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A new species of *Zygaspis* (Reptilia: Squamata: Amphisbaenidae) from north-eastern Mozambique

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Abstract.—We describe a new species of *Zygaspis* based on a single specimen collected in north-eastern Mozambique and deposited in the Natural History Museum of Zimbabwe, Bulawayo. The new species is characterised by its extensively black pigmentation, 191 body annuli, discrete preoculars, four parietals, two postoculars, three supralabials, and two post-supralabials. Phylogenetic analysis of mtDNA suggests that it is a sister taxon to the widespread *Zygaspis quadrifrons*.

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Introduction

Amphisbaenians are elongate, limbless lizards commensurate with their subterranean lifestyle. This elongate form and the resultant internal and external morphological adaptations to burrowing originally led taxonomists to place the group as a suborder within the squamates. However, molecular evidence has now shown this group to be sister to the Lacertidae (Müller *et al.* 2011; Townsend *et al.* 2004). This underlines the importance of both morphological and molecular evidence for taxonomy in this group, an approach which we endorse and extend in the description of a new species in the southern African genus *Zygaspis*.

In his revision of the African Amphisbaenidae, Loveridge (1941) assigned all round-snouted taxa to the genus *Amphisbaenia* Linnaeus (except for two species of *Placogaster* [= *Cynisca*] in West Africa), but Vanzolini (1951) erected two new genera, *Loveridgea* for *A. phylofiniens* Tornier, and *Shrevea* for *A. quadrifrons* Peters (he also tentatively assigned *A. dolichomenta* Witte & Laurent to this genus). Subsequently, Vanzolini (1953) indicated that *Zygaspis* Cope 1885 had priority as the generic name for *Z. quadrifrons*.

Broadley & Gans (1969) described *Z. niger* [= *Z. nigra*] from western Zambia where it is sympatric with *Z. quadrifrons*. Saiff (1970) reviewed geographic variation in *Z. quadrifrons* and *Z. nigra* and noted character displacement in sympatry. Broadley & Gans (1978) transferred *Amphisbaena violacea* to *Zygaspis* and placed *A. vandami*

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FitzSimons in synonymy with *Z. violacea*. Broadley & Broadley (1997) revised *Zygaspis,* described two new species, *Z. ferox* and *Z. kafuensis,* recognised *Z. dolichomenta* as a full species, and reinstated *Z. vandami,* with a new subspecies *Z. v. arenicola.* A molecular phylogeny (Measey & Tolley 2013) showed *Z. vandami* (*Z. violacea* complex) to be the sister taxon of the *Z. quadrifrons* complex, represented by *Z. quadrifrons* and *Z. nigra.* The last species of *Zygaspis* named was nearly 20 years ago (Broadley & Broadley 1997).

Here we describe a new species in the genus *Zygaspis* based on newly collected material recently deposited in the Natural History Museum of Zimbabwe, Bulawayo. We use tissue collected from the same individual to place the new species into an existing phylogeny of some species of the genus (Measey & Tolley 2013) using fragments of two genes of mitochondrial DNA.

Materials and Methods

The specimen used in this account was deposited in the Natural History Museum of Zimbabwe, Bulawayo (NMZB). Specimens used for comparison are listed in Appendix 1. Snout-vent length measurements were performed using a ruler (to the nearest 1 mm), while other measurements were taken with a digital calliper (to the nearest 0.1 mm). We used Broadley & Broadley (1997) for comparing existing species with the new species.

Phylogenetic Analysis

Total genomic DNA was extracted from liver tissue using standard salt extraction methodology (see Bruford *et al.* 1992). Fragments from two mitochondrial genes (16S and ND2) were amplified, following Measey and Tolley (2013). We used published primers for a selected fragment of the 16S ribosomal rRNA gene and for NADH dehydrogenase subunit 2 (Measey & Tolley 2013). Amplification conditions were the same as those detailed in Measey and Tolley (2013). Products were sequenced by Stellenbosch University Central Analytical Facility. Sequences were checked and aligned in GeneiousPro v. 4.8 (Drummond *et al.* 2007). Uncorrected p-distances for the 16S fragment were calculated in MEGA 6 (Tamura *et al.* 2013). All new sequences generated have been deposited in the EMBL Nucleotide Sequence Database (LT558090; LT558091).

We included African representatives from Measey and Tolley (2013: Table 1) as well as *Trogonophis wiegmanni* as an outgroup taxon. We chose the outgroup taxa because of its known close sister relationship with the ingroup taxa (Pyron *et al.* 2013). We used jModelTest (v 2.1.6; Darriba *et al.* 2012) which selected (GTR+I+G for 16S and ND2) as the best of 88 models tested. Phylogenetic hypotheses were recovered under two different optimality criteria—maximum likelihood and Bayesian inference. The Bayesian analysis was run using MrBayes 3.2.3 (Ronquist & Huelsenbeck 2003) and maximum likelihood using RAxML (v8.0.24 Stamatakis 2014) as implemented in the CIPRES Science Gateway v3.3 (www.phylo.org/portal2/). The Bayesian analysis employed 20 million generations, sampling every 1000 generations, with the first 4500 trees removed as burn-in. The remaining trees were used to construct a 50% majority rule tree, where nodes with >0.95 posterior probability were considered to be supported. The maximum likelihood search was started from a random tree with all parameters estimated.

Results

Zygaspis maraisi sp. nov.

Holotype.—NMZB 18009 (Figure 1) from Pioneer Camp, Afungi Peninsula, Cabo Delgado Province, Mozambique, 10°50′17″ S, 40°30′53″ E, and elevation 17 m (Figure 2). Collected by Johan Marais, 24 November 2013.

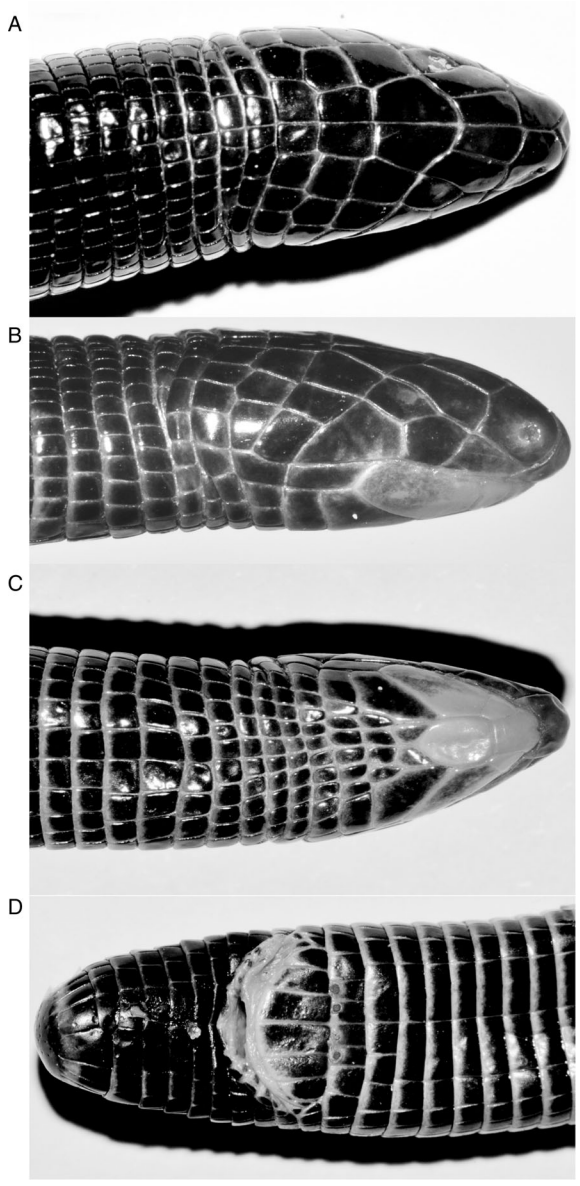

Figure 1. The holotype of *Zygaspis maraisi* sp. nov. (NMZB 18009) from Pioneer Camp, Afungi Peninsula, Cabo Delgado Province, Mozambique. A dorsal; B lateral; C ventral; D cloacal region.

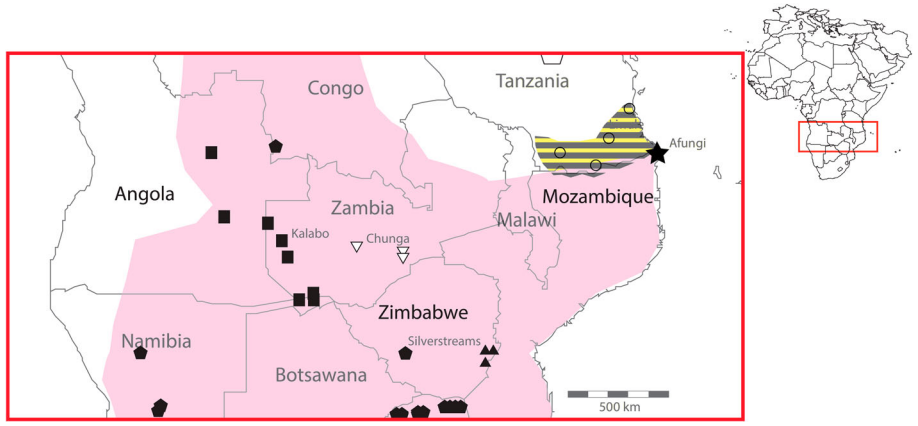

Figure 2. Sampling site of the holotype of *Zygaspis maraisi* sp. nov. (black star) relative to other species of *Zygaspis* with their type localities identified: *Z. ferox* (black triangle), *Z. kafuensis* (inverted white triangle), *Z. quadrifrons* (black pentagons) and *Z. nigra* (black square). Note that with the description of *Z. maraisi* sp. nov., the polygons for the genera *Zygaspis* (grey) and *Loveridgea* (striped) (*Loveridgea ionidesi* (white circles)) are now adjacent. Inset: African continent showing position of detailed section.

Diagnosis.—Similar to other species of *Zygaspis*, the diagnostic feature of *Z. maraisi* **sp. nov**. is its extensive black pigmentation, with only the chin area being white (*Z. nigra,* the only partly-coloured species, develops increasing black pigment as it grows, but adults have only the anterior 90% of each dorsal segment black, while no ventral segments are more than 50% black). It agrees with the *Z. quadrifrons* complex in having paired discrete preoculars. The new species further differs from *Z. nigra,* in having the third supralabial followed by two temporals and two post-supralabials. It differs from most *Z. quadrifrons* in having body annuli less than 200. It differs from *Z. kafuensis* in lacking a short blind sulcus extending from the rear edge of the nasal towards the nostril. It differs from *Z. ferox* in having only 32 segments in a mid-body annulus, and being more slender, with a smaller head. It differs from *Z. dolichomenta* in having the postmental barely longer than the mental.

Loveridge (1941) recorded 221–242 body annuli in *Zygaspis* [*Amphisbaena*] *q. quadrifrons* and 198–221 in *A. q. capensis* (now synonymised with *Z. quadrifrons*), and these figures were repeated by Fitzsimons (1943), who reported an 'overlap' between the two subspecies in the Kalahari. Jacobsen (1989) recorded 190–228 (mostly 200–220) body annuli for *Z. quadrifrons* in the North-West and Limpopo Provinces (formerly Transvaal). Broadley and Broadley (1997) recorded 195–245 (usually 207–245) body annuli for *Z. quadrifrons* throughout its range ($N = 982$).

Etymology.—The name is a patronym honouring the collector, Johan Marais, in recognition of his many contributions to southern African herpetology. The name is constructed in the masculine genitive.

Description.—A large species of *Zygaspis* with discrete preoculars, four parietals, two postoculars, three supralabials, and two post-supralabials. Postmental only slightly longer than mental; two plus four postgenials; eight postmalars; three infralabials, second much the largest.

Annuli on body 191, tail autotomised at sixth caudal annulus, with 18 dorsal and 14 ventral segments in a midbody annulus, the lateral sulci are well defined. There are four precloacal pores on the last body annulus, six precloacal and 10 postcloacal segments. SVL/diameter ratio 34; SVL/head length ratio 24.

Size.—Holotype 207 mm snout-vent length, 7 mm tail length (autotomised), diameter 6 mm, head length 8.5 mm.

Colouration.—Black above and below, except for the mental, postmental and infralabials, which are white.

Genetics.—The maximum likelihood and Bayesian analyses produced identical topologies, and the same arrangement of genera as previous investigations (e.g. Measey & Tolley 2013). As previously, the analyses could not resolve the placement of the genus *Zygaspis*. Within the genus our analyses produced maximum support for *Z. vandami* as the sister species to other *Zygaspis*, and for the close relationship between *Z. quadrifrons* and *Z. maraisi* sp. nov.; although the relationship between this clade and *Z. nigra* was supported only by Bayesian analysis (Figure 3).

Habitat.—*Strychnos madagascarensis–Xylothecia tettensis* Short Open Woodland on deep grey/white sands.

Distribution.—Although the species is known only from the holotype collected on the Afungi peninsula, this taxon may be more widespread in the coastal sandy soils of north-eastern Mozambique.

Conservation.—Under the current International Union for Conservation of Nature guidelines (IUCN 2014), this species should be regarded as Data Deficient.

Discussion

The addition of *Z. maraisi* sp. nov. to the genus *Zygaspis* means that it now constitutes eight species. Outstanding taxonomic work is needed on the two subspecies of *Z. vandami* to elucidate whether they represent full species (Measey 2014). The cryptic habits of these subterranean taxa make it likely that there are more species awaiting description. Approximately 28% of squamate reptiles are associated with the soil (Measey 2006), and a global analysis on the conservation status of the world's reptiles revealed that amphisbaenians, like other subterranean reptiles, are particularly poorly known (Böhm *et al.* 2013). Threats to amphisbaenians are thought to include soil compaction and reduction in leaf litter (Measey *et al.* 2009).

Zygaspis maraisi sp. nov. becomes the fourth large species of *Zygaspis* without fusion of the dorsal head shields. Two of these species inhabit forests—*Z. dolichomenta* in lowland forests of the western Congo basin, and *Z. ferox* in submontane forests of south-eastern Zimbabwe (Broadley & Broadley 1997), whereas *Z. kafuensis* and *Z. maraisi* sp. nov. inhabit sandy substrates. Our description of *Z. maraisi* sp. nov. extends the distribution of the genus into the far northeast of Mozambique, suggesting that populations may be found throughout the coastal areas of the Mozambique plain. To date, there are no records of *Z. quadrifrons* from northern Mozambique; the nearest

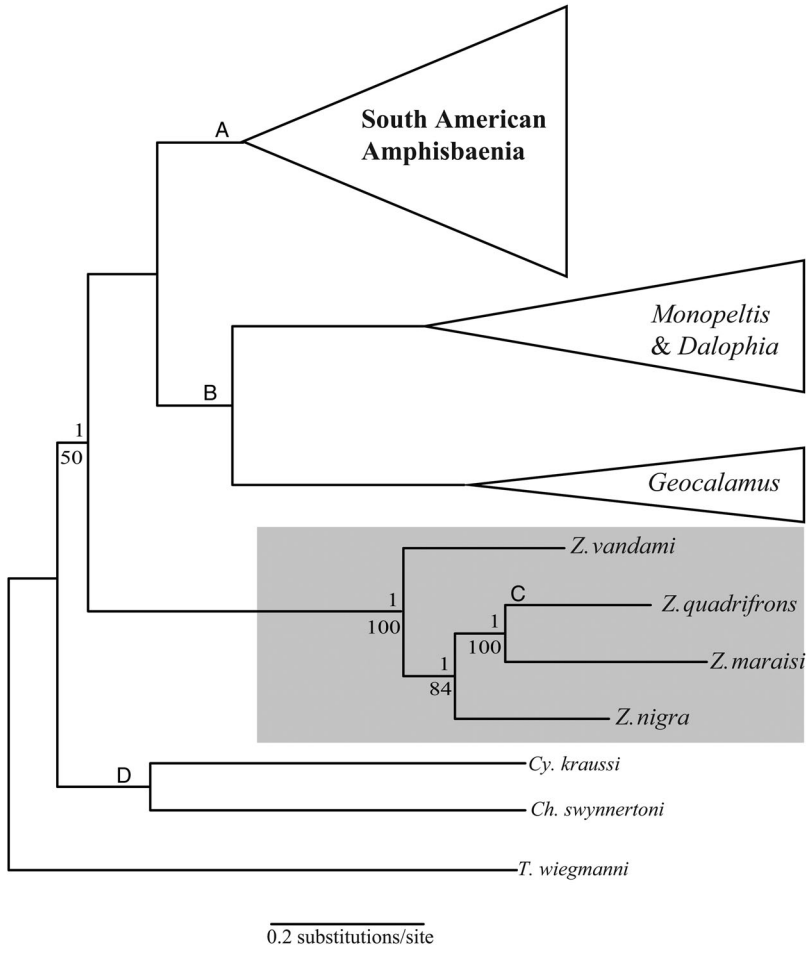

Figure 3. A Bayesian topology based on two mtDNA gene fragments (467 bp 16S and 640 bp ND2) showing the relationship of *Zygaspis maraisi* sp. nov. to other African amphisbaenians (see Measey & Tolley 2013). Numbers above the line represent Bayesian posterior support, while those underneath are likelihood bootstrap values. Note that there are no sequences for many species within the genus *Zygaspis* or the proposed sister taxon *Loveridgea*. (Generic assignments: *Ch, Chirindia; Cy, Cynisca; T, Trogonophis; Z, Zygaspis.*)

specimens known are from Qualamaine (870 km south-southeast) and Inhaminga (1030 km southeast) (Broadley & Broadley 1997; Saiff 1970). This new species is the eastern-most in the genus and occurs in close proximity to *Loveridgea ionidesi* north of the Rovuma River (Figure 2), and it is possible that these are sister genera (Measey & Tolley 2013).

Our phylogeny places *Z. maraisi* sp. nov. unambiguously as the sister species to the most geographically-widespread taxon, *Z. quadrifrons*, but in the absence of samples of *Z. dolichomenta, Z. ferox* and *Z. kafuensis* (Figure 3). Uncorrected p-distances suggest that this species is between 12.7 and 8.1% different from other members of the genus for which 16S sequences exist (Table 1). Although this is the smallest genetic distance we record, it is much larger than normal distances within 16S for other members of the

Table 1. Uncorrected p-distances between species in the genus *Zygaspis* from a 467 bp fragment of 16S.

	Z. vandami	*Z. quadrifrons*	*Z. nigra*
Z. quadrifrons	0.098		
Z. nigra	0.101	0.094	
Z. maraisi sp. nov.	0.112	0.081	0.127

family Lacertidae (e.g. Guo *et al.* 2011). While the monophyly of the clade containing all *Zygaspis* receives full support, inclusion of this taxon does not help place the genus within the larger phylogeny of the Amphisbaenidae. The position of the genus in relation to South American amphisbaenians, as well as the other African genera, including the unsampled members of the genus *Loveridgea*, remains ambiguous.

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Appendix 1. Comparative Material Examined

Zygaspis ferox: NMZB-UM 8333, 8974 (Holotype) Silverstreams, Zimbabwe; *Zygaspis kafuensis:* NMZB-UM 30039 (Holotype) and NMZB-UM 30040-41 Chunga, Kafue National Park, Zambia; *Zygaspis nigra:* NMZB-UM 4817-8, 6698 (Holotype), 6706 Kalabo, Zambia; NMZB 10105 Gago Coutinho District, Angola. *Zygaspis quadrifrons:* NMZB-UM 4822-3, 6725, 6729 Kalabo, Zambia; *Zygaspis vandami arenicola:* NMZB-UM 17731-2 Gonarezhou National Park, Zimbabwe; NMZB-UM 24236 Katima Mulilo, Caprivi, Namibia; NMZB 1822 Mavuradonha Mts, Zimbabwe; NMZB-UM 20631 Mparamanga Gorge, Malawi; NMZB-UM 20633 Nkula Falls, Malawi; NMZB-UM 21915 Inhamitanga, Mozambique. These last three *Zygaspis* localities are the nearest to the Afungi Peninsula. *Zygaspis violacea:* NMZB 10006 Magaruque Island, Mocambique (Holotype of *Z. longicauda*).