MNHN 8797 from south Tunisia is red-brown above and purple below. Only a 446 mm male from Sfax (FMNH 83646) has a distinct throat band covering ventrals 8–18. Specimens from Libya are similar (see photo in Schleich *et al.*, 1996, plate 49) but a 1370 mm male from Kouf National Park (FMNH 214914) is brown with yellow flecks above and shows

faint banding, while a 610 mm male from near Misurata (FMNH 83058) has black throat bands covering V 3–6 and 11–25. Kramer & Schnurrenberger (1963) reported that Libyan juveniles are cream with dark dorsal crossbands, black head and neck and a broad black throat band.

**FIGURE 4.** Holotype of *Naja senegalensis* (MNHN 2008.0074) in dorsal and ventral view

**FIGURE 5**. Side view of head of holotype of *Naja senegalensis* (MNHN 2008.0074).

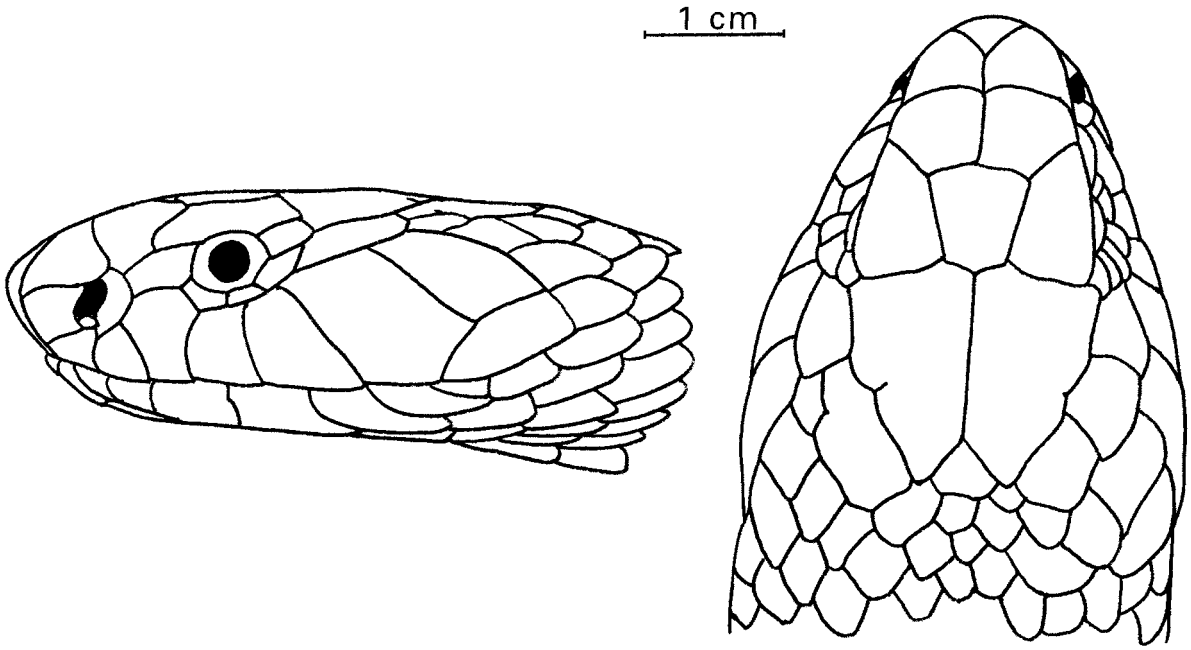


**FIGURE 6**. Sketch of head scalation of holotype of *Naja senegalensis*.

**FIGURE 7.** Juvenile specimen of *Naja senegalensis* from Medina Djikoye, Sine Saloum, Senegal . Note the clearly defined white blotch on the neck.

- Egyptian specimens are yellow to brown above, often mottled, and the head often darker, with faint darker edges on the head scales and an indication of a “teardrop” marking. FMNH 171897 from Hahîg, Matruh, has 3 yellow bands on the posterior body and 3 on the tail (3 + 3), and FMNH 75232 from northwest of Cairo has 4 + 2 similar bands. Most Egyptian cobras have a single dark throat band covering ventrals ca. 15–25. See photos in Saleh (1997, p. 175), Baha El Din (2006, fig. 109) and Dobiey & Vogel (2007, p. 66).

**FIGURE 8.** Adult specimen of *Naja senegalensis* from near Bamako, Mali, photographed at the house of a local snake catcher. Note the very obvious hood marking, reminiscent of some Asiatic *Naja*.

- No material has been examined from northern Sudan, but most specimens from the south resemble those from Egypt. However, FMNH 190325, a female from Kassala, has a dark brown dorsum with pale streaks and 9 + 2 yellow bands on body and tail, these extend ventrally. FMNH 58468, a 412 mm female from Torit, has two black neck bands on V 12–13 and 15–29, while NMK 3231, a 1880 mm male from Sennar, has brown bands on V 1–9 and 13–27.

- Ethiopian specimens are usually brown with numerous scattered patches of yellow scales, sometimes with a divided yellow band on the neck, but AAU H.664, a male from north of Gondar, has yellow blotches coalescing to form bands caudad, while the venter is blackish with 9 + 2 distinct yellow bands. Dobiey & Vogel (2007, p. 65) illustrate a specimen from Keren, NW Eritrea, which displays a striking pattern of dark brown or black marbling on a creamy-white background. It is unclear whether this is an individual aberrance or a characteristic of the local population.

- Ugandan specimens are yellow to grey-brown, the head and neck often darker and frequently with a faint yellow band on the neck. MUZM (un-numbered), a 900 mm female from Soroti, is black above with 7 + 2 yellow bands, and two juveniles (NMZB-UM 5236–7) from this area show faint banding on the dorsum. A black throat band usually covers ca. v 12–24.

- Kenyan specimens are usually mottled brown and yellow above, with contrasting facial and supralabial markings, sometimes with a yellow band on the neck, rarely other bands caudad. Usually a dark throat band covers ca. V 16–25, rest of venter yellow with brown blotches. Tanzanian specimens are similar, but KMH 3184, a 1063mm female from Mangola, is grey-brown above, with one yellow band on the nape, three on the posterior body and two on the tail.

- There are few records of *N. haje* from the north-eastern Democratic Republic of Congo (DRC) and northern Central African Republic (CAR). One of us (LC) collected four specimens in northern CAR, which

do not differ from those of northern Cameroon, where all the specimens collected by one of us (LC) are very dark, grey or black but not brown, with a pale throat, and superficially similar to Moroccan specimens (photograph in Chirio & LeBreton, 2007, p. 579).

*Naja senegalensis* differs from *N. anchietae*, *N. annulifera* and *N. arabica* in having consistently higher scale row numbers around the neck (23 or more vs. 21 or fewer in the three other species). Additionally, *N. anchietae* and *N. annulifera* differ in having a pointed, enlarged rostral scale, fewer ventral scales (males: maximum 201; females: maximum 206, vs. minimally 205 and 219, respectively, in *N. senegalensis*) and, with few exceptions, 19 or fewer midbody dorsal scale rows.

*Naja arabica* also differs from *N. senegalensis* in colour pattern, which is highly variable (Gasperetti, 1988; Egan, 2007). Specimens from south-west Saudi Arabia and Yemen may be blackish-brown above and below, or with the head and neck black, the rest of the body yellow, the venter often dark mesially. Gasperetti (1988) noted that some individuals were dull black, copper coloured, or various shades of brown or yellow, with blackish top of head and tail, and Egan (2007) additionally noted entirely orange specimens with yellow heads. An adult female from Dhofar, Oman (BMNH 1976.1487) is yellow-brown, but with a black head and neck and becoming black caudad and with a black venter. A 418mm male from the same region (BMNH 1977.1198) has a brown head, yellow-brown dorsum and yellow venter, and van der Kooij (2001, p. 59) illustrates a largely black specimen with coppery lower sides and described a "copper coloured ventral surface", although this is not evident in the photo.

**Description of holotype (Fig. 4–6).** The holotype (MNHN 2008.0074, previously IRD S-8549) is an adult male of the following dimensions: total length 1430 mm, snout-vent length 1175 mm, tail length 255 mm, ratio total length: tail length 5.3.

Head broad and short, weakly distinct from the neck, which is partly dilated. Snout rounded. Eye small, pupil round. Rostral as broad as high, clearly visible from above. The nostril is large and entirely divides the nasal. Two internasals, two prefrontals. The frontal is slightly longer than the prefrontals and internasals, their greatest width is similar. Loreal absent. A single rectangular preocular, twice as long as wide, between eye and nasal. Two postoculars on the left, three on the right. Two suboculars on the left and three on the right entirely separate the eye from the supralabials. 1+ 2 temporals on right, 1+3 on left. Seven supralabials, sixth is largest. Eight infralabials, the first four contact the anterior chin shields. No cuneates. The posterior chin shields are as long as but narrower than the anterior ones. Dorsal scales smooth and oblique, in 25 rows around the neck, 21 around midbody and 15 ahead of the vent. Vertebral row not enlarged. 211 ventrals, anal single. 65 subcaudals, all divided except the second to the ninth, which are single. Stomach content: one *Bufo xeros*.

Upper side of head, body and tail entirely grey-brown. Lower flanks lighter on first two dorsal scale rows, except at anterior and posterior end of body, where they are of the same colour as the dorsum. Lower side of head is grey-brown, similar to the upper side. Underside includes a dark grey area extending from the fifth to the 30th ventral scale, excluding the 12th and 17th ventrals, which are partially light. From the 31st ventral, the dominant colour of the ventrals is yellowish, with dark spots that become fainter towards the posterior part of the body. Subcaudals entirely yellowish, except on the terminal third of the tail, where they become progressively darker.

**Description of paratypes.** The 31 paratypes include 17 males and 14 females. The largest male (IRD S-3429) measured 2065 mm, the largest female (IRD S-1640) 2315 mm in total length. Mean length of males was 1035 mm (SD = 699 mm), of females 1110 mm (SD = 654 mm). The total length: tail length ratio ranged from 5.7 to 6.6 in males (mean: 6.2; SD: 0.2) and from 6.1 to 6.8 in females (mean 6.4, SD 0.3). Midbody dorsal scale rows 21 in males and 21 ($N$ = 13) or 23 ($N$ = 1) in females. The number of scale rows around the neck is 23 (3 males, 1 female), 24 (1 female), 25 (13 males and 9 females), 26 (1 female) or 27 (2 males, 2 females). Ventrals 205–216 (mean 211.7, SD 2.7) in males, 219–225 (mean 222.3, SD 1.6) in females. Subcaudals 59–65 in males (mean 61.5, SD 1.5) and 56–64 in females (mean 59.9, SD 2.2), all or mostly divided. Nasal always fully divided, loreal always absent. Preocular always single, elongate and rectangular. 1–3 postoculars, 1–3 suboculars, the total number of scales around the eye varying from 5 to 7. Supralabials

always 7, except in one specimen with 8 on one side. Temporals 1+2 (*N* = 5), 1+3 (*N*=18), or a combination of the above (*N* = 9). Nuchals 7 (*N* = 19), 8 (*N*=9) or 9 (*N* = 3). Cuneates number 0 (*N* = 6), 1 (*N* = 20), or 1 on one side only (*N* = 5). When present, cuneate always between 4th and 5th infralabials. The examination of stomach contents revealed *Bufo xeros* and *Rhamphiophis oxyrhynchus* in two specimens.

Juveniles (17 specimens measuring under 1000 mm in total length) are pale greyish to greyish-brown dorsally, except the top of the head, the neck and the anterior body, which are dark grey or blackish. On all but one of the juveniles, a white blotch was present on the neck, contrasting strongly with the dark or black colour of the rest of the forebody (Fig. 7). The venter is largely pale beige or yellowish, except for a band of approximately 20 ventrals, usually situated between the 5th and the 30th ventral, which is entirely black. In adults, the dorsal coloration is dark brown or greyish brown, the top of the head and the forebody often being slightly darker, this representing a remnant of the juvenile coloration. A paler neck blotch is sometimes visible. The venter is slightly paler than the dorsum (larger specimens) or yellowish (medium-sized specimens), with the exception of approximately 20 scales under the anterior body, which are dark brown or dark grey. Underside of head usually dark in large specimens.

**Description of other specimens.** The other specimens examined present the same general characteristics as the type series, both in terms of scalation and juvenile and adult coloration. The largest specimen is a female from Mali, measuring 2450 mm (IRD 805-M). The largest male measures 2205 mm (IRD S-343, Senegal). The eye is consistently separated from the supralabials by one or several suboculars. The midbody scale rows number 21, rarely 23, and ventral and subcaudal counts agree with the type series. In males from Mali, ventrals number 211–216, and subcaudals 60–64, and in females, the corresponding counts are 222–225 and 56–64. The number of dorsal scale rows around the neck ranges from 25 (*N*=22) to 27 (*N* = 2) in the 24 specimens from Mali and Niger in which this character has been recorded.

**Etymology.** The name of the new species refers to the country of origin of the type series.

**Distribution and ecology.** *Naja senegalensis* is widely distributed in the savannas of western Africa from Senegal east to south-western Niger, Benin and western Nigeria (Fig. 9). In Senegal, it appears to be found throughout most of the country, although records are lacking from the arid northeast. It is widespread in southern Mali, and has also been recorded from north-western Guinea, Burkina Faso, south-western Niger and northern Benin (Roman, 1973, 1980; Trape & Mané, 2006). The eastern limits of its range are poorly documented. The material from Burkina Faso reported by Roman consisted entirely of *N. senegalensis*, with the apparent exception of a single specimen from Dori in the extreme northeast of the country (LC, observation). A single specimen is known from Shagunu, on the shores of Kainji Reservoir, western Nigeria (CM 92607), whereas a number of specimens of the *N. haje* complex imported from Nigeria by the Liverpool School of Tropical Medicine are referable to *N. haje*, as is material from northern Cameroon and the Central African Republic (Chirio & Ineich, 2006) examined by one of us (LC). The distribution of *N. haje* extends to the north and east of that of *N. senegalensis* at least as far as Tombouctou, Mali (NMZB 13981).

*Naja senegalensis* occupies a variety of savanna habitats. In northern Benin and eastern Burkina Faso, it seems to show a predilection for riparian situations: in the villages around W National Park, villagers know it well and state that it always lives near water. One of us (LC) found some burrows with shed skins near small temporary streams, and the preserved specimens were found in the same biotopes (one was even caught in a fisherman's net). This species seems to be excluded from the banks of larger, permanent rivers by *N. melanoleuca* in W National Park, western Niger. On the other hand, in western Senegal (Sine-Saloum), author JFT has not observed any tendency for the species to be associated with water bodies.

**Biogeography.** The historical biogeography of the *Naja haje* complex is of considerable interest because of its widespread and fragmented distribution. Wüster *et al.* (2007) used molecular dating to infer the historical biogeography of the cobras. The divergence between the *Naja haje* group and *N. nivea* was estimated at approximately 12 Mya, and that between *N. annulifera* and *N. haje* at approx. 7 Mya, albeit with wide confidence intervals. This estimated time of origin for the *N. haje* complex corresponds closely to the late Miocene expansion of C4 grasslands (Cerling *et al.*, 2006), which may have favoured the spread of this clade of open-habitat cobras.

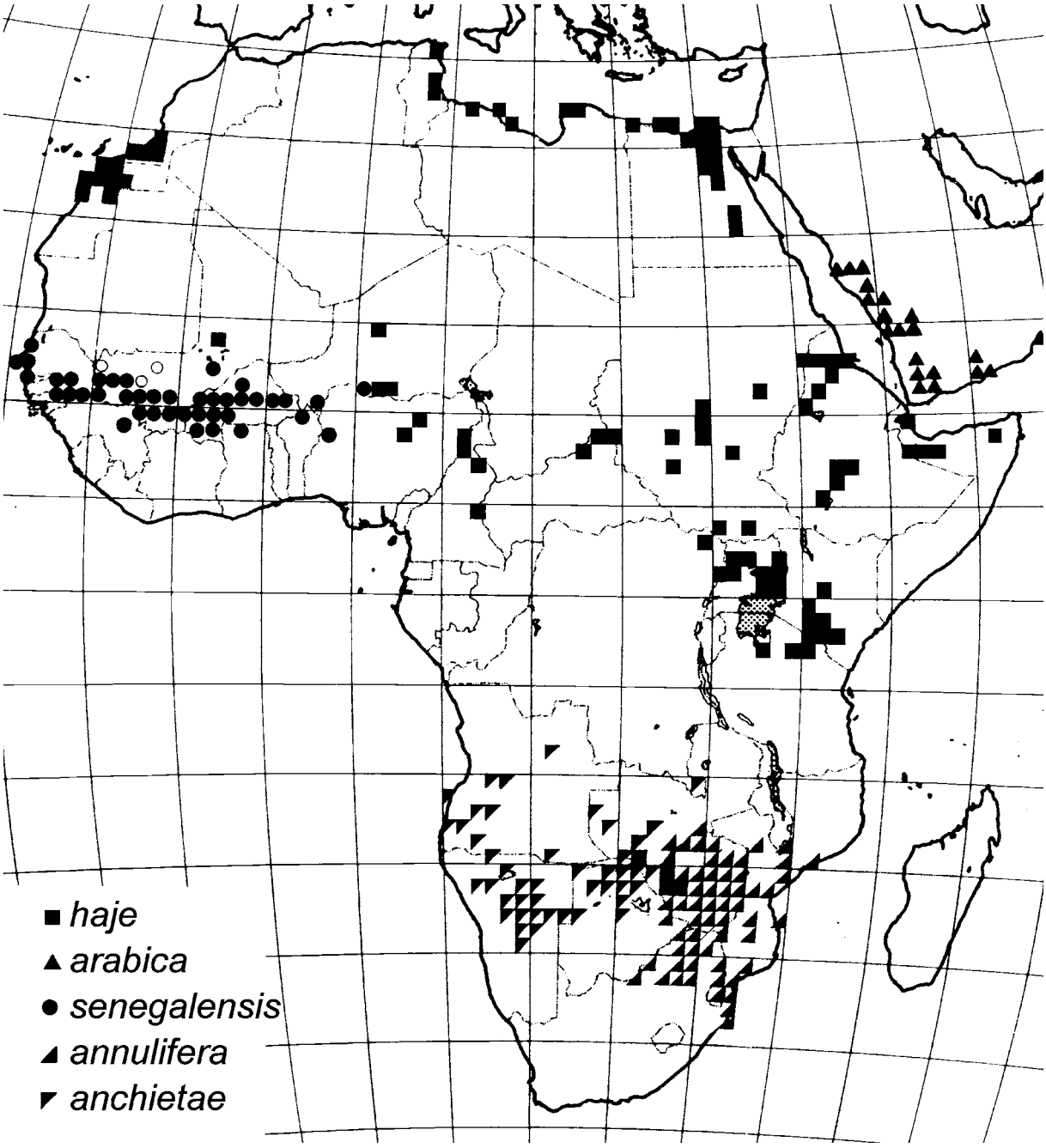


**FIGURE 9**. Distribution of the constituent species of the *Naja haje* complex. Hollow symbols represent unverified literature records.

*Naja senegalensis* joins the list of long-isolated species that appear to be endemic to the Sudano-Sahelian savannas of West Africa, exemplified also by snake species such as *Naja katiensis* (Wüster *et al.*, 2007), *Atractaspis dahomeyensis* and *A. micropholis* (Trape & Mané, 2006), *Hemorrhois dorri* (Trape & Mané, 2006), and lizards such as the gekkonid *Hemitheconyx caudicinctus* and the agamid *Agama sankarica*. Some other species, such as *Psammophis praeornatus* (Trape & Mané, 2006; Kelly *et al.*, 2008) and *Echis ocellatus* (Pook *et al.*, in press) also have primarily West African savanna distributions, although their ranges extend further east to the Central African Republic. The reasons for the apparent isolation of multiple co-distributed West African savanna forms remain unclear, because, irrespective of Plio-Pleistocene fragmentation and expansion of the equatorial forests of Africa, a savanna connection would almost certainly have persisted between the Sudan and Sahel savannas of West Africa and the open formations of East Africa. Additional

molecular dating studies would be needed to ascertain whether these co-distributed West African savanna isolates result from a common event, or whether these distributions were established at different times.

The origin of the Arabian Peninsula populations of the complex appears to be a rather more recent event, most likely dating back to the late Pliocene/early Pleistocene, long after the initial opening of the Red Sea in the late Oligocene/early Miocene, or later Miocene land connections across the southern Red Sea (Bosworth *et al.*, 2005; Fernandes *et al.*, 2006). Past phylogeographic studies have proposed a variety of different ages for trans-Red Sea relationships. Amer & Kumazawa (2005) estimated an age of 11–15 My for the divergence between Arabian and African clades of *Uromastyx*, Pook *et al*. (in press) estimated the split between African and Arabian clades of the *E. pyramidum* complex at 8 Mya, whereas Winney *et al.* (2004) estimated a mid–late Pleistocene crossing of the Red Sea to explain the presence of the hamadryas baboon (*Papio hamadryas*) in Arabia. The origin of *Naja arabica* appears to lie between these extremes, and was dated at approximately 1.75 Mya by Pook *et al.* (in press). The reciprocal monophyly of *N. arabica* and all African *N. haje* does not allow the route of colonization from Africa to Arabia to be inferred: *N. haje* occurs both in Egypt and in the Horn of Africa, so that dispersal either across the Sinai Peninsula into north-western Arabia or across the Bab-El-Mandab into southern Arabia remain tenable hypotheses. Additional mtDNA data from populations from the Horn of Africa might shed further light on the question of the origin of the Arabian populations of this complex (Ineich, 2001).

**Key to the species of the *Naja haje* species complex**

1. West African savannas; 25 (rarely 23 or 27) scale rows around the neck; head and supralabial region dark, without pattern in adults; juveniles normally with a strongly contrasting white blotch on the dark neck..... *N. senegalensis*
- Elsewhere; 23 or fewer scale rows around neck; head and supralabial region often contrastingly patterned; juveniles without white blotch on neck ..... 2
2. Africa south of Tanzania and Congo forests; rostral enlarged, snout pointed; normally 19 or fewer midbody dorsal scale rows..... 3
- Africa from Tanzania northward, Arabian Peninsula; rostral not enlarged; often 21 midbody dorsal scale rows..... 4
3. Western southern Africa; 17 midbody dorsal scale rows, 15 dorsal scale rows around neck ..... *N. anchietae*
- Eastern southern Africa; 19 midbody dorsal scale rows, 19 dorsal scale rows around neck ..... *N. annulifera*
4. Arabian Peninsula; normally over 65 subcaudals; often 19 scale rows around neck, supralabials usually unpatterned ..... *N. arabica*
- Africa from Tanzania northward; normally 65 or fewer subcaudals, rarely fewer than 21 scale rows around neck, supralabials often contrastingly patterned with “teardrop” marking, except in very dark animals..... *N. haje*

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## Appendix 1

Material of *Naja haje* and *Naja arabica* examined by the authors. * = specimens examined for PCAs. Collection acronyms follow Leviton *et al.* (1985), with the following addition: KMH = University of Dar es Salaam, Tanzania. Countries are listed from north to south. Material of *Naja annulifera* and *Naja anchietae* is listed in Broadley & Wüster, 2004.

*Naja haje*: MOROCCO. No precise locality MNHN 2308, 3208*; 12km N of Tiznit, Agadir CM 55257*. TUNISIA. No precise locality MNHN 8797*, 22-244*; USNM 56718; NW of Gabes CM 56575, 56660; USNM 195384*; Mareth FMNH 75970*; Sfax FMNH 83646*; Salammbo (Cartago) ZMUC 6540*; Tunis ZMB 14855*. LIBYA. No precise locality ZMB 10807*, 28532; Kouf National Park FMNH 214914*; Misurata FMNH 83058*. EGYPT. No locality ZMB 4349*, 4350*; Abu Ghalib FMNH 75234*; USNM 131257-58*; Aswan ANSP 19436*; Bahig FMNH 171897*-98*; Beni Suef FMNH 75097*-98*, 164708; Biba FMNH 69255*; Birquash FMNH 75230*; Burgel Arab USNM 195487*; 179km NW of Cairo FMNH 75232*; Damanhur FMNH 75229*; Delta Barrage USNM 13025*-26*-27; El Badshein NMZB-UM 6899*, 6900*; El Daba FMNH 68812*; El Mansuriya FMNH 129881*-82*; Helwan FMNH 75233*; Kom-o-Shin, Faiyum FMNH 72026*; Mersa Matruh TMP 20845*; 20km W of Sidi Barrani FMNH 75231*; Sinnuris FMNH 153045*; Tamiya FMNH 171896; Thebes MCZ 881* (2). SUDAN. Aweil ZFMK 29574; Sennar NMK 3251*-32*; Telaweit, Kassala FMNH 190325*; Torit FMNH 58466*, 58467*-8*. ETHIOPIA. No locality USNM 218680; Arba Minch AAU 488*; 90km N of Gondar AAU 664*; Gura ANSP 25206*; Koka Dam, Awash AAU 660* ; 10km N of Lake Lagano PEM 8584*; Lake Shalla AAU 805*. KENYA. Athi River NMK 562*, 740*, 886*, 887*, 1506, 1544*, 3050* + 3 un-numbered **; Embakasi NMK 2853*; Kajiado NMK 2476*; Karen – Langata NMK 1825; Lukenya Hills NMK 2703*; Mtito Andei USNM 48592*. TANZANIA. Arusha CM 37171*; USNM 76611*; Longido NMK 1477; Mangola KMH 3184*. UGANDA. Bugoma Forest LACM 39019; Gulu MCZ 47835*; Lira, Lango MCZ 47808 (2)**; NMZB-UM 5336*; Soroti NMZB-UM 5337. DEMOCRATIC REPUBLIC OF CONGO. Faradje AMNH 12326; Kasenyi ANSP 20782. NIGERIA. Katsina area BMNH 1975.651*, 652*, 653*, 654*, 655; NIGER. Cissia (13°52'N/10°25'E) IRD 248-N, IRD 672-N; Tekhé (14°01'N/06°01'E) IRD 690-N* ; Tahoua (14°54'N/05°16'E) IRD 832-N*. MALI. Tombouctou NMZB 13981*; ZMUC 6501. WEST AFRICA. No precise locality CM 7217*; ZMB 2820* (type of *Naja haje* var. *viridis* Peters 1873).

*Naja arabica*: No locality ZMB 2805. SAUDI ARABIA. Abha CAS 148565*; An Numas BMNH 1985.744*; Barahard CAS 136529*; Dalaghan BMNH 1985.745; Hakimah CAS 140491*; Hijla BMNH 1985.911*; Khamis Mushayt CAS 148558, 148588*; 20km W of Sayal al Kabir CAS 148040; Wadi Amagk CAS 139801*; Wadi Mahra CAS 145323*. OMAN (DHOFAR). Khadrafi BMNH 1977.1198*; Wadi Darbat BMNH 1976.1487*.