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Phylogeny and evolution of unique skull morphologies in dietary specialist African shovel-snouted snakes (Lamprophiidae: *Prosymna*)

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Prosymna is a specialized African snake genus lacking close relatives. The evolutionary relationships and history within *Prosymna* are poorly understood. Here we assembled a multi-gene data set including representatives for 11 of 16 species to investigate the phylogenetic relationships of this group. Our analyses support the monophyly of *Prosymna* and are congruent with species groups previously recognized on the basis of external morphology. Divergences among extant *Prosymna* began in the mid-Cenozoic, with the earliest divergence splitting northern from southern lineages. High-resolution computed tomography scans confirm that a specialized skull morphology is found across the genus and was probably present in the common ancestor of *Prosymna*. This specialization is exemplified by dentition featuring reduced anterior but greatly enlarged, blade-like posterior maxillary teeth and an unusually high degree of fusion of cranial bones. One species, *P. visseri*, has a hammer-like maxilla unlike that of any other known snake. Evidence for oophagy in *Prosymna* and the possible roles of morphological specializations in egg-slitting or egg-crushing feeding mechanisms are discussed.

ADDITIONAL KEYWORDS: biogeography – Colubroidea – computed tomography – cranium – CT – egg-eating – Elapoidea – oophagy – systematic.

INTRODUCTION

The genus *Prosymna* includes 16 species of small-bodied, semi-fossorial (burrowing) or rock-dwelling snakes that occur in savannas across sub-Saharan Africa (FitzSimons, 1962; Branch, 1998; Alexander & Marais, 2007; Spawls *et al.*, 2018; Uetz *et al.*, 2019). *Prosymna* snakes are morphologically distinctive, with small wedge-shaped heads terminating in a pointed, sometimes upturned rostrum shielded by a single large scale, and reduced dentition compared to most other caenophidian snakes (Broadley, 1980). This appearance gives *Prosymna* their common name of African shovel-snouted snakes. The evolutionary history of *Prosymna* is of interest because of their broad geographical distribution and unique anatomical traits. A well-supported phylogenetic hypothesis would allow for identification of historical biogeographical patterns that may have contributed to speciation in *Prosymna*, and permit comparison to other broadly distributed African groups. More intriguingly, a resolved phylogeny in concert with physical trait data permits the study of functional evolutionary patterns of the unique morphology of these snakes.

The distinct head morphology of *Prosymna* extends to the underlying skull. Bourgeois (1968), in an analysis of the skulls of 73 caenophidian African snake taxa, included *Prosymna ambigua* and noted a

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variety of idiosyncratic characteristics. These include an extremely compact shape, tight sutures between elements (i.e. a more solid skull with reduced kinesis), extremely reduced dentition (except the two rearmost maxillary teeth are greatly enlarged), an anteriorly reduced maxilla with corresponding posterior elongation of the premaxilla, a reduced palatine, an extremely elongate supratemporal and participation of the prefrontal in the orbit (Fig. 1). Most of these traits have not been observed in any other species, while the last is shared with another burrowing snake, *Scaphiophis albopunctatus* (Bourgeois, 1968).

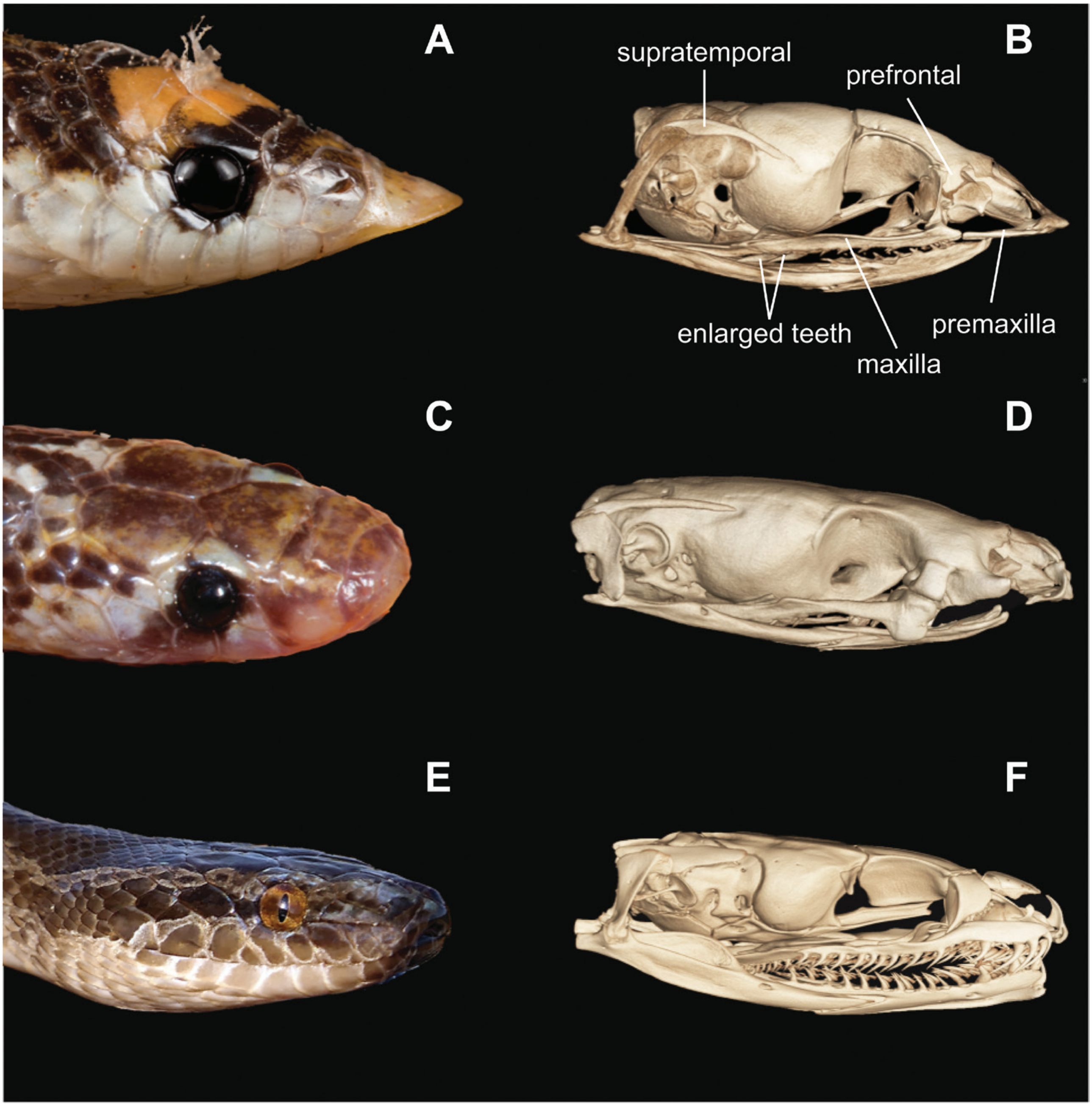

Figure 1. Unique head morphology of *Prosymna* compared to the typical lamprophiid snake *Boaedon capensis*. Characteristic features of *Prosymna* identified by Bourgeois (1968) are labelled. *Prosymna bivittata*: A, wild specimen, Musina, South Africa; B, CAS 165604, lateral view of skull. *Prosymna visseri*: C, wild specimen, Epupa Falls, Namibia; D, CAS 214753, lateral view of skull. *Boaedon capensis*: E, wild specimen, Tanzania; F, CAS 85747, lateral view of skull. Photo credits: Luke Verburgt (Enviro-Insight; A), Johan Marais (African Snakebite Institute; C); André Van Hecke (E).

A *Prosymna stuhlmanni* skull figured in Kirchhof *et al.* (2016) shares these traits. However, because these are the only available detailed descriptions of *Prosymna* skulls, it is unclear whether these traits are shared across *Prosymna*. The one exception is dentition, which can be observed externally and has therefore been documented for all species. Based on external observation, all *Prosymna* have reduced dentition, but there is variation in the number of teeth, including the number of enlarged posterior maxillary teeth (Parker, 1949; Loveridge, 1958; Broadley, 1980).

Dentition has a functional link to diet. Some studies have speculated that *Prosymna* species, like many other semi-fossorial snakes, feed on invertebrates (e.g. FitzSimons, 1962; Jacobsen, 1982), with direct evidence of insect ingestion having been observed on at least one occasion (Miller, 2009). However, studies of gut contents have consistently documented squamate eggs and small, possibly embryonic lizards as primary dietary items for at least six species of *Prosymna* (Broadley, 1979; Spawls *et al.*, 2018). These observations make *Prosymna* one of several lineages of snakes in which eggs form a key dietary component, along with species of *Boiga* (formerly *Elachistodon*), *Cemophora*, *Dasypeltis*, *Oligodon*, *Phyllorhynchus* and *Salvadora* (Gans, 1952; Gans & Williams, 1954; Scanlon & Shine, 1988; Coleman *et al.*, 1993; Trauth, 1993; Gardner & Mendelson, 2003).

Oophagous snake lineages typically have modified dentition. In *Boiga* and *Dasypeltis*, the teeth are small to vestigial and processes on the vertebral column are used to crush hard-shelled bird eggs (Gans & Williams, 1954; Mohan *et al.*, 2018). Other egg-eating snakes that feed mainly upon leathery squamate eggs tend to have small or even missing anterior teeth but one or more enlarged posterior teeth (Coleman *et al.*, 1993; Trauth, 1993; Gardner & Mendelson, 2003). In *Prosymna*, the enlarged posterior teeth are laterally flattened and decurved (Boulenger, 1894; Loveridge, 1958; Bourgeois, 1968). This contrasts with the needle-like teeth of rear-fanged snakes used for venom delivery. Snakes with this type of dentition use their enlarged teeth to slit eggs during feeding (Minton, 1966; Broadley, 1979; Coleman *et al.*, 1993). However, hard-shelled gecko eggs have also been identified in the gut contents of some *Prosymna*, suggesting that there may be some variation in feeding mechanism (Broadley, 1979).

For a number of reasons, both inter- and intrageneric evolutionary relationships of *Prosymna* are poorly understood. The inconspicuous behaviour of *Prosymna* makes focused study *in situ* difficult. Individuals are most commonly encountered serendipitously during general faunal surveys, with one species having an 80-year gap in observations (Kalumanga, 2007). The wide geographical range of the genus also means that only a fraction of species occur in any one area, making it difficult to broadly sample the group. Beyond these factors, the relative distinctiveness of *Prosymna* has hindered identification of their closest relatives, causing difficulty in determining whether traits are ancestral or derived within the group.

Molecular data are invaluable in such situations. Published molecular phylogenetic analyses have confirmed that *Prosymna* is a member of the superfamily Elapoidea (Vidal *et al.*, 2008; Kelly *et al.*, 2009; Zaher *et al.*, 2009), which is typically divided into the families Elapidae (including cobras, kraits, coral snakes, etc.) and Lamprophiidae (including *Prosymna* and many other mostly African caenophidian snakes). Some of these analyses suggest that Elapidae is deeply nested within a paraphyletic Lamprophiidae, and that *Prosymna* represents the most divergent taxon in the superfamily, which is consequently placed in its family (Prosymnidae). Another recent study weakly supported *Buhoma* as the closest relative of *Prosymna* (Figueroa *et al.*, 2016). All confirm that the genus has no close relatives. These studies have focused on broader relationships and typically only included one or a few *Prosymna* species. The most comprehensive studies (Pyron *et al.*, 2013; Figueroa *et al.*, 2016; Zaher *et al.*, 2019) included five *Prosymna* species, but even these had no common data for all five taxa, making it impossible to confidently infer relationships among *Prosymna* species. The only published broad analysis of evolutionary relationships within *Prosymna* is that of Broadley (1980), who based his analysis not on molecular data but on 26 morphological characters mostly related to scalation, dentition or colour pattern. Due to the lack of an obvious close outgroup to polarize traits, Broadley performed a phenetic rather than cladistic analysis, grouping taxa based on minimum average trait distance. This analysis resulted in recognition of three species groups: the *ambigua* group (*ambigua*, *ornatissima*, *semifasciata*, *stuhlmanni*), the *meleagris* group (*greigerti*, *meleagris*, *ruspolli*, *somalica*) and the *sundevalli* group (*bivittata*, *lineata*, *sundevalli*). The remaining species – *angolensis*, *frontalis*, *janii*, *pitmani*, *visseri* – were variously linked to these species groups but without strong support.

To fill this gap in knowledge, we seek in this study to determine the evolutionary relationships among *Prosymna* species using a multi-gene molecular data set. Our assembled data set includes most described species and captures the full extent of geographical and morphological variation known in the genus. We use computed tomography (CT) scanning to obtain osteological data for a subset of species. These data in combination allow us to address the following research questions: (1) Do the species groups identified by Broadley (1980) represent natural evolutionary groupings? (2) Do the historical biogeographical patterns observed in *Prosymna* match those in other widespread sub-Saharan African squamate lineages? (3) Does the skull osteology of *Prosymna* show evolutionary trends related to dietary or habitat adaptations? We hypothesize that our phylogeny will corroborate the work of Broadley (1980), because the species groups he defined are consistent with multiple lines of evidence: they were identifed based on external morphology but are also geographically coherent.

MATERIAL AND METHODS

Molecular phylogeny

Ethanol-preserved tissue samples were obtained for 30 *Prosymna* specimens, with additional sequence data obtained from GenBank for six specimens (Table 1). Total ingroup sampling of 36 specimens includes representatives of 11 of 16 described species and multiple taxa from each of the species groups recognized by Broadley (1980). Outgroup samples were obtained from GenBank and include nine taxa representing the major non-*Prosymna* lineages in Elapoidea identified in previous broad phylogenetic studies (Vidal *et al.*, 2008; Kelly *et al.*, 2009; Zaher *et al.*, 2009; Figueroa *et al.*, 2016) plus one colubrid. For samples sequenced in this study, DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit under the manufacturer's protocol. Fragments of the mitochondrial genes 16S (519 bp), *CytB* (1117 bp) and *ND2* (1032 bp) and the nuclear genes *CMos* (702 bp), *Enc-1* (962 bp) and *RAG-1* (999 bp) were targeted for amplification and sequencing. PCR was performed in 25-mL reactions under the following standard conditions: 95 °C initial denaturation followed by 40 cycles of 95 °C denaturation for 30 s, 50 °C annealing for 30 s and 72 °C extension for 60 s. Primers used are listed in Table 2. Amplified products were purified using either AxyPrep magnetic beads or agarose gel extraction, with PCR products sequenced at either Villanova University or the University of Michigan. Chromatogram inspection, sequence assembly and translation to ensure no stop codons appeared in protein-coding sequences were performed using MEGA software (Kumar *et al.*, 2018).

Sequences were aligned using ClustalX (Larkin *et al.*, 2007) under default parameters. We used a greedy search algorithm in PartitionFinder 2.1.1 (Lanfear *et al.*, 2012) to identify subsets and best-fitting models in the concatenated alignment, considering each gene and each codon position of protein-coding genes as potentially different partitions. Based on this analysis, 11 subsets and their best-fitting models were identified (Table 3) and these models or their closest available analogues were applied in downstream phylogenetic analyses. We estimated phylogenetic relationships among taxa using maximum likelihood and Bayesian analyses. The likelihood analysis was performed using IQ-TREE 1.5.5 (Nguyen *et al.*, 2014), with branch support assessed using 1000 rapid bootstraps. The Bayesian analysis was performed in BEAST 1.10.4 (Suchard *et al.*, 2018). The likelihood tree topology estimated in IQ-TREE was used as the starting topology, with starting branch lengths generated using the 'chronopl' penalized likelihood function in ape 5.3 (Paradis & Schliep, 2018), setting the lambda value to 1. Divergence time estimates were made by calibrating the root height using a normal prior (mean = 49, SD = 5), based on the time of divergence of Elapoidea + Colubridae estimated in previous studies (Kelly *et al.*, 2009; Vidal *et al.*, 2009; Pyron & Burbrink, 2012; Zheng & Wiens, 2016). The BEAST analysis was run for 100 million generations, sampling every 10 000 generations. The first 10% of samples were discarded as burn-in. Effective sample sizes of parameters were estimated in Tracer 1.5 to ensure adequate chain length; relevant values were >600 for all parameters.

Morphology

We obtained skull osteological data for eight species, all of which were included in the phylogeny: *Prosymna ambigua*, *P. bivittata*, *P. greigerti*, *P. lineata*, *P. ruspolii*, *P. stuhlmanni*, *P. sundevalli* and *P. visseri*. These taxa include exemplars of all species groups identified by Broadley (1980) and exemplars of all geographical regions inhabited by the genus, including West Africa, the Horn of Africa, Central Africa, south-eastern Africa and south-western Africa. Data were obtained via high-resolution CT scanning performed at GE Inspection Technologies, LP Technical Solutions Center in San Carlos, CA, using a Phoenix V|Tome|X S scanner. Current, voltage and detector-time were modified to optimize the greyscale range and the heads of the specimens were scanned to maximize the resolution (Supporting Information, Table S1). The raw data were processed using GE's proprietary datos|x software v.2.3 to produce a series of tomogram images, which were viewed, sectioned, measured and analysed using VGSTUDIO MAX 3.3 (Volume Graphics, Heidelberg, Germany) and Avizo lite version 2019.2 (Thermo Fisher Scientic, Waltham, MA, USA). Individual skeletal elements were reconstructed separately for each scan, to facilitate osteological analysis. Tomograph stacks and 3D models have been deposited in MorphoSource (Boyer *et al.*, 2016, see supplementary data for DOIs). Comparative data for additional lamprophiid species were derived from MorphoSource and the literature,

Table 1. Specimens used in phylogenetic analyses.

Species	Specimen number	Locality	16S	*ND2*	*RAG1*	*ENC-1*	*C-MOS*	*CYT-B*
Aparallactus werneri	n/a	n/a	AY188045			JN881241	AF471116	AF471035
Atractaspis corpulenta	n/a	n/a	AY611837			JN881242	AY611929	AY612020
Boaedon fuliginosus	n/a	n/a	KX249802		KM519725	JN881267	AF544686	KM519712
Buhoma procterae	n/a	n/a	AY611818				AY611910	AY612001
Lampropeltis getula	n/a	n/a	KX694649	MG672874	MG673016	JN881266	KX694811	MG672798
Leioheterodon madagascarensis	n/a	n/a	AY188061	AY059010			AF544685	AY188022
Naja kaouthia	n/a	n/a	KX277260	AY059008	JF412633	JN881273	AY058938	AF217835
Oxyrhabdium leporinum	n/a	n/a					DQ112081	AF471029
Prosymna ambigua	CAS 258670	Angola: Malanje, Cangandala NP	MT453136	MT460633	MT460655		MT460596	
Prosymna ambigua	GJ 2762	Angola: Malanje	MT453137	MT460634	MT460656		MT460597	
Prosymna ambigua	PEM R16763	Tanzania: Mara, Gnimeti River, Klein's Camp, Loliondo Game Controlled Area	MT453112		MT460635		MT460579	
Prosymna bivittata	AMB (53F6)	no locality	MT453113	MT460610				
Prosymna bivittata	PEM R17431	South Africa: KwaZulu-Natal, Mkhuze Falls Private Game Reserve	MT453114	MT460611	MT460636			
Prosymna bivittata	PEM R17432	South Africa: KwaZulu-Natal, Mkhuze Falls Private Game Reserve	MT453115	MT460612	MT460637	MT460598	MT460580	MT482412
Prosymna bivittata	PEM R17433	South Africa: KwaZulu-Natal, Mkhuze Falls Private Game Reserve	MT453116	MT460613	MT460638		MT460581	MT482413
Prosymna cf. *frontalis*	MCZ R193166	Namibia: Erongo, Farm Omandumba	MT453117	MT460614	MT460639			
Prosymna cf. *frontalis*	PEM R17996	Angola: Namibe Province, Espinheira	MT453118	MT460615	MT460640	MT460599	MT460582	MT482414
Prosymna cf. *frontalis*	MCZ R184857	Namibia: Hobatere Lodge	MT453119		MT460641			
Prosymna frontalis	MCZ R185112	Namibia: Karas, Farm Oas	MT453120	MT460616				
Prosymna frontalis	MCZ R185003	Namibia: Karas, Geister Schlucht, Klein Aus Vista	MT453121	MT460617	MT460642		KR814680	KR814693
Prosymna greigerti	E 107.19	n/a (Genbank)	JF340124					
Prosymna greigerti	E 124.1	n/a (Genbank)	JF340125					
Prosymna greigerti	UTEP 22161	Uganda: Northern Region, Pian Upe Wildlife Reserve, Hyena Hill		MT460632	MT460654		MT460595	
Prosymna janii	JM 1045	South Africa: KwaZulu-Natal, St. Lucia	MT453122	MT460618	MT460643			MT482415

Table 1. Continued

Species	Specimen number	Locality	16S	*ND2*	*RAG1*	*ENC-1*	*C-MOS*	*CYT-B*
Prosymna janii	MCZ Z37878	South Africa: KwaZulu-Natal	MT453123					
Prosymna janii	AMB (SNH8)	South Africa: KwaZulu-Natal	MT453124	MT460620	MT460644	MT460601	MT460583	MT482417
Prosymna janii	PEM R12072	South Africa, KwaZulu-Natal, Kosi Bay Nature Reserve	FJ404222				FJ404293	FJ404319
Prosymna janii	PEM R17372	South Africa: KwaZulu-Natal, Greater St. Lucia Wetland Park		MT460619		MT460600		MT482416
Prosymna lineata	MCZ R184472	South Africa: Limpopo, 7.5km E of Musina on Tishipe Rd.	MT453126	MT460622	MT460646	MT460602	MT460585	MT482419
Prosymna lineata	JM 1816	South Africa: Limpopo, Khamai	MT453127	MT460623	MT460647		MT460586	
Prosymna lineata	MBUR 394	South Africa: Limpopo, Makgabeng area	MT453128	MT460624		MT460603		MT482420
Prosymna lineata	AMB (53F8)	South Africa: Limpopo, Blouberg	MT453125	MT460621	MT460645		MT460584	MT482418
Prosymna meleagris	E 107.22	n/a (Genbank)	JF340122					
Prosymna meleagris	E 113.2	n/a (Genbank)	JF340123					
Prosymna meleagris	MVZ245380	Ghana: Greater Accra Region, Shai Hills	MT453135	MT460631	MT460653		MT460594	
Prosymna ruspolii	CMRK316	Tanzania					DQ486171	DQ486347
Prosymna stuhlmanni	LHM-000270	South Africa: Limpopo, 3.2 km W of Lesheba Wilderness Reserve		MT460625			MT460587	MT482421
Prosymna stuhlmanni	PEM R17402	South Africa: Kwazulu-Natal, Mkhuze Game Reserve	MT453129	MT460626	MT460648	MT460604	MT460588	MT482422
Prosymna sundevalli	MCZ R184401	South Africa: Eastern Cape, Farm Newstead	MT453130	MT460627	MT460649	MT460605	MT460589	MT482423
Prosymna sundevalli	MCZ R184512	South Africa: Limpopo, 23.9km NE Vaalwater on Rd. to Melkrivier	MT453131	MT460628	MT460650	MT460606	MT460590	MT482424
Prosymna visseri	CAS 214753	Namibia: Kunene Region, Opuwo Dist. ca 2 km N of Sesfontein	MT453132	MT460629	MT460651	MT460607	MT460591	MT482425
Prosymna visseri	PEM R17994	Angola: Namibe, Espiheira	MT453133	MT460630	MT460652	MT460608	MT460592	MT482426
Prosymna visseri	MG 302	Namibia: Kunene Region, Farm Kaross	MT453134			MT460609	MT460593	MT482427
Psammophis condanarus	n/a	n/a	Z46479	AY058991			AF471104	AF471075
Pseudaspis cana	n/a	n/a	AY611898	AY058992			AY611989	AY612080

Museum acronyms are as follows, following Sabaj (2016): California Academy of Sciences (CAS); Museum of Comparative Zoology, Harvard University (MCZ); Museum of Vertebrate Zoology, University of California (MVZ); Port Elizabeth Museum (PEM); University of Texas-El Paso, Biodiversity Collections (UTEP). For unaccessioned specimens, tissue sample acronyms are as follows: Aaron Bauer (AMB); Greg Jongsma (GJ); Johan Marais (JM); Marius Burger (MBUR); Mike Griffin (MG); Vincent Egan (LHM).

Table 2. Primers used for PCR

Primer	Gene	Reference	Sequence
16SA	16S	Palumbi *et al.* (1991)	5′-CGCCTGTTTATCAAAAACAT-3′
16SB	16S	Palumbi *et al.* (1991)	5′-CCGGTCTGAACTCAGATCACGT-3′
CMOSS77	*CMos*	Lawson *et al.* (2005)	5′-CATGGACTGGGATCAGTTATG-3′
CMOSS78	*CMos*	Lawson *et al.* (2005)	5′-CCTTGGGTGTGATTTTCTCACCT-3′
proCMOSF1	*CMos*	this study	5′-RATGCACGKCCCTGCAGTAG-3′
proCMOSR1	*CMos*	this study	5′-GCTGAMCCTTTAACAKCTGCAGTTAG-3′
Cytb F.1	*CytB*	Whiting *et al.* (2003)	5′-TGAGGACARATATCHTTYTGRGG-3′
Cytb R.2	*CytB*	Whiting *et al.* (2003)	5′-GGGTGRAAKGGRATTTTATC-3′
Cytb-WWF	*CytB*	Broadley *et al.* (2006)	5′-AAAYCAYCGTTGTWATTCAACTAC-3′
Cytb-WWR	*CytB*	Broadley *et al.* (2006)	5′-TGGCCRATGATRATAAATGGG-3′
H16064	*CytB*	Burbrink *et al.* (2000)	5′-CTTTGGTTTACAAGAACAATGCTTTA-3′
L14910	*CytB*	Burbrink *et al.* (2000)	5′-GACCTGTGATMTGAAAACCAYCGTTGT-3′
ENC1_f4	*Enc-1*	Wiens *et al.* (2010)	5′-TCBTGGAGRATGTGYCTYAGCAA-3′
ENC1_r3	*Enc-1*	Wiens *et al.* (2010)	5′-GTCACRTCTCCCACYTTAGTCCACTGG-3′
H5934a	*ND2*	Arévalo *et al.* (1994)	5′-AGRGTGCCAATGTCTTTGTGRTT-3′
L4437B	*ND2*	Macey *et al.* (1997)	5′-AAGCAGTTGGGCCCATACC-3′
L5002	*ND2*	Macey *et al.* (1997)	5′-AACCAAACCCAACTACGAAAAAT-3′
proND2F1	*ND2*	this study	5′-ACMTGACARAAAATYGCACC-3′
TRPR3a	*ND2*	Greenbaum *et al.* (2007)	5′-TTTAGGGCTTTGAAGGC-3′
proRAG1F1	*RAG-1*	this study	5′-TCBTGGAGRATGTGYCTYAGCAA-3′
proRAG1R1	*RAG-1*	this study	5′-GTCACRTCTCCCACYTTAGTCCACTGG-3′
R13	*RAG-1*	Groth & Barrowclough (1999)	5′-TCGAATGGAAATTCAAGCTGTT-3′
R18	*RAG-1*	Groth & Barrowclough (1999)	5′-GATGCTGCCTTCGGCCACCTTT-3′
RAG1F700	*RAG-1*	Bauer *et al.* (2007)	5′-GGAGACATGGACACAATCCATCCTAC-3′
RAG1R700	*RAG-1*	Bauer *et al.* (2007)	5′-TTTGTACTGAGATGGATCTTTTTGCA-3′

Table 3. Data partitions and models used in phylogenetic analyses

Subset	Model applied
16S	GTR+I+G
CytB position 1	GTR+I+G
CytB position 3	GTR+I+G
ND2 position 1	GTR+I+G
ND2 position 3	K81+G
CytB position 2 + *ND2* position 2	TVM+I+G
Enc-1 position 1	TrN+I
Enc-1 position 2	TVM+I
Cmos position 1 + *RAG-1* position 1	TVM+G
Cmos position 2 + *RAG-1* position 2	HKY+I+G
Cmos position 3 + *RAG-1* position 3 + *Enc-1* position 3	HKY+G

particularly Bourgeois (1968), who surveyed and figured the cranial osteology of 73 African snake species, including 32 lamprophiids and five elapids. Observations were focused on skull characters potentially associated with dietary habits, but we also identified fusions of elements, tooth counts, number and types of braincase foramina, and significant variation in the size or shape of individual elements. The terminology used for individual elements is provided in Figure S1.

We used stochastic mapping, implemented in phytools (Huelsenbeck *et al.*, 2003; Revell, 2012), to estimate ancestral states for seven morphological traits that vary among *Prosymna* and may have phylogenetic signal: (1) presence of a postorbital, (2) presence of an elongate premaxillary process of the maxilla, (3) parietal–braincase fusion, (4) dermatocranial (nasal/frontal/parietal) fusion, (5) presence of a hammer-like maxillary process, (6) prefrontal shape (wider than tall) and (7) presence of a rostral bony shelf on the premaxilla. Data were coded as missing for four taxa without available CT scans: *Prosymna frontalis*, *P. janii*, *P. meleagris* and *Buhoma procterae*. Traits were mapped onto the BEAST maximum clade credibility tree after pruning to include only one node per *Prosymna* species. For each trait, 1000 simulations were run under a symmetrical model.

RESULTS

Phylogeny

Prosymna forms a well-supported monophyletic group (Fig. 2). The deepest divergence is between two geographically coherent clades. A northern clade distributed from West Africa across the Sahel and Sudanian savanna to the Horn of Africa includes *P. greigerti*, *P. meleagris* and *P. ruspolii* (Fig. 3). This corresponds to the *meleagris* group of Broadley (1980). The remaining species form a southern clade ranging across central, eastern and southern Africa. Within the northern clade, one specimen identified as *P. meleagris* is grouped with a number of individuals identified as *P. greigerti* rather than other specimens assigned

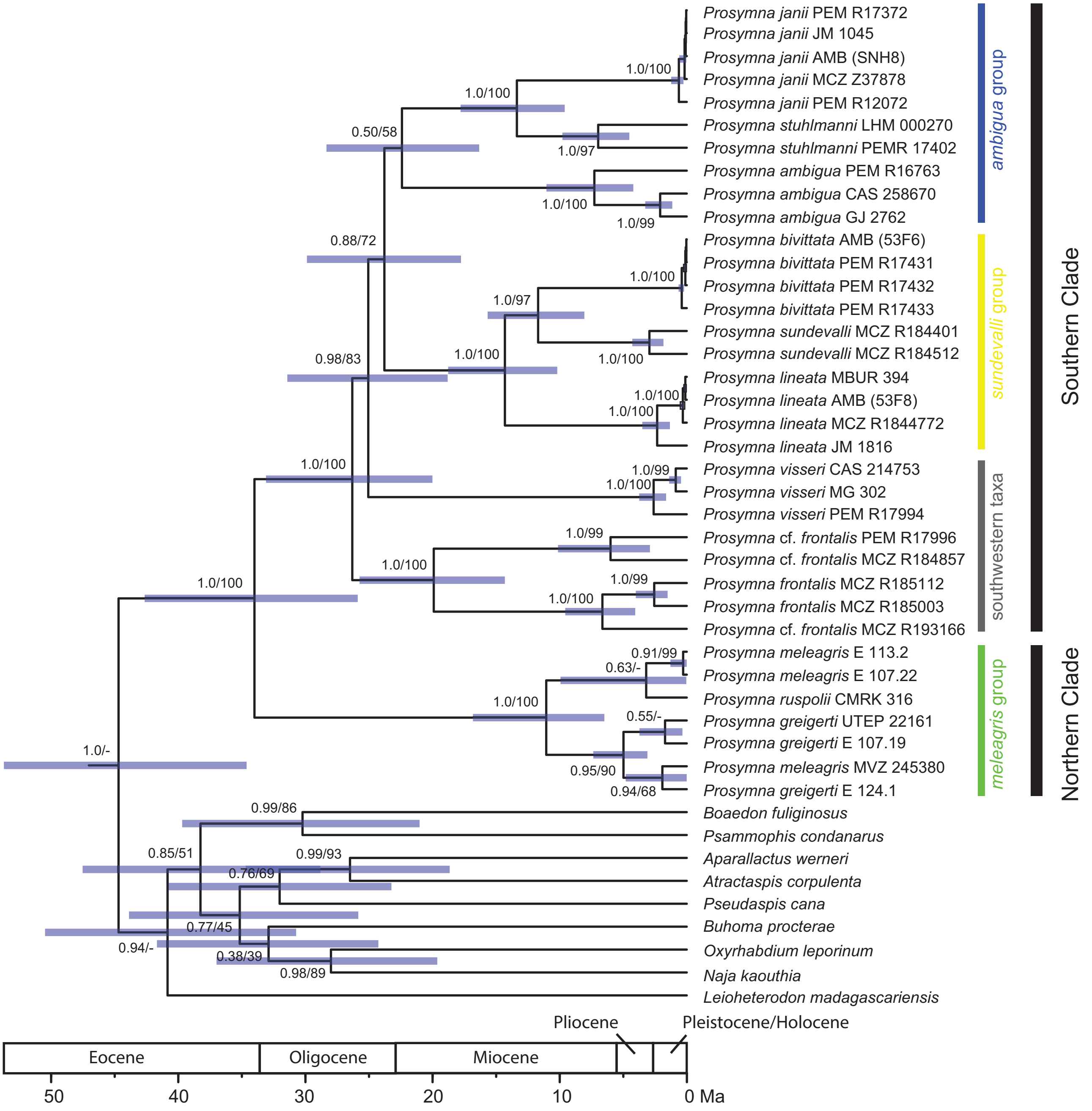

Figure 2. Time-calibrated phylogeny of *Prosymna*. Support values (Bayesian posterior probabilities/maximmum-likelihood bootstrap) and 95% highest posterior density intervals of divergence time estimates are given at nodes. The tree is rooted with *Lampropeltis getula* (not shown).

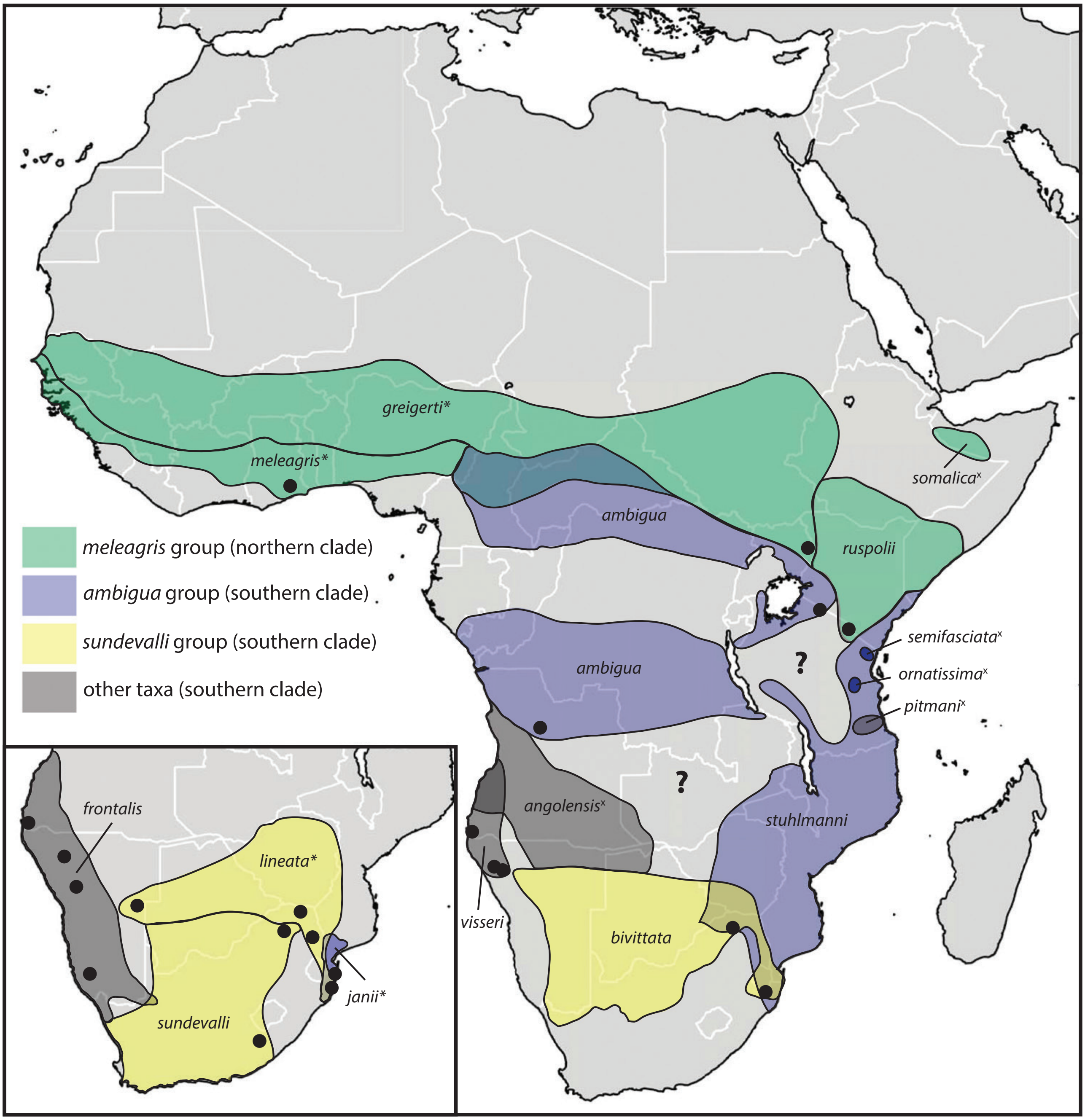

Figure 3. Geographical distributions of *Prosymna* species. For species included in the phylogenetic analysis, known sampling localities are indicated with black dots. Species included in the phylogeny with one or more unknown localities are indicated with an asterisk (*), and those not included in the phylogeny are indicated with an 'x' (^{x}).

to *meleagris*. This specimen (MVZ 245380) is from a locality in which only *P. meleagris* is documented to occur (Chirio *et al.*, 2011), suggesting that species limits in this complex need to be re-assessed. Within the southern clade, the deepest divergences have exceptionally short branches and are poorly resolved, indicating a rapid radiation around 25 Mya. Some well-supported subgroupings are apparent, however. The species *P. bivittata*, *P. lineata* and *P. sundevalli* form one such group, and *P. janii* + *P. stuhlmanni* form another. Both of these groupings are geographically coherent, with the former concentrated in south-central Africa and the latter in south-eastern Africa. The two south-western African species included in the phylogeny, *P. frontalis* and *P. visseri*, are not closely related to one another or to any other *Prosymna* species. The phylogeny is almost fully consistent with the species groups proposed by Broadley (1980) based on his analysis of external morphology. The only exception is that *P. janii*, which Broadley did not place in any species group, is embedded with species belonging to the *ambigua* group.

Divergence time estimates place the split between the northern and southern clades of *Prosymna* in the mid-Cenozoic, 34 (43–26) Mya (Fig. 2). A series of rapid splits in the southern clade is estimated to have taken place around the Oligocene–Miocene boundary, ~25 Mya. In contrast, divergences in the northern clade are estimated to have been much more recent, beginning 10 Mya. Within a few species, including *P. ambigua*, *P. frontalis*, *P. stuhlmanni* and *P. visseri*, relatively deep divergences estimated at >5 Mya may indicate the existence of cryptic taxa. This is especially true of *P. frontalis*, within which the deepest divergence was estimated to have occurred ~19 (26–13) Mya.

MORPHOLOGY

Dentition is variable among *Prosymna* species (Table 4). Maxillary tooth number varies from eight to 11; the posteriormost two to three teeth are greatly enlarged and flattened (Figs 4–6). Most species have three to five palatine teeth, but *P. bivittata* has only one per side and *P. sundevalli* has none. Dentary teeth range from five to ten in number, except in *P. visseri*, which has none. The premaxilla has a strong ascending nasal process that lies between the ventral laminae of the nasals (Bourgeois, 1968). In some species the anterior portion of the premaxilla has a tall profile that gradually slopes anterodorsally (*P. ambigua*, *P. greigerti*, *P. ruspolii*; Fig. 4), other species have a tall profile with an abrupt slope that forms a convex profile (*P. stuhlmanni*, *P. visseri*; Figs 4, 5), and in other species, the premaxillary profile is low with a gradual slope forming a dorsal concavity that ends in a narrow tip (*P. bivittata*, *P. lineata*, *P. sundevalli*; Fig. 5).

The frontals form a tubular structure and are paired; in *P. visseri* these bones are fused to the parietals and the remaining bones of the braincase (including the basisphenoid), a fusion pattern that is exceptional among squamates. Fusion of roofing bones is not restricted to *P. visseri*. In *P. bivittata*, *P. lineata* and *P. sundevalli*, the frontals and parietals are not fused, but the parietal is fused to the supraoccipital and the rest of the basicranial bones (although the parabasisphenoid remains separated from the braincase on *P. lineata* and *P. bivittata*). This is the first documented occurrence of braincase and roofing bone fusion in squamates (Cundall & Irish, 2008; Evans, 2008; Ollonen et al., 2018; Watanabe *et al.* 2019), and is exceptional considering the different origins of the bones of these units (intermembranous ossification in roofing bones, and endochondral in the braincase). *Prosymna lineata* seems to exhibit some incomplete fusion, but the specimen we studied may be distorted due to fractures. This fusion of bones is not seen in other members of *Prosymna* sampled. *Prosymna ambigua* and *P. stuhlmanni* have unfused braincases, whereas in *P. ruspolii* and *P. greigerti* the fusion is complete. Several prominent foramina pierce the braincase, but number and position vary (Figs 4–5; Supporting Information, Table S2). Because we only examined one specimen per species, we cannot be certain if the different fusion patterns are due to ontogenetic variation, although all animals are adult-sized. What is certain is that *P. bivittata*, *P. lineata*, *P. sundevalli* and *P. visseri* show a tendency towards more consolidated (i.e. peramorphic) skulls. In contrast to the widespread pattern of skull element fusion, the frontonasal suture is loose and practically transverse in all species, indicating some prokinesis that allows the anterior part of the snout to be hinged, although there are slight variations that might show differential mobility.

The maxilla of *P. visseri* is very different from the rest of the species studied, being hammer-shaped rather than blade-like, while the premaxilla is greatly reduced (Fig. 6). The maxilla varies considerably in the relative size of the prefrontal process; it is most developed in *P. ambigua*, *P. lineata*, *P. sundevalli*

Table 4. Dental formulae of *Prosymna* species included in this study; for each column tooth count is given as (left/right)

Species	Specimen number	Maxillary	Palatine	Dentary
P. ruspolii	CAS 111900	10/11	4/4	10/10
P. greigerti	CAS 136067	8/8	3/3	6/7
P. ambigua	CAS 16948	8/8	4/4	9/9
P. stulmanni	CAS 169883	10/11	5/4	8/8
P. visseri	CAS 214753	9/9	4/4	n/a
P. bivitata	CAS 165604	8/9	1/1	5/6
P. lineata	CAS 183262	9/10	3/3	7/7
P. sundevalli	CAS 111684	9/9	n/a	7/8

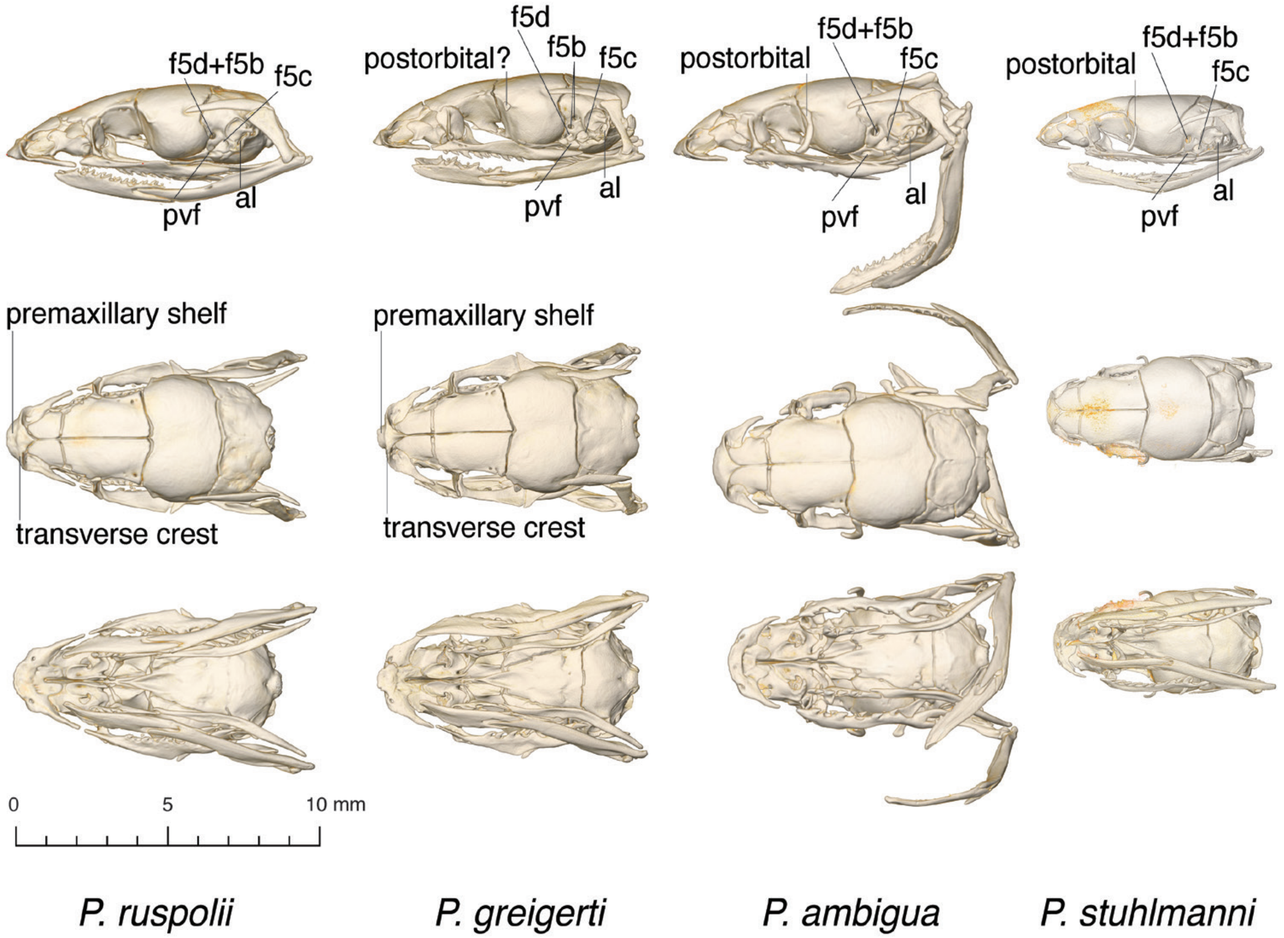

Figure 4. Lateral, dorsal and ventral views of *Prosymna* skulls, depicting variation among species in the *meleagris* and *ambigua* groups. Foramina are indicated as follows: apertura lateralis (al), foramina of trigeminal nerve branches (f5b, c, d), proximal vascular foramen (pvf); see Figure S1 for complete labelling of skull elements.

and *P. visseri*, moderate in *P. bivittata*, and faint in *P. greigerti*, *P. ruspolii* and *P. stuhlmanni*. The length and width of the palatine process is also variable, being very broad and short in *P. visseri*, but longer and narrower in the other species (Fig. 6). Additional details of skull morphology are described in the Supporting Information (SI Text).

Stochastic mapping supports unambiguous shifts in character state for several traits (Supporting Information, Fig. S2). The hammer-shaped maxilla and complete dermatocranial fusion are both estimated to have evolved in the ancestor of *Prosymna visseri*. A bony shelf of the premaxilla is estimated to have evolved in the common ancestor of the *meleagris* group. Fusion of the parietal and braincase is estimated to have evolved in the common ancestor of the *sundevalli* group. The history of other traits is more ambiguous. For example, it is equally probable that an elongate premaxillary process of the maxilla evolved twice in the *sundevalli* group or that it evolved once with subsequent loss in *P. sundevalli*.

DISCUSSION

The phylogenetic relationships of *Prosymna* species support a conservative pattern of evolution in which clades have biogeographical and morphological coherence. The deep biogeographical split between northern and southern taxa of *Prosymna* is similar to patterns in a variety of other broadly distributed groups, and is one of the fundamental biogeographical patterns of African reptiles. For example, a cluster analysis of biogeographical regions for all African reptiles (Linder *et al.*, 2012) identified one regional cluster (Ethiopian, Somalian, Sudanian regions) that corresponds to areas inhabited by the northern clade of *Prosymna*, and another regional cluster

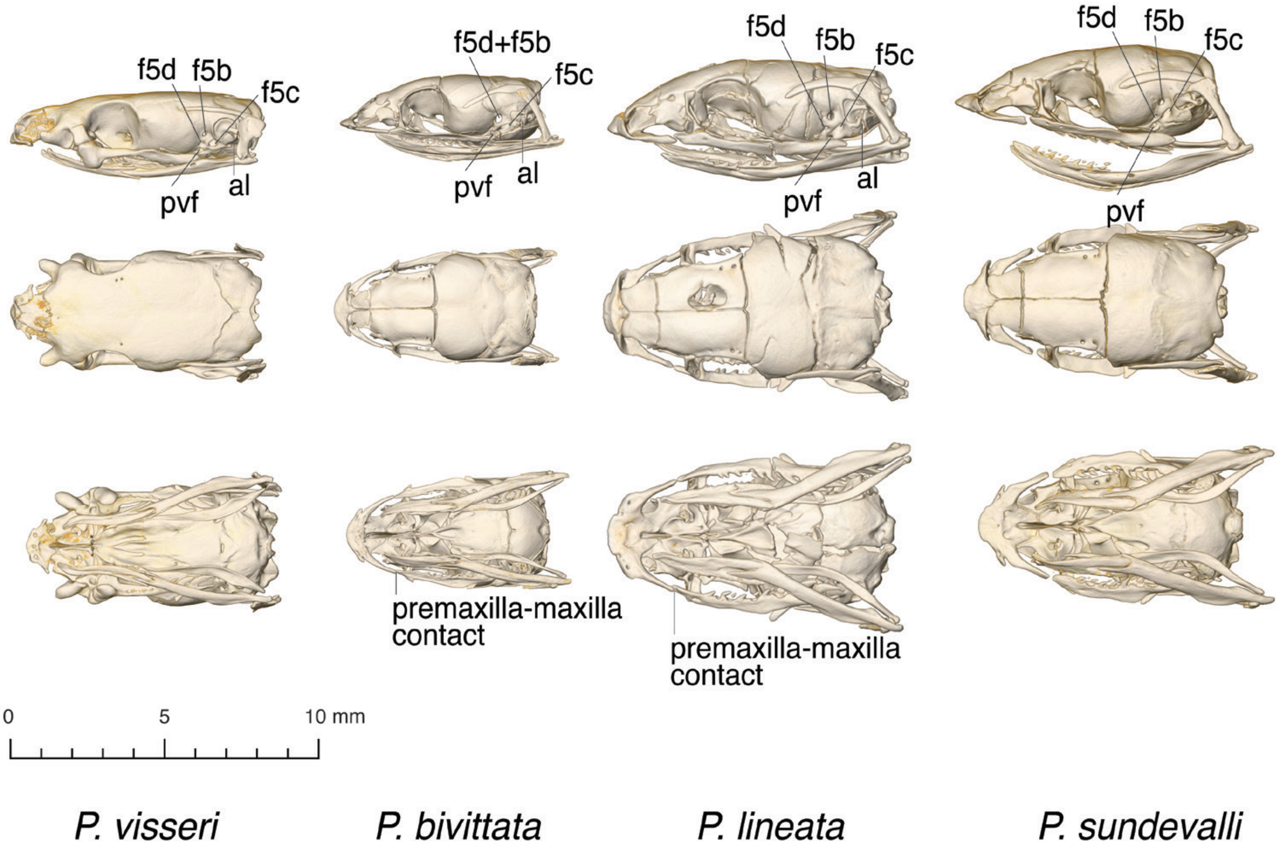

Figure 5. Lateral, dorsal and ventral views of *Prosymna* skulls, depicting variation among *P. visseri* and species in the *sundevalli* group. Foramina are labelled as in Figure 4; see Figure S1 for complete labelling of elements.

(Namibian, South African, Zambezian regions) corresponding to areas inhabited by the southern clade. The most significant physiographical barriers to north/south movement in Africa are the East African Rift System and the Congo Basin, both of which have undergone dynamic change initiated by tectonic uplift in East Africa in the mid-Cenozoic, 25–40 Mya (Burke, 1996; Ebinger & Sleep, 1998; Goudie, 2005; Stankiewicz & de Wit, 2006; Roberts *et al.*, 2012). The ranges of the northern and southern clades of *Prosymna* correspond broadly to these barriers, although southern clade species *P. ambigua* ranges marginally across the Congo Basin and northern clade species *P. ruspolii* ranges marginally across the Kenyan Rift.

Many widespread reptile groups have similar north/south splits. Examples include *Agama* lizards, *Panaspis* skinks, Nile monitors (*Varanus*) and many snakes: centipede-eaters (*Aparallactus*), puff adders (*Bitis*), herald snakes (*Crotaphopeltis*), Egyptian and spitting cobras (*Naja*), and cat snakes (*Telescopus*; Wüster *et al.*, 2007; Trape *et al.*, 2009; Barlow *et al.*, 2013; Leaché *et al.*, 2014; Dowell *et al.*, 2016; Medina *et al.*, 2016; Portillo *et al.*, 2018; Šmíd *et al.*, 2019; Engelbrecht *et al.*, 2020). This common pattern does not mean that a common process drove divergences in all of these groups. While our estimate of the divergence time between northern and southern *Prosymna* corresponds to early uplift in East Africa, north/south splits in other groups occurred at other times. The initial major north/south divergence in *Panaspis* probably occurred earlier (~50 Mya; Medina *et al.*, 2016). Major north/south splits in *Agama*, *Aparallactus* and *Telescopus* are estimated to have occurred later (~20 Mya; Leache *et al.*, 2014; Portillo *et al.*, 2018; Šmíd *et al.*, 2019), and north/south phylogeographical-level divergences in *Bitis*, *Crotaphopeltis*, *Naja* and *Varanus* are much more recent (Wüster *et al.*, 2007; Trape *et al.*, 2009; Barlow *et al.*, 2013; Dowell *et al.*, 2016; Engelbrecht *et al.*, 2020). Thus, while the development of major physical barriers may have played a role in permitting divergences among all these taxa, different events would have been involved in each case. It is also likely that climate and vegetation have played a role as significant as physiographical barriers in divergences.

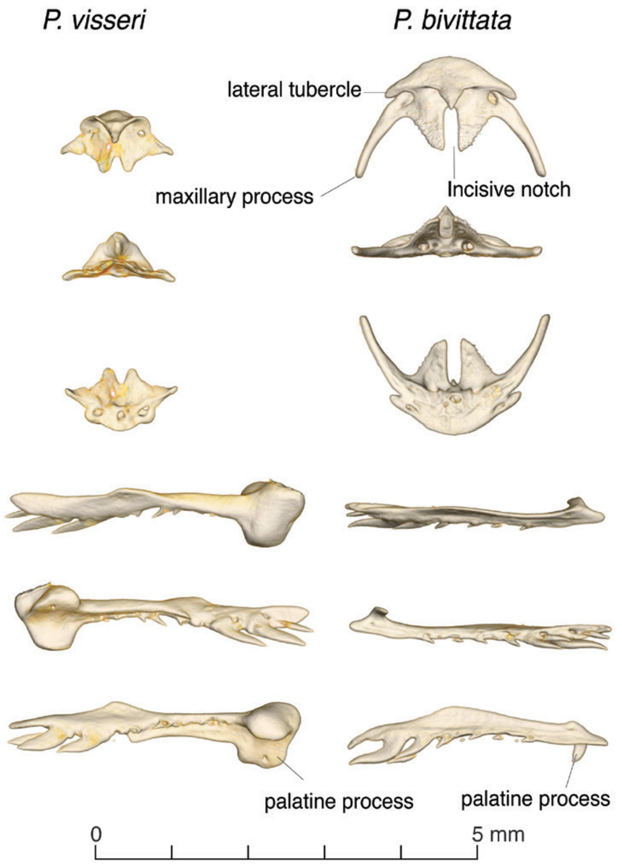

Figure 6. Above: dorsal, posterior and ventral ventral views of the premaxilla in *Prosymna visseri* and *P. bivittata*. Below: lateral, medial and ventral views of the right maxilla in *Prosymna visseri* and *P. bivittata*. Key distinguishing features are labelled. The maxilla of *P. visseri* was better preserved in the left side, and therefore it was mirrored to facilitate anatomical comparisons.

A general but not constant trend of aridification has persisted in Africa since the mid-Cenozoic, resulting in major vegetational changes (Feakins & Demenocal, 2010; Jacobs *et al.*, 2010). While under current climatic conditions central African rainforests form a barrier separating northern and southern *Prosymna*, drier conditions have prevailed in the past resulting in forest contraction and fragmentation (Bonnefille *et al.*, 2007; Senut *et al.*, 2009; Duminil *et al.*, 2015). Forest contraction permits dispersal of savanna taxa such as *Prosymna*, and may have been involved in the mid-Cenozoic divergence between northern and southern *Prosymna*. More recently, the range of *P. ambigua*, which consists of two blocks separated north and south of the Congo rainforest, is probably the result of Pleistocene forest fragmentation followed by subsequent expansion.

A coherent pattern of morphological evolution within *Prosymna* is exemplified by the consistency of our results with the phenetic classification of species groups based on external morphology (Broadley, 1980). The one exception, *P. janii*, has a number of unique aspects of its scalation, including being the only *Prosymna* species with keeled scales, so it is not surprising that a phenetic classification would not place this species in a broader species group. Shared osteological traits are evident for several clades. The sampled members of each species group are comparable in skull shape and patterns of fusion. Each species group also displays one or more shared derived features, although stochastic mapping indicates that some of these features may have evolved more than once (Supporting Information, Fig. S2). Species in the *ambigua* group have unfused braincases and are the only species possessing a postorbital bone. Members of the *meleagris* group have fused braincases and unique premaxillae bearing a horizontal shelf. Species in the *sundevalli* group exhibit braincase fusion plus partial fusion of roofing elements, and possess elongate maxillary processes of the premaxillae, rectangular nasals and three frontal foramina. The one *Prosymna* we studied osteologically that does not have any close relatives, *P. visseri*, has the most unique skull, exemplified by its extreme level of fusion and dramatically modified maxilla.

Although clade-specific traits demonstrate the variation among the sampled species in skull morphology, more notable are the features shared across *Prosymna* that set the genus apart from all other African snakes (Bourgeois, 1968). All sampled species have compact, generally rigid skulls (exemplified by extensive fusion of elements), anterior reduction of the maxilla (although not in *P. visseri*), reduced dentition overall, enlargement of the posterior maxillary teeth, reduced palatine, elongate supratemporal and participation of the prefrontal in the orbit. All except *P. visseri* have a posteriorly elongated premaxilla. Thus, the basic morphological plan of the skull had probably already evolved in the mid-Cenozoic common ancestor of *Prosymna*, with consistent functional pressures maintaining these phenotypic traits over time.

Evolutionary maintenance of shared skull traits in *Prosymna* species may be due to functional importance for burrowing or feeding specializations. The consolidated, rigid skulls of *Prosymna* are unique among African caenophidians, but similar consolidation occurs in other burrowing squamate lineages (Savitzky, 1983; Lee & Scanlon, 2002; Shine & Wall, 2008). We interpret anterior reduction of the maxilla and palatine as manifestations of general skull consolidation, whereas participation of the prefrontal in the orbit (also seen in the burrowing genus *Scaphiophis*) may provide cranial reinforcement and protection to the eyes. The enlarged premaxilla creates a spade-like surface that may facilitate locomotion in loose soil. The overall shape of the skull of *Prosymna* most closely resembles that of other snakes adapted for digging in a loose substrate, such as *Heterodon*, rather than the more ram-like skulls of scolecophidian snakes or amphisbaenians dwelling in compact substrates (Wake, 1983; Watanabe *et al.*, 2019). Snout kinesis is used for digging in other snakes that burrow in soft substrate (Deufel, 2017). The loose frontonasal suture of *Prosymna* may facilitate comparable locomotion.

In contrast to *Prosymna* skull elements that resemble those of other burrowing squamates, supratemporals are typically reduced in burrowing snakes (Savitzky, 1983), while those of *Prosymna* are greatly elongated. The snake supratemporal participates in connecting the mandible and the cranium via its articulation with the quadrate. It is functionally involved in the feeding process as it facilitates lateral and ventral extension of the lower jaw (e.g. Gans, 1952, 1961; Kardong, 1977; Greene, 1983). Significant rotation of the supratemporal has been implicated in the kinematics of egg-feeding in the Asian snake genus *Oligodon*, which slits eggs using enlarged posterior maxillary teeth and requires coordinated movements at several joints to effect the cutting action of the maxillary teeth (Coleman *et al.*, 1993). Although the kinematics of egg-feeding in *Prosymna* have not been studied, the greatly enlarged posterior maxillary teeth are similar to those of other egg-slitting snakes (Coleman *et al.*, 1993; Trauth, 1993; Gardner & Mendelson, 2003) and therefore a similar cutting mechanism can be inferred. In *Oligodon*, rotation of the maxilla itself is primarily responsible for positioning the enlarged maxillary teeth to produce the cut. The overall rigidity of the skull in *Prosymna* probably reduces its range of motion in the maxilla, but the elongate supratemporal and quadrate may compensate for this by increasing the range of ventrolateral motion in the lower jaw and, therefore, permitting a large enough gape to bring the cutting points of the posterior maxillary teeth into contact with an egg being ingested.

Prosymna skull morphology reaches more extreme levels of specialization in the south-western African species *P. visseri*. The most striking features differentiating *visseri* from all other *Prosymna* include a flattened premaxilla giving the skull a blunt rather than shovel shape, extreme ossification with fusion of most cranial elements, anterior projections of the septomaxillae, a shorter, stout quadrate, and the hammer-shaped, toothless anterior portion of the maxilla, which contacts an enlarged prefrontal. Differences in overall skull shape may be partly related to habitat differences, as *P. visseri* is a rock-dweller inhabiting fissures whereas most other *Prosymna* are burrowers. The hammer-shaped maxilla, however, is probably related to innovation in feeding mode. Several genera of south-western African geckos commonly use rock fissures as egg deposition sites (Branch, 1998; Alexander & Marais, 2007), and gecko eggs have been inferred to be the primary dietary item for *P. visseri* (McLachlan, 1987; Bauer *et al.*, 2001). Unlike the leathery eggs of other squamates, gecko eggs harden after laying. Several aspects of the skull of *P. visseri* appear adapted for crushing these hard-shelled dietary items. The hammer-shaped maxilla of *P. visseri*, reinforced by an enlarged prefrontal, provides a crushing surface, the short, stout quadrate should increase leverage, and complete fusion of the cranium may ensure that none of the force of jaw closure is lost to kinesis at sutures. Even with these additional specializations, *P. visseri* does maintain some traits of other *Prosymna* that suggest an egg-slitting ancestor, including enlarged posterior maxillary teeth and an elongate supratemporal. The time of divergence between *P. visseri* and other *Prosymna* was 26 (32–20) Mya. This corresponds to the time frame in which rock-dwelling geckos were rapidly diversifying in south-western Africa (Heinicke *et al.*, 2017), so the unique morphology of *P. visseri* may have evolved in response to a newly opened niche for a gecko egg-eating specialist. This egg-crushing jaw morphology is unlike that of any other known snake, including the other *Prosymna* we studied. However, *P. frontalis* has been noted to have a swollen, toothless anterior maxilla (Broadley, 1980), and this species is also a rock-dweller (rather than burrower) from south-western Africa. More detailed morphological examination of this species therefore might reveal another *Prosymna* species with less pronounced adaptations for egg-crushing.

The overall evolutionary pattern in *Prosymna* suggests that the common ancestor of the genus already possessed specializations for burrowing and feeding by egg-slitting in the mid-Cenozoic. While the overall morphology of the skull is a major departure from other African snake lineages, there are clear morphological parallels in other independently evolved oophagous snakes. There has been only limited subsequent change from the ancestral *Prosymna* form in most extant lineages. Subsequent diversification appears to have largely occurred through a series of divergences into geographically distinct clusters of species, the earliest of which was between the northern *meleagris* group on the one hand and all other *Prosymna* on the other. The exception from this general pattern is *P. visseri*, which has skull morphology unlike that of any other snake, evolving a specialized egg-crushing skull from an already specialized ancestor.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. *Boaedon capensis* (CAS85747) in dorsal (A) and right lateral (B) views. *Prosymna bivittata* (CAS165604) in dorsal (C) and right lateral (D) views. Abbreviations: bo, basioccipital; col, columella; cp, compound bone; d, dentary; ecp, ectopterygoid; exo, exoccipital; f, frontal; mx, maxilla; na, nasal; p, parietal; pmx, premaxilla; po, postorbital; prf, prefrontal; pro, prootic; psp, parasphenoid; pt, pterygoid; q, quadrate; so, supraoccipital; st, supratemporal.

Figure S2. Density plots based on stochastic mapping of morphological traits. Colour corresponds to the posterior probability of the plotted morphological character state, with red indicating a probability of 1.0 and blue indicating a probability of 0. Plotted traits are as follows: A, postorbital bone present; B, elongate maxillary process of the premaxilla present; C, parietal fused to braincase; D, dermatocranial bones fused; E, hammer-like maxillary process present; F, prefrontal wider than tall; G, bony rostral shelf on premaxilla present.

Table S1. Specimens used for CT scans.

Table S2. Foramina of the braincase in *Prosymna*. Homologies are based on position of foramina, size and direction of the opening.