

Rediscovery, range extension, habitat and phylogenetic relation of the endemic Scaled Sandveld Lizard *Nucras scalaris* Laurent, 1964 (Sauria: Lacertidae) in the central Angolan plateau

Ninda L Baptista , Krystal A Tolley , Marc Bluhm , Manfred Finckh & William R Branch

To cite this article: Ninda L Baptista , Krystal A Tolley , Marc Bluhm , Manfred Finckh & William R Branch (2020): Rediscovery, range extension, habitat and phylogenetic relation of the endemic Scaled Sandveld Lizard *Nucras scalaris* Laurent, 1964 (Sauria: Lacertidae) in the central Angolan plateau, African Journal of Herpetology, DOI: [10.1080/21564574.2020.1778108](https://doi.org/10.1080/21564574.2020.1778108)

To link to this article: <https://doi.org/10.1080/21564574.2020.1778108>

 View supplementary material 

 Published online: 08 Jul 2020.

 Submit your article to this journal 

 View related articles 

 View Crossmark data 



Rediscovery, range extension, habitat and phylogenetic relation of the endemic Scaled Sandveld Lizard *Nucras scalaris* Laurent, 1964 (Sauria: Lacertidae) in the central Angolan plateau

Ninda L Baptista ^{a,b,c}, Krystal A Tolley ^{d,e}, Marc Bluhm^f, Manfred Finckh ^f and William R Branch^{g,h}

^aInstituto Superior de Ciências da Educação da Huíla (ISCED-Huíla), Rua Sarmento Rodrigues, Lubango, Angola; ^bCIBIO/InBio – Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus de Vairão, Vairão, Portugal; ^cFaculdade de Ciências, Universidade do Porto, Porto, Portugal; ^dSouth African National Biodiversity Institute, Kirstenbosch Research Centre, Cape Town, South Africa; ^eCentre for Ecological Genomics and Wildlife Conservation, Department of Zoology, University of Johannesburg, Auckland Park, Johannesburg, South Africa; ^fBiodiversity, Ecology and Evolution of Plants, Institute of Plant Science and Microbiology, University of Hamburg, Hamburg, Germany; ^gPort Elizabeth Museum (Bayworld), Humewood, South Africa; ^hResearch Associate, Department of Zoology, Nelson Mandela University, Port Elizabeth, South Africa

ABSTRACT

The Scaled Sandveld Lizard *Nucras scalaris* Laurent, 1964 is a poorly known lacertid endemic to north-eastern Angola and is only known from the type series collected more than half a century ago. The original description provided a comprehensive morphological description, but there was a lack of information regarding its evolutionary relationships and habitat associations. A recent discovery of *N. scalaris* from Cusseque, Bié Province, on the central Angolan plateau provided the opportunity to address some knowledge gaps of this species. A phylogenetic analysis confirmed its distinctiveness at the species level. Despite a limited dataset, the analysis suggests that *N. scalaris* is sister to *N. broadleyi* rather than *N. lalandii*, the latter having been assumed to be the sister species, because of morphological similarity between them. The new record of *N. scalaris* represents a range extension of more than 350 km to the southwest and extends the elevation range to 1 570 m above sea level, compared to previous known localities at about 1 300 m above sea level (Alto Cuilo and Alto Chicapa, in Lunda Sul Province). The specimen also provides new information on live coloration, as well as the habitat association of grassy shrubland that is dominated by geoxylic suffrutices (the so-called ‘underground forests’) characteristic of the Angolan Miombo Woodlands. This new information raises the question whether poor survey data have led to an under-estimation of faunal diversity in this peculiar and overlooked vegetation type, and reinforces the need for further surveys which could highlight the importance of this habitat.

ARTICLE HISTORY

Received 10 February 2020
Accepted 1 June 2020

KEYWORDS

Angolan Miombo
Woodlands; Bié Province;
geoxylic suffrutex; Ongote;
Reptilia; underground forests

CONTACT Ninda L Baptista  nindabaptista@gmail.com

© 2020 Herpetological Association of Africa

 Supplemental data for this article can be accessed at <https://doi.org/10.1080/21564574.2020.1778108>

African Journal of Herpetology is co-published by NISC Pty (Ltd) and Informa Limited (trading as Taylor & Francis Group)

RESUMO

O Lagarto-da-areia-de-laurent *Nucras scalaris* Laurent, 1964 é um lacertídeo endêmico do nordeste de Angola sobre o qual se sabe muito pouco, sendo apenas conhecido da série tipo, colhida há mais de meio século. A descrição original da espécie fornece bastante detalhe a nível morfológico, mas até agora não havia nenhuma informação acerca das relações filogenéticas ou do habitat a que a espécie está associada. Uma redescoberta recente do lagarto no Cusseque, na província do Bié, no planalto central de Angola, permitiu preencher algumas das lacunas de conhecimento que existiam relativamente a esta espécie. Uma análise filogenética confirmou que *N. scalaris* é distinto a nível específico de todos os outros *Nucras*. Embora os nossos dados sejam limitados, *N. scalaris* parece ser a espécie-irmã de *N. broadleyi* e não de *N. lalandii*, como se pensava anteriormente, devido a semelhanças morfológicas entre ambas. O novo registo de *N. scalaris* representa uma extensão da sua área de ocorrência de mais de 350 km para sudoeste, e de um aumento de altitude para 1570 m acima do nível do mar quando comparado com as localidades de onde era conhecido anteriormente a cerca de 1300 m acima do nível do mar (Alto Cuílo e Alto Chicapa, na província da Lunda Sul). (Alto Cuílo e Alto Chicapa, na província da Lunda Sul). Está agora disponível mais informação sobre a coloração do animal vivo, e a espécie parece estar associada ao ongote, as 'florestas subterrâneas' características da Mata de Miombo Angolana. Esta descoberta levanta a questão sobre se a escassez de levantamentos terão levado a subestimar a diversidade faunística associada a este tipo de vegetação peculiar e negligenciado, e reforça a necessidade de mais levantamentos que possam realçar a importância deste habitat.

PALAVRAS-CHAVE

Mata de Miombo Angolana; Província do Bié; geófilas; Anharas de Ongote; Reptilia; florestas subterrâneas

Introduction

The genus *Nucras* Gray, 1838 (Squamata: Sauria: Lacertidae) contains twelve species that occur across southern and eastern Africa (Bauer et al. 2019; Branch et al. 2019b). In southern Africa, *Nucras* are distributed mainly in the arid and mesic savanna on sandy soils, although some species enter fynbos, montane grassland and Karoo scrub (Branch 1998; Alexander and Marais 2007). Two endemic species of *Nucras* are currently known to occur in Angola (Marques et al. 2018; Branch et al. 2019b; Branch et al. 2019c): *Nucras scalaris* Laurent, 1964, from the northeast, and *Nucras broadleyi* Branch, Conradie, Vaz Pinto and Tolley, 2019 from the southwest. *Nucras broadleyi* is within the larger *N. tessellata* + *lalandii* species complex that occurs in the arid regions of southern Africa, although it is the most genetically distinct and geographically distant species in that complex (Branch et al. 2019b). Very little information exists about the Scaled (or Laurent's) Sandveld Lizard, *N. scalaris* from Angola. It is only known from the type series of four specimens, three males (no. 5401, 5481 and 5485) and one female (no. 5346) collected in June/July 1954 from the type locality Alto Chicapa, and from Alto Cuílo, both in Lunda Sul Province of north-eastern Angola (Figure 1). The type specimens are in the collection at the Museu Regional do Dundo, Angola, except for paratype no. 5485, which is deposited in the Museum of Comparative Zoology, Harvard (accession no. MCZ Herp R74121).

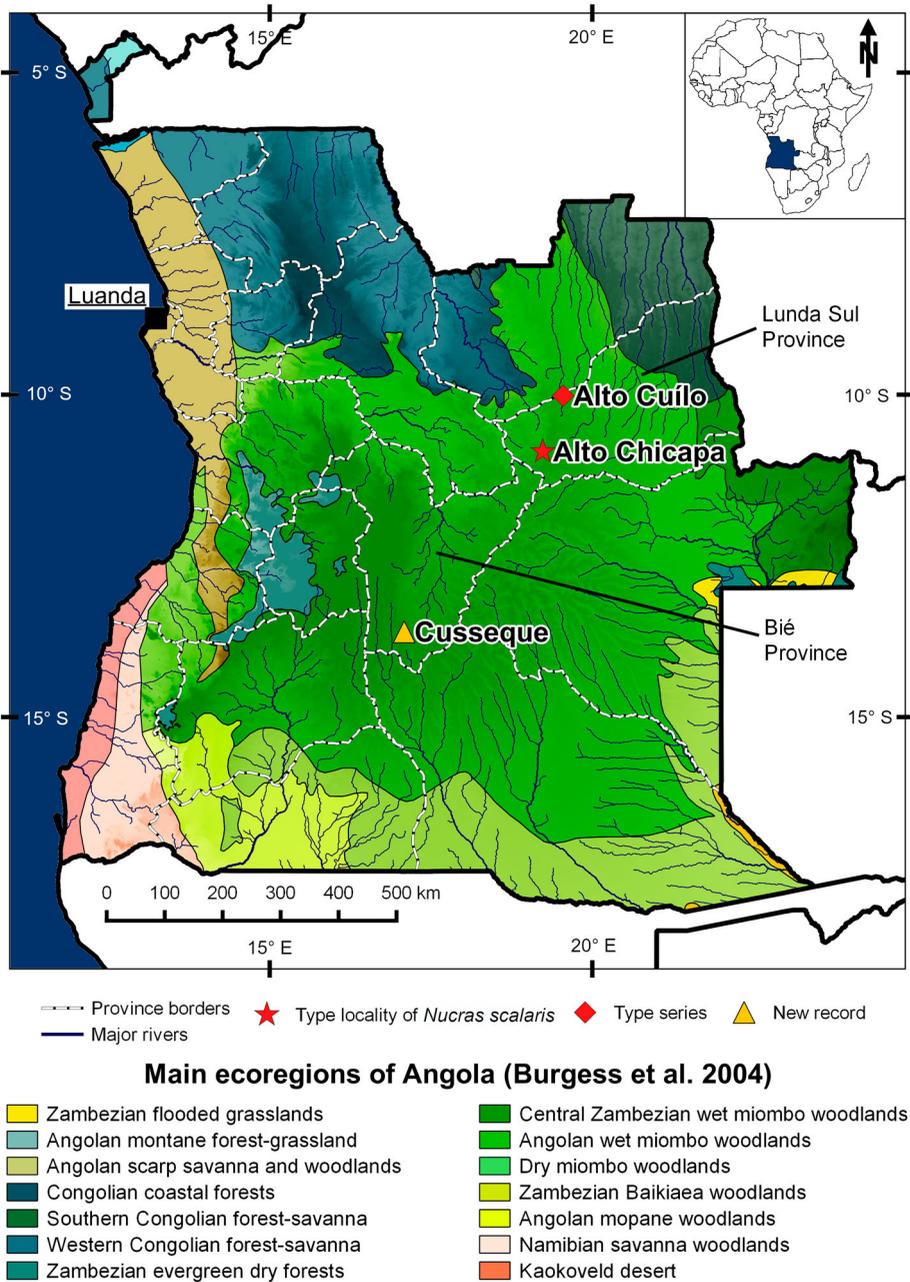


Figure 1. Map of Angola showing ecoregions according to Burgess et al. (2004). Records of the *Nucras scalaris* type series depicted in red, and the new record in orange.

Laurent (1964) provides a detailed description of the species, which resembles *N. lalandii* (Milne-Edwards, 1829) from south-eastern Africa, but has consistent morphological differences that place it as a separate taxon. Features distinguishing *N. scalaris* from *N. lalandii* are a larger number of dorsal midbody scale rows (41–49 v 34–42, respectively), a larger

number of lamellae under the fourth toe (21–27 v 16–22), the existence of enlarged scales under the forearm in *N. scalaris* (absent in *N. lalandii*), and the dorsal coloration consisting of fewer transversal bars that are more complete and regular in *N. scalaris* than in *N. lalandii* (Laurent 1964). Until now, no information was available about the evolutionary relationships or habitat associations of *N. scalaris* although it has been regarded as the sister species to *N. lalandii*, because of their morphological similarity (Laurent 1964).

Here, we report on the first record of this species since the original description more than 50 years ago and provide live photographs and details on habitat for the first time. In addition, we included *Nucras scalaris* in a phylogenetic analysis, to assess whether *N. scalaris* does represent a separate species, if it might be within the *N. lalandii* + *tessellata* complex, and/or whether it is a sister species to *N. lalandii*. Lacertids, including *Nucras*, are considered to be labile in their morphology, with their similarities linked to ecology rather than shared ancestry (e.g. Edwards et al. 2012; Edwards et al. 2013). Thus, the similar morphological features between *N. scalaris* and *N. lalandii* might not reflect a recent common ancestor, and *N. scalaris* might be more closely related to geographically proximate species, such as *N. broadleyi*. Notably, *Nucras scalaris* and *N. lalandii* are currently separated by at least 1 700 km. For these two species to have shared a recent common ancestor, a major vicariance event would be necessary to explain that relationship. An alternative, and possibly more likely scenario would be that *N. scalaris* diverged from species that are geographically closer to it than *N. lalandii*.

Materials and methods

Study area and sampling

Within the Southern African Science Service Centre for Climate Change and Adaptive Land Management (SASSCAL) project, biodiversity monitoring and surveys have been carried out across Angola (Baptista et al. 2018; Jürgens et al. 2018; Baptista et al. 2019). During herpetological surveys in the Cusseque observatory (approximately -13.683°S , 17.085°E ; 1 572 m above sea level) in the Municipality of Chitembo, Bié Province (Figure 1), an adult female *Nucras scalaris* was captured on 14 June 2018. The observatory is located in the upper valley of the Cusseque River, on the southern slopes of the central Angolan plateau. In Cusseque, vegetation varies from closed miombo woodlands to different types of grasslands and peatlands (Revermann et al. 2013). The climate is semi-humid, with a pronounced rainy season between November and April (Weber 2013). The lizard was caught in a pitfall trap set along a drift fence placed on an open sandy strip. In the ecotone, this sandy strip separates peatlands in a small tributary of Cusseque River (downslope), from geoxylic suffrutex-dominated grassy shrublands ('underground forests' locally called 'Anharas de Ongote') and open miombo woodlands (upslope) (Figure 2).

These grassy shrublands and woodlands grow on pasmmo-ferrallitic soils, with a sandy topsoil of several decimetres depth overlaying a strongly weathered, batholithic substrate. During the rainy season, precipitation infiltrates in the sandy soils and percolates between sand and underlying batholithic substrates into the peatlands. The greyish-white sandy strip (Figures 2B and 2C), where the pitfall trap was placed, corresponds to the zone where interflow waters seep out during strong rains and flows into the adjacent peatlands.

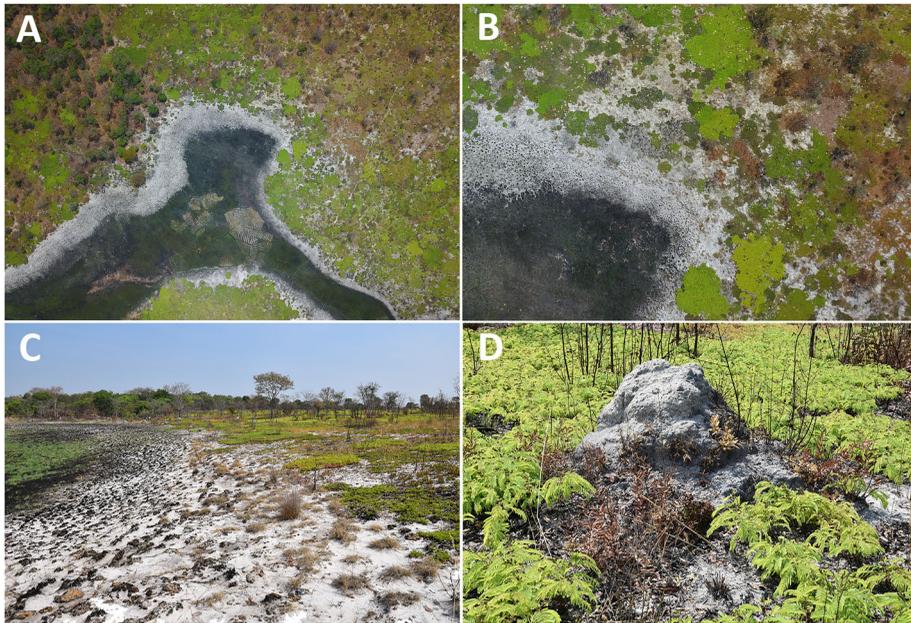


Figure 2. Habitat in Cusseque observatory (A) Aerial overview of habitat. *Nucras scalaris* was captured on the strip of greyish-white sand separating the peatlands (dark gray) from the surrounding geoxylic suffrutex-dominated grassy shrublands and miombo woodlands (light green and reddish patches). (B) Aerial view of patchy configuration dominated by *Brachystegia russelliae*. Trees, shrubs, termite mounds (light grey) and lines of molehills (white dots) clearly discernible. (C) Sandy strip where drift fence was set. (D) Termite mounds. All images taken in the end of the dry season, 4 September 2019. Photo credit: A, B: P Zigelski; C, D: M Finckh.

The downslope peatlands are dominated by tuft grasses (e.g. *Monocymbium ceresiiforme*, *Tristachya huillensis*), sedges and bracken (*Pteridium* sp.), and are used for shifting, small-scale, dry season local farming. For most of the year the soils of the peatland are water saturated, drying out only for a short period at the end of the dry season. The lizard was collected and euthanized by hypothermia and then freezing (Lillywhite et al. 2017), preserved in 70% ethanol, and deposited in the herpetological collection in Instituto Superior de Ciências da Educação da Huíla (ISCED-Huíla), Lubango, Angola, with the following accession no. NB1110. Tissue for DNA extraction was collected after preservation.

Morphological analysis

Measurements of the specimen were taken with a digital calliper to the nearest 0.1 mm following Laurent (1964), Edwards et al. (2013) and Branch et al. (2019b). These included: snout-vent length (SVL): tip of snout to anterior edge of cloaca, tail length: tip of tail to posterior edge of cloaca, total length: combined SVL and tail length, head length: from anterior edge of occipital/parietal scale to tip of snout, head width: width of head (just behind eye), head height: height of head at the posterior part of the cranium, snout length: from anterior corner of eye to tip of snout, foot length: from distal tip of fourth toe to back of heel, eye length: horizontal diameter of eye, ear-eye length: from posterior

corner of eye to anterior edge of ear opening. Laurent (1964) used several body ratios in the original description of *N. scalaris*, but Broadley (1972) discussed their use in the genus *Nucras*, concluding that there was too much overlap in these features, precluding their usefulness for species identification. Accordingly, we opted to only use full measurements in the present study. Characterisation of scalation and other features followed Laurent (1964), Broadley (1972) and Branch et al. (2019b) for upper labials: anterior and posterior to subocular, lower labials, indicating the position of the largest, dorsal midbody scale rows, transverse rows of ventrals, longitudinal rows of ventrals, supraciliaries, granules between supraciliaries and supraoculars, number of subdigital lamellae under 4th toe, number of scales in collar, presence or absence of enlarged scales under forearm, the presence of interparietal and whether it was in contact with occipital, number of femoral pores. All counts were performed on both left and right sides. This new material was compared with the detailed description of historical type material of the species provided in literature and with information taken from the photographs available in MCZBASE (2020) (Table 1).

Phylogenetic analysis

To examine whether *N. scalaris* is within the *N. tessellata* + *lalandii* complex, we placed the recently collected *N. scalaris* individual in a phylogenetic context using data downloaded from GenBank (*ND4*, *16S* rRNA and *RAG1* genes) for a final dataset that included 10 of the 12 described species (Table 2), and an outgroup with three species (*Australolacerta australis*, *Ichnotropis capensis*, *Meroles suborbitalis*). The only tissue sample in existence of *N. scalaris* is muscle that was excised from the whole specimen previously preserved in 70% ethanol. DNA template was extracted from this tissue using multiple methods (NaCl, phenol/chloroform, Qiagen Micro kit, Qiagen DNeasy kit, Zymo extraction kit), but each yield a poor-quality degraded (100–500 bp) template (concentration $<10 \mu\text{g } \mu\text{l}^{-1}$, $A_{260}/_{280} > 2.2$ and $A_{260}/_{230} > 1.8$). We made numerous attempts to sequence all three genes with multiple different primer combinations and PCR conditions, including trials for internal primers, but only the *16S* rRNA gene amplified.

Amplification of 431 base pairs (bp) of the *16S* rRNA gene was carried out with primers 16Sa and 16Sb (Palumbi 1996) with the PCR profile: denaturation for 4 min at 95 °C, followed by 35 cycles of denaturation (94 °C, 45 s), annealing (52 °C, 45 s), and extension (72 °C, 1 min) and a final extension at 72 °C for 10 min. PCR product was inspected through electrophoresis on a 0.8% agarose gel. Sanger sequencing was carried out at Macrogen (Amsterdam, Netherlands) using the forward primer for three separate PCR products to ensure sequence consensus of this problematic sample. The new sequence was aligned in Geneious v.11 (Kearse et al. 2012) with the data downloaded from GenBank.

Phylogenetic analyses were run using maximum likelihood and Bayesian approaches on a dataset of 41 taxa, including the outgroup for a total of 2 016 base pairs (Table 2). In addition, a *16S*-only tree was run for the same group of taxa following the same analysis protocols as the full dataset. The Bayesian analysis was run using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001) at the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway v3.3 (Miller et al. 2010). Data were partitioned by marker, with a final alignment containing 893 bp for *RAG1*, 678 bp for *ND4* and 446 bp for *16S* (exclusions made for 36 bases of hypervariable region as aligned to the outgroup taxa). jModelTest v.1 (Posada 2008) was used to assess the evolutionary model that best fitted

Table 1. Morphometric, meristic, coloration and habitat data for *Nucras scalaris* (newly collected specimen, data from Laurent 1964 and MCZBASE 2020), *Nucras broadleyi* (data from Branch et al. 2019b), and *Nucras lalandii* (data from FitzSimons 1943; Laurent 1964; Branch 1998; Edwards et al. 2013). All measurements in mm. N/A – not available in literature.

	NB1110 (left/right)	<i>Nucras scalaris</i>	<i>Nucras broadleyi</i>	<i>Nucras lalandii</i>
Snout-vent length	88.7	83–88	36–74	75–110
Tail	165	141–182	90–144+	N/A
Total	253.7	229–265	130–218	N/A
Head length	16	N/A	9.5–13.8	15.8 ± 1.9
Head width	10.9	N/A	5.9–6.4	10.0 ± 1.6
Head height	9.3	N/A	N/A	8.4 ± 1.2
Snout length	6.1	N/A	3.8–4.9	N/A
Foot length	12.4	N/A	N/A	N/A
Eye length	3.5	N/A	2.1–2.4	N/A
Ear-eye length	6.6	N/A	3.5–4.2	N/A
Upper labials	4(2)/4(2)	4(2)/(N/A)	4(2)	6*
Lower labials	6(3)/6(3)	6–8*	6(3)	5–7
Dorsal midbody scale rows	38	41–49	38–50	34–42
Ventrals (transverse rows)	8	8	8	8
Ventrals (longitudinal rows)	31	30–35	27–33	32–38
Supraciliaries	7/6	6–8	5–7	5–7
Granules between supraciliaries and supraoculars	1/1	1–3	0–6	1–3 (usually 1)
Subdigital lamellae under 4 th toe	23/23	21–27	23–29	16–22 (usually 18–20)
Femoral pores	11/11	11–14	12–16	10–15 (usually 12–14)
Number of scales in collar	8	9–11	7	7–14 (usually 8–10)
Interparietal Y/N	Y	Y	Y	Y
Interparietal contact with occipital?	In contact	In contact except for one specimen	Majority in contact. Varying from separate to broad contact	Usually in contact behind with a small occipital thus separating parietals. Exceptionally not reaching occipital, and parietals in contact
Enlarged scales under forearm?	Present	Present	Present	Absent
Dorsal coloration pattern	12 complete and regular transversal bars	12–13 complete and regular transversal bars	Longitudinal stripes; pale longitudinal stripes broken up posteriorly with flanks spotted and broken paravertebral stripes	Incomplete and irregular transversal bars
Habitat	<i>Geoxylic suffrutex</i> dominated grassy shrubland on sandy soils within Angolan Miombo Woodlands	N/A	Mopane woodlands, dry savannas, and semi-desert shrublands.	Montane and temperate grassland.

each of the partitions using the Akaike's Information Criterion Corrected (AIC), and this was incorporated into the Bayesian analysis (16S and ND4, $nst = 6 + G$ (GTR + G); RAG1, $nst = 2 + G$ (HKY + G)). The Markov Chain Monte Carlo (MCMC) was run for 20 million generations twice in parallel, with a burn-in of 10%. A ML analysis was run using RAxML (Stamatakis 2014) through the CIPRES portal. The dataset was partitioned by marker applying the GTR + I + G model for each partition with 1 000 bootstrap replicates. For all analyses, Tracer v1.6 (Rambaut et al. 2014) was used to verify that the effective sample size (ESS)

Table 2. Samples included in the phylogenetic analysis with the field/museum numbers and GenBank accession numbers for *16S*, *ND4* and *RAG1*. Dashes indicate no data available. CAS: California Academy of Sciences, NMB: National Museum, Bloemfontein, PEM: Port Elizabeth Museum.

Genus	Species	Field ID	Museum accession no.	<i>16S</i>	<i>ND4</i>	<i>RAG1</i>
<i>Nucras</i>	<i>boulengeri</i>	JM02169		HG005184	HG005212	HG005233
<i>Nucras</i>	<i>broadleyi</i>	AG 18	PEM R24005	MN265869	–	MN265872
<i>Nucras</i>	<i>broadleyi</i>	AG 166	PEM R24157	MN265870	MN265871	MN265873
<i>Nucras</i>	<i>holubi</i>	F-MCZ-38793	CAS 234138	HG005186	HG005214	HG005235
<i>Nucras</i>	<i>holubi</i>	RSP420	PEM R18647	HG005187	HG005215	HG005236
<i>Nucras</i>	<i>holubi</i>	MBUR 00260	PEM R22814	HG005188	HG005216	HG005237
<i>Nucras</i>	<i>intertexta</i>	RSP277	PEM R18661	HG005190	HG005218	HG005239
<i>Nucras</i>	<i>intertexta</i>	RSP030	PEM R18257	HG005191	HG005219	HG005240
<i>Nucras</i>	<i>intertexta</i>	F-MCZ-38872	CAS 234212	HG005192	HG005220	HG005241
<i>Nucras</i>	<i>intertexta</i>	MB 20952		HG005193	HG005221	HG005242
<i>Nucras</i>	<i>intertexta</i>	MB 21183		HG005194	HG005222	–
<i>Nucras</i>	<i>lalandii</i>	HB124		HF951553	HF951532	HF951537
<i>Nucras</i>	<i>lalandii</i>	HB037		HF951554	HF951533	HF951538
<i>Nucras</i>	<i>lalandii</i>	HZ246		HF951555	HF951534	HF951539
<i>Nucras</i>	<i>lalandii</i>	MBUR 00414	PEM R22815	HG005195	HG005223	HG005243
<i>Nucras</i>	<i>lalandii</i>	MBUR 00483		HG005196	HG005224	HG005244
<i>Nucras</i>	<i>lalandii</i>	MB 20982		HG005197	HG005225	HG005245
<i>Nucras</i>	<i>livida</i>	MBUR 00670	PEM R22821	HG005198	–	HG005246
<i>Nucras</i>	<i>livida</i>	MBUR 00687		HG005199	HG005226	–
<i>Nucras</i>	<i>livida</i>	KTH08-071	PEM R18747	HG005200	HG005227	HG005247
<i>Nucras</i>	<i>livida</i>	MB 21176		HG005201	HG005228	HG005248
<i>Nucras</i>	<i>livida</i>	MB 21225		HG005202	HG005229	HG005249
<i>Nucras</i>	<i>ornata</i>	MBUR 01226	NMB R10907	HG005203	–	HG005250
<i>Nucras</i>	<i>ornata</i>	MBUR 01230	NMB R10658	HG005204	–	HG005251
<i>Nucras</i>	<i>ornata</i>	AMB8635	PEM R17591	HG005206	–	HG005252
<i>Nucras</i>	<i>scalaris</i>	NB1110	NB1110	MT431951	–	–
<i>Nucras</i>	<i>taeniolata</i>	HZ250		HG005207	–	HG005253
<i>Nucras</i>	<i>taeniolata</i>	HZ251		HG005208	HG005230	HG005254
<i>Nucras</i>	<i>taeniolata</i>	HZ252		HG005209	–	HG005255
<i>Nucras</i>	<i>taeniolata</i>	PEM R18080	PEM R18080	HG005210	HG005231	HG005256
<i>Nucras</i>	<i>tessellata</i>	MB 20161	NMB R11574	HF951558	–	HF951542
<i>Nucras</i>	<i>tessellata</i>	MB 20650	PEM R16873	HF951556	HF951535	HF951540
<i>Nucras</i>	<i>tessellata</i>	MB 20687	PEM R16872	HF951557	HF951536	HF951541
<i>Nucras</i>	<i>tessellata</i>	AMB5584	CAS 206725	HG005211	HG005232	HG005257
<i>Nucras</i>	<i>tessellata</i>	KTH08-069	PEM R18745	HF951559	–	HF951543
Outgroup						
<i>Australolacerta</i>	<i>australis</i>	MH0531		DQ871152	FR751398	DQ871208
<i>Australolacerta</i>	<i>australis</i>	GW08		HF547772	HF547725	HF547691
<i>Ichnotropis</i>	<i>capensis</i>	AMB 6001		DQ871148	HF547732	DQ871206
<i>Ichnotropis</i>	<i>capensis</i>	AMB 6067	CAS 209602	DQ871149	HF547733	DQ871207
<i>Latastia</i>	<i>longicaudata</i>			AF080358	–	EF632229
<i>Meroles</i>	<i>suborbitalis</i>	SVN-049		HF547800	HF547759	HF547718

was above 200 for all parameters. Finally, pairwise uncorrected net p-distances between *N. scalaris* and other members of the genus were estimated for *16S* using MEGA v.7 (Kumar et al. 2016) with no missing data in the alignment.

Results

Morphology

Meristic and scalation data are summarized in Table 1. The new specimen (NB1110) has a serpentiform shape, with an elongated and cylindrical body a thick and long tail (186% of SVL), and short limbs (Figures 3A and 3B). Nostrils oriented laterally in a

short (68% longer than wide) and tall head (85% wider than tall), rounded snout (Figures 4B and 4C). Six upper labials, four anterior to subocular and two posterior to subocular; and six lower labials with the third being the largest. Seven and six supraciliaries on the left and right side, respectively, and one granule between supraciliaries and supraoculars on both sides. Its dorsal scalation consists of small oval or rounded granular scales, smooth, juxtaposed and more elongated in the front, imbricated and less elongated and polygonal in the back. Anterodorsal surface of the forearm has two series of scales larger than the adjacent scales; one or two series of plates clearly larger than the adjacent scales on the proximal part of the inferior surface of the forearm (Figure 4F); dorsal surface of the tibia covered on granular scales; large scales on the ventral surface of the tibia. Tail scales keeled, forming regular elongated rings, pointing obtusely to the back; middorsal scales barely larger than adjacent, ventral scales slightly larger than dorsal scales and more weakly keeled, but sharper. It has a conspicuous dorsal coloration pattern, consisting of a series of twelve continuous dark transversal bars in the trunk, and over forty interrupted bars in the tail (Figure 3A). The colouration and body shape are essentially identical to the plate showing the *N. scalaris* paratype (Ang. 5481, see Laurent (1964), p 58, Figure 17), although the tail is considerably thicker in NB1110. The new specimen is also morphologically identical to Ang. 5485 (MCZ Herp R-74121) from the type series, as concluded after comparison with photographs available in the Museum of Comparative Zoology on-line database (MCZBASE 2020). Ventral coloration is plain white (Figure 4A). The specimen is assigned to *N. scalaris* based on strong morphological agreement with the species' original description (Laurent 1964) in meristic, morphometric and coloration features (Table 1). The only exceptions to this are the collar formed by eight scales and 38 midbody scale rows with ranges of 9–11 and 41–49, respectively (Laurent 1964; Figure 4D). These small discrepancies are expected given that the species was described from only four specimens, and intra-specific variation probably has not been fully captured.



Figure 3. Live *Nucras scalaris*, adult female (NB1110) from Cusseque, Bié Province, Angola. (A) Dorsal view of lizard in pitfall trap where it was captured, note coloration consisting of conspicuous transversal bands and long and thick tail. (B) Lateral view. Photo credit: A,B: M Bluhm.

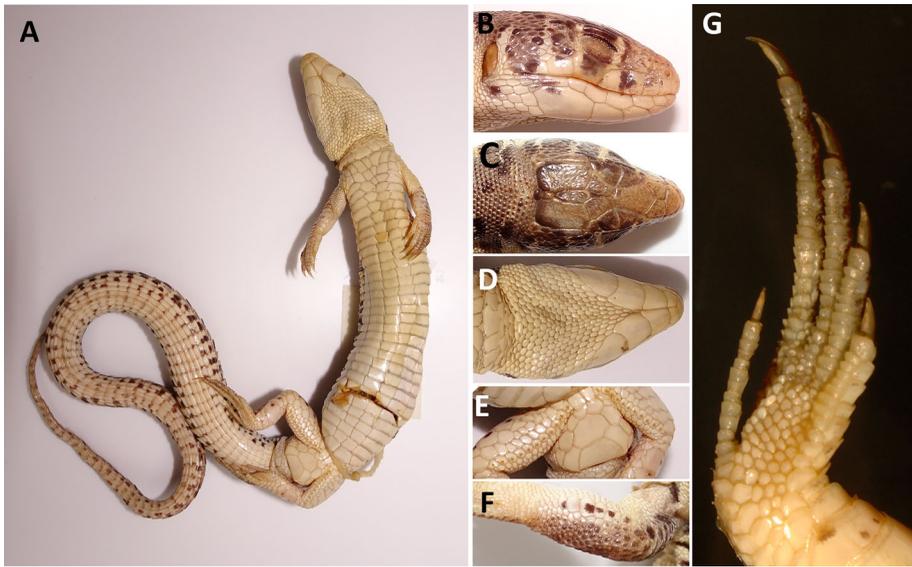


Figure 4. Scalation details of *Nucras scalaris*, adult female (NB1110) from Cussequ, Bié Province, Angola. (A) Ventral view of entire body. (B) Head in lateral view. (C) Head in dorsal view. (D) Head in ventral view, note collar with 8 scales. (E) Cloacal region with 11 femoral pores in each leg. (F) Forearm with enlarged scales. (G) Ventral view of foot, showing 23 lamellae under fourth toe. Photo credit: N Baptista.

Phylogenetic analyses

Similar to previous phylogenetic analyses (e.g. Edwards et al. 2013; Bauer et al. 2019; Branch et al. 2019b) there were two main clades of *Nucras* (excluding *N. boulengeri*). The *N. tessellata* + *lalandii* species complex are all from southern Africa, either endemic to the arid or semi-arid regions of South Africa (*N. lalandii*, *N. livida* and *N. taeniolata*) or Namibia, Botswana and South Africa (*N. tessellata*). There is a separate, well-supported clade with species (*N. intertexta*, *N. ornata* and *N. holubi*) from the more mesic east (southern Zimbabwe and Mozambique, and north-western South Africa). Finally, there is a separate, distinct lineage with one species (*N. boulengeri*) from East Africa that is sister to all other *Nucras*.

The new specimen of *N. scalaris* is confirmed as a separate taxon, because it does not fall within any of the other *Nucras* species for either the three gene or the 16S phylogeny (Figure 5; Supplementary material Figure S1). Furthermore, pairwise uncorrected net 16S p-distances between *N. scalaris* and other *Nucras* were 7–12% (Table 3) further substantiating its species status. The three gene analysis supports *Nucras scalaris* as being within the larger *N. tessellata* + *lalandii* + *broadleyi* complex from arid southern Africa. The phylogeny suggests there is a sister relationship between *N. scalaris* and *N. broadleyi*. Although the node is well-supported by the Bayesian analysis (0.97 pp), the maximum likelihood bootstrap value is moderate (66%).

It should be noted that the 16S-only analysis has essentially no support at deeper internal nodes, resulting in a large polytomy for the major clades (Supplementary material Figure S1). Regardless, each of the terminal clades (i.e. species) are well-supported and

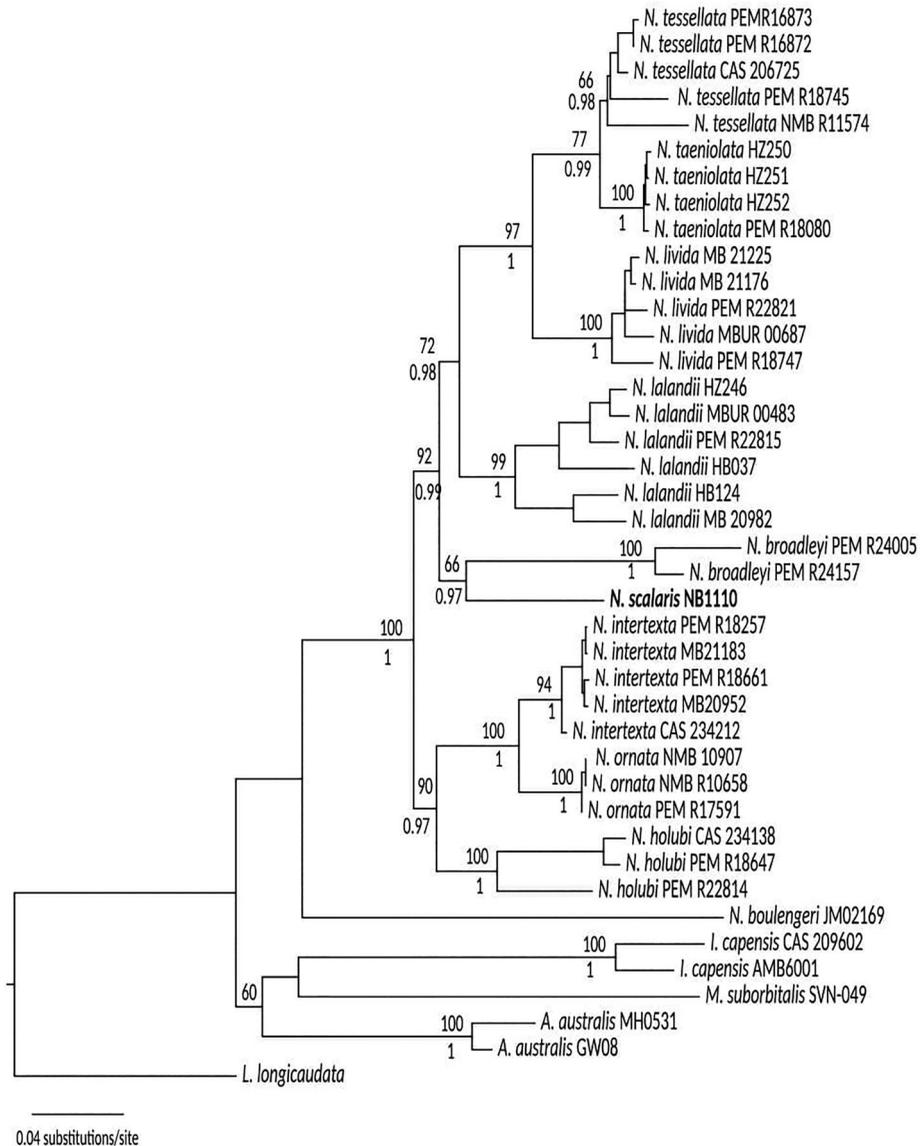


Figure 5. Maximum likelihood topology for the three gene dataset of *Nucras* with bootstrap values (above) and Bayesian posterior probabilities (below) at each node. Intra-specific support values are not shown, and bootstrap (<60%) and posterior probabilities (<0.95) are not shown for any node.

N. scalaris remains a separate taxon in this 16S topology. The sister relationship between *N. scalaris* and *N. broadleyi* does not receive support in the 16S-only analysis, although *N. scalaris* also does not appear to be sister to *N. lalandii*.

Habitat and ecology

The geoxylic suffrutex-dominated grassy shrublands ('underground forests') are likely to be the main habitat of *Nucras scalaris*. Between August and December, these grassy

Table 3. Pairwise uncorrected net p-distances for species of *Nucras* for the 16S gene, with intra-specific values on the diagonal. Intra-specific values were not estimated (na) for species with only one individual in the dataset.

	<i>N. scalaris</i>	<i>N. broadleyi</i>	<i>N. boulengeri</i>	<i>N. holubi</i>	<i>N. intertexta</i>	<i>N. lalandii</i>	<i>N. livida</i>	<i>N. ornata</i>	<i>N. taeniolata</i>	<i>N. tessellata</i>
<i>N. scalaris</i>	na									
<i>N. broadleyi</i>	0.086	0.047								
<i>N. boulengeri</i>	0.119	0.095	na							
<i>N. holubi</i>	0.077	0.075	0.079	0.043						
<i>N. intertexta</i>	0.101	0.078	0.098	0.040	0.004					
<i>N. lalandii</i>	0.073	0.086	0.101	0.040	0.066	0.046				
<i>N. livida</i>	0.099	0.091	0.112	0.061	0.086	0.061	0.013			
<i>N. ornata</i>	0.092	0.080	0.108	0.049	0.040	0.071	0.088	0.000		
<i>N. taeniolata</i>	0.088	0.099	0.110	0.059	0.085	0.055	0.076	0.082	0.000	
<i>N. tessellata</i>	0.084	0.072	0.101	0.051	0.076	0.044	0.031	0.080	0.069	0.025

shrublands are dominated by geoxylic suffrutices, such as *Brachystegia russelliae*, *Cryptosepalum exfoliatum* ssp. *suffruticans*, *Syzygium guineense* ssp. *huillense*, *Euclea crispa* and *Combretum platypetalum*, forming a dense covering at about 30 to 50 cm above the ground. From the end of December to the start of the dry season, the inflorescences of typical savanna grasses, such as *Tristachya nodiglumis*, *Aristida* spec. div., and *Ctenium concinnum* dominate, forming a more open layer at about 1–1.5 m height lasting four to six months. Man-made fires run through the grassland in July or August, leaving bare soil for a few weeks before the geoxyles begin to resprout.

In this heterogeneous habitat, additional structure is provided by multi-stemmed shrubs and a scattering of larger trees, such as *Monotes africana*, *Protea gagedii*, *Swartia madagascariensis*, *Strychnos cocculoides*, *Burkea africana*, and *Ochna pulchra*. The habitat also includes burnt tree stumps and a considerable amount of deadwood. There are biogenous structures, such as termite mounds of 1–1.5 m height (Figure 2D) and lines of mole hills (ca 20–30 cm high) made by Mechow's mole-rats (*Fukomys mechowii*). These structures interrupt the geoxylic matrix and provide open spots where lizards can bask. Given there are ample termite mounts in this habitat, these insects probably are an important prey item for *N. scalaris*.

Discussion

The new *Nucras* specimen from the central Angolan plateau morphologically fits the description of *Nucras scalaris*, making this the first record since the original description more than half a century ago. Although the genetic data are limited, we confirm that *N. scalaris* is a separate species that falls within the larger clade containing both *N. broadleyi* and the *N. tessellata* + *lalandii* complex. It is likely that *N. scalaris* is sister to *N. broadleyi* not *N. lalandii*, but this is based on a single gene and one individual. Although the relationship received moderate to good support in the three gene phylogeny, it was not supported in the 16S-only analysis. Therefore, confirmation of this relationship will have to be verified through additional samples for phylogenetic analyses.

A morphometric and meristic comparison among *N. scalaris*, *N. broadleyi* and *N. lalandii* show overlap between several characters (Table 1). The presence of enlarged scales on the surface of the arm (Figure 4F) that distinguishes *N. scalaris* from *N. lalandii*, does not distinguish *N. scalaris* from *N. broadleyi*. However, a number of morphological features can distinguish these two species. The dorsal coloration is cream with twelve transversal and continuous dark brown bands in *N. scalaris* (Figure 3) versus a dorsum with a series of longitudinal pale stripes and bright orange tail in *N. broadleyi* (Branch et al. 2019b). Furthermore, *Nucras scalaris* has a much larger and robust body than *N. broadleyi*. These species occupy different habitats, because *N. scalaris* is associated with the 'underground forests' in the Angolan Miombo Woodlands in the plateau in central and north-eastern Angola, whereas *N. broadleyi* inhabits mopane woodlands, dry savannas, and semi-desert shrublands from southwestern Angola (Branch et al. 2019b).

Although Sandveld lizards are thought to be relatively abundant, they are rarely observed (Branch 1998; van der Meer et al. 2010; Branch et al. 2019b). Unlike other lacerids, they tend to avoid the open spaces where detectability would be high, making them difficult to record. It is interesting to note that all specimens of *N. scalaris* were recorded in the dry season (July 1954 and June 2018). However, the few records collected to date does

not allow for an assessment whether lizards are more active in the dry season, or whether the very limited sampling has resulted in a bias, because of random events. However, only one specimen of *N. scalaris* was captured in a pitfall trap from a trap line that was left for four days in the dry season. During the same period, we commonly caught other lizards, such as *Panaspis*, *Trachylepis* and *Ichnotropis*. Active searches in the wet season (March 2019), 25 km north of the type locality, did not produce any additional records of *N. scalaris* (Vaz Pinto, pers. comm.), nor did targeted surveys in Cusseque during the late dry season in September 2019. Other lizards recorded in Cusseque include *Acanthocercus* sp., *Agama aculeata*, *Gerrhosaurus bulsi-multilineatus* complex, *Ichnotropis capensis*, *Panaspis* sp., *Trachylepis binotata*, *Sepsina angolensis*, and *Zygaspis quadrifrons*. Consequently, although other species appear to be relatively easy to record in the same area, *N. scalaris* does not appear to be abundant.

All three known localities for *N. scalaris*, Alto Chicapa, Alto Cuílo and Cusseque, are located in the broad Angolan Miombo Woodlands Ecoregion (Burgess et al. 2004). The characterization of this ecoregion does not include the open geoxylic suffrutex-dominated grassy shrubland ('underground forests'), although it is an extensive and locally important habitat type in this area (Barbosa 1970). Indeed, it is the typical vegetation type across vast areas of the Angolan plateau (Zigelski et al. 2019), including Alto Cuílo and Alto Chicapa (Vaz Pinto, pers. comm.). Overall, these findings provide another piece to the puzzle regarding the biogeography of Angolan reptiles, an area of work that has been previously highlighted as crucial (see Branch et al. 2019c). If the distribution of this species is limited to the Angolan plateau, its status as an Angolan endemic would be reinforced. It will be important, however, to understand whether this perceived endemism is the result of under-sampling or a poor appreciation of the ecology of the species, or whether it is truly an Angolan endemic.

This new record extends the distribution of *Nucras scalaris* to Bié Province, approximately 380 km southwest of the previously known range (Figure 1) and increases the known elevation of occurrence from 1 300 m to 1 570 m above sea level. The record also corresponds with the first instance of any Sandveld lizard associated with 'underground forest' habitats. The cylindrical and elongated body shape possibly reflect a propensity to shelter within and beneath the short and dense vegetation (Figures 2C and 2D), which might explain why the species is seldom encountered. Although the ecology and life history of *N. scalaris* is still relatively unknown, we can infer that termites are probably an important prey item for *N. scalaris*, as with other species of Sandveld lizards (Branch 1998; Alexander and Marais 2007; van der Meer et al. 2010). Despite there being many unknowns regarding this species, the single specimen has advanced our knowledge of this poorly known Angolan endemic reptile. The finding has also prompted the species to be removed from the IUCN Red List Data Deficient category and allowed for a full assessment to be carried out. The species has now been evaluated as Least Concern given the large range extension covering an area that is not currently under any major concerning threats (Baptista et al. forthcoming). However very little is still known about this species' real distribution, ecology and abundance, and additional studies might provide a more precise assessment.

The discovery of this species in the 'underground forest' begs the question whether other undocumented species diversity could be associated with this peculiar and often overlooked vegetation type. The new record of *N. scalaris* adds to a recent series of

rediscoveries of endemic Angolan herpetofauna, including frogs (Conradie et al. 2013; Baptista et al. 2018), lizards (Stanley et al. 2016; Vaz Pinto et al. 2019), amphisbaenians (Branch et al. 2018) and snakes (Branch et al. 2019a; Gonçalves et al. 2019). These discoveries are progressively contributing to our understanding of these poorly known species, as well as providing a more comprehensive understanding of Angolan herpetofauna and biogeography.

Acknowledgements

This research was carried out in the framework of the Southern African Science Service Centre for Climate Change and Adaptive Land Management (SASSCAL) project, sponsored by the German Federal Ministry of Education and Research (BMBF) under promotion number 01LG1201M. Collecting of specimen and export of tissue took place under the Memorandum of Understanding between the Angolan Ministry of Environment (MINAMB/INBAC) and Instituto Superior de Ciências de Educação da Huíla (ISCED-Huíla). The National Research Foundation of South Africa (grant #119470) funded laboratory work. NLB is currently supported by FCT contract SFRH/PD/BD/140810/2018. MB collected the specimen during fieldwork for his MS thesis. This work benefited from logistical, administrative and laboratory support, discussions on the subject, photographs, and revisions of the manuscript for which we thank alphabetically Anja le Grange, Brian J Huntley, CTM staff, Fernanda Lages, John Mendelsohn, Javier Lobon Rovira, José Luís Alexandre, Miguel Carretero, Pedro Vaz Pinto, Raquel Godinho, Telmo António and Werner Conradie. Paulina Zigelski produced the map. William R Branch (1946–2018) passed away before seeing this work published. Bill's enthusiasm and encouragement led to invaluable developments in Angolan herpetology, and his co-authors on this paper were privileged and honoured to have worked with him.

ORCID

Ninda L Baptista  <http://orcid.org/0000-0002-2859-6606>

Krystal A Tolley  <http://orcid.org/0000-0002-7778-1963>

Manfred Finckh  <http://orcid.org/0000-0003-2186-0854>

References

- Alexander G, Marais J. 2007. *A guide to the reptiles of southern Africa*. South Africa: Penguin Random House.
- Baptista N, António T, Branch WR. 2018. Amphibians and reptiles of the Tundavala region of the Angolan escarpment. *Biodivers Ecol*. 6:397–403.
- Baptista N, Ceriaco LMP, Conradie W. *Nucras scalaris*. The IUCN Red List of Threatened Species. Forthcoming.
- Baptista NL, António T, Branch WR. 2019. The herpetofauna of Bicular National Park and surroundings, southwestern Angola: a preliminary checklist. *Amph Rept Cons*. 13(2) [Special Section]:96–130 (e203).
- Barbosa LAG. 1970. *Carta Fitogeográfica de Angola*. Luanda: Instituto de Investigação Científica de Angola.
- Bauer AM, Childers JI, Broeckhoven C, Mouton LN. 2019. A new *Nucras* Gray, 1838 Squamata: Lacertidae) from the Strandveld of the Western Cape, South Africa. *Zootaxa*. 4560(1):149–163.
- Branch WR, Baptista N, Vaz Pinto P. 2018. Angolan amphisbaenians: rediscovery of *Monopeltis luandae* Gans 1976, with comments on the type locality of *Monopeltis perplexus* Gans 1976 (Sauria: Amphisbaenidae). *Herpetol Notes* 11:603–606.

- Branch WR, Baptista N, Keates C, Edwards S. 2019a. Rediscovery, taxonomic status, and phylogenetic relationships of two rare and endemic snakes (Serpentes: Psammophiinae) from the southwestern Angolan plateau. *Zootaxa*. 4590(3):342–366.
- Branch WR, Conradie W, Vaz Pinto P, Tolley KA. 2019b. Another Angolan Namib endemic species: a new *Nucras* Gray, 1838 (Squamata: Lacertidae) from south-western Angola. *Amph Rept Cons*. 13(2) [Special Section]:82–95(e199).
- Branch WR, Vaz Pinto P, Baptista N, Conradie W. 2019c. The Reptiles of Angola – History, updated checklists, endemism, hot spots, and future directions for research. In: Huntley BJ, Ferrand N, Russo V, Lages F, editors. *Biodiversity of Angola. Science and Conservation: A Modern Synthesis*. Switzerland: Springer. https://doi.org/10.1007/978-3-030-03083-4_13.
- Branch WR. 1998. Field guide to snakes and other reptiles of southern Africa. Cape Town: Struik Publishers;
- Broadley DG. 1972. A review of the *Nucras tessellata* group (Sauria: Lacertidae). *Arnoldia*. 20:1–35.
- Burgess N, Hales JD, Underwood E, Dinerstein E, Olson D, Itoua I, Schipper J, Ricketts T, Newman K. 2004. *Terrestrial ecoregions of Africa and Madagascar – a conservation assessment*. Washington (DC): Island Press.
- Conradie W, Branch WR, Tolley KA. 2013. Fifty Shades of Grey: giving colour to the poorly known Angolan Ashy Reed Frog (Hyperoliidae: *Hyperolius cinereus*), with the description of a new species. *Zootaxa*. 3635(3):201–223.
- Edwards S, Vanhooydonck B, Herrel A, Measey GJ, Tolley KA. 2012. Convergent evolution associated with habitat decouples phenotype from phylogeny in a clade of lizards. *PLoS One*. 7(12):e51636.
- Edwards S, Tolley KA, Vanhooydonck B, Measey GJ, Herrel A. 2013. Is dietary niche breadth linked to morphology and performance in Sandveld lizards *Nucras* (Sauria: Lacertidae)? *Biol J Linn Soc Lond*. 110(3):674–688.
- FitzSimons VF. 1943. The lizards of South Africa: Systematic account of the lizards (Sauria) of Southern Africa. *Transv Mus Mem*. 1(1):1–6.
- Gonçalves FM, Braine D, Bauer AM, Valério H, Marques MP, Ceríaco LM. 2019. Rediscovery of the poorly known Angolan Adder, *Bitis heraldica* (Bocage, 1889) (Serpentes: Viperidae): New records, live photographs and first case history of envenomation. *Herp Rev*. 50(2):241–246.
- Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*. 17(8):754–755.
- Jürgens N, Strohbach B, Lages F, Schmiedel U, Finckh M, Sichone P, Hahn L-M, Zigeliski P. 2018. Biodiversity observation – an overview of the current state and first results of biodiversity monitoring studies. *Biodivers Ecol*. 6:382–396.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*. 28(12):1647–1649.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 33(7):1870–1874.
- Laurent RF. 1964. Reptiles et amphibiens de l'Angola (Troisième contribution). *Publ Cult Comp Diam Ang*. 67:1–165.
- Lillywhite HB, Shine R, Jacobson E, Denardo DF, Gordon MS, Navas CA, Wang T, Seymour RS, Storey KB, Heatwole H, et al. 2017. Anesthesia and euthanasia of amphibians and reptiles used in scientific research: should hypothermia and freezing be prohibited? *Bioscience*. 67(1):53–61.
- Marques MP, Ceríaco LMP, Blackburn DC, Bauer AM. 2018. Diversity and Distribution of the Amphibians and Terrestrial Reptiles of Angola Atlas of Historical and Bibliographic Records (1840–2017). *Proc Cal Acad Sci*. 4(65) Supplement II:1–501.
- MCZBASE. 2020. The Database of the Zoological Collections. Harvard University: Museum of Comparative Zoology. <https://mczbase.mcz.harvard.edu/guid/MCZ:Herp:R-74121>. [Accessed 8 March 2020].
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA. pp 1–8.

- Palumbi SR. 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK, editors. *Molecular Systematics*. Sunderland (MA): Sinauer Associates, Inc.
- Posada D. 2008. jModelTest: phylogenetic model averaging. *Mol Biol Evol.* 25(7):1253–1256.
- Revermann R, Maiato F, Gomes A, Lages F, Finckh M. Cusseque – Vegetation. *Biodivers Ecol.* 2013;5:59–63.
- Stamatakis A. 2014. RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics.* 30(9):1312–1313.
- Stanley EL, Ceríaco LM, Bandeira S, Valério H, Bates MF, Branch WR. 2016. A review of *Cordylus machadoi* (Squamata: Cordylidae) in southwestern Angola, with the description of a new species from the Pro-Namib desert. *Zootaxa.* 4061(3):201–226.
- van der Meer MH, Whiting MJ, Branch WR. 2010. Ecology of southern African sandveld lizards (Lacertidae, *Nucras*). *Copeia.* 2010(4):568–577.
- Vaz Pinto P, Veríssimo L, Branch WR. 2019. Hiding in the bushes for 110 years: rediscovery of an iconic Angolan gecko (*Afrogecko ansorgii* Boulenger, 1907, Sauria: Gekkonidae). *Amph Rept Cons.* 13(2) [SpecialSection]:29–41(e182).
- Weber T. 2013. Cusseque – Climate. *Biodivers Ecol.* 5:37–46.
- Zigelski P, Gomes A, Finckh M. 2019. Suffrutex Dominated Ecosystems in Angola. In: Huntley BJ, Ferrand N, Russo V, Lages F, editors. *Biodiversity of Angola. Science and Conservation: A Modern Synthesis*. Switzerland: Springer. https://doi.org/10.1007/978-3-030-03083-4_7.