Received: 18 May 2018 | Revised: 4 February 2019 | Accepted: 1 March 2019

DOI: 10.1111/jbi.13578

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RESEARCH PAPER

Journal of Biogeography | WILEY

Ancient habitat shifts and organismal diversification are decoupled in the African viper genus *Bitis* (Serpentes: Viperidae)

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Funding information
Systematics Research Fund; Natural Environment Research Council

Editor: Serban Procheş

Abstract

Aim: The expansion of open habitats during the mid-Miocene has been hypothesized as a driver of allopatric speciation for many African taxa. This habitat-dependent mode of diversification has been implicated in the shift from C_3 (e.g. forest/woodland) to C_4 dominated systems (i.e. open savanna, grasslands) in a number of African squamates. We examined this hypothesis using a genus of African viperid snakes (*Bitis*) with both open habitat and forest-dwelling representatives.

Location: Africa.

Methods: A comprehensive multilocus dataset was used to generate a calibrated species tree using a multispecies coalescent model. Individual gene trees and patterns of nuclear allele sharing were used to assess species monophyly and isolation. To test the habitat-dependent evolution hypothesis, we generated an ancestral character state reconstruction for open and closed habitats using the dated phylogeny. This was related to the timing of open habitat expansion and forest/woodland contraction in Africa.

Results: The genus *Bitis* originated in the Oligocene, with species level diversification in the late Miocene/Pliocene. Four well-supported clades correspond to the recognized subgenera *Bitis*, *Keniabitis*, *Macrocerastes* and *Calechidna*. Several previously unrecognized lineages potentially represent cryptic species.

Main conclusions: Habitat-dependent evolution does not appear to have been a main driver for generic level viperine diversification: the ancestral state for *Bitis* was open habitat and at least one clade moved into forest in the Miocene, long after forest had contracted and fragmented. Forest-dependent species diversified only in the late Miocene, presumably as forest became further reduced in extent, fitting an allopatric model of speciation. Although our results do not favour a general pattern of habitat-dependent diversification in *Bitis*, cladogenesis within the subgenus *Calechidna* for "arenicolous" species (*Bitis caudalis* complex) and "rupicolous" species (*B. atropos-cornuta* complex), corresponds to the aridification of southwest Africa.

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This suggests there are subtleties not captured in the broad open habitat category, which are relevant for understanding the role of habitat-dependent evolution.

KEYWORDS
multilocus phylogenetics, multispecies coalescent, reptiles, snakes, sub-Saharan Africa

1 | INTRODUCTION

In broad terms, sub-Saharan African faunal lineages can be segregated into those that occupy closed or dense canopy forest/woodland ecosystems ("forest" lineages) and those that occupy structurally more open ecosystems such as grassland, heathlands, open savanna and desert ("open-habitat" lineages: e.g. deMenocal, 1995, 2004; Maslin et al., 2014; Tolley, Chase, & Forest, 2008; Tolley et al., 2011). Through the Palaeogene (66–23 Ma) dense woodland/forest was widespread across sub-Saharan Africa, and was gradually displaced by open ecosystems through the Oligocene and early Miocene as the tropical climate aridified (Coetzee, 1993; Kissling et al., 2012; Linder, 2017; Morley, 2007). During the Oligocene, forest/woodland became reduced in extent, contracting from North Africa and the Southern & Zambezian region into central Africa presumably leaving substantial patches in central Africa (see Morley, 2007; Figures S1 & S2), possibly as a mosaic with more open vegetation types (Linder, 2017). From the Mid to Late Miocene, beginning *c*. 10 Ma, open habitats expanded markedly, with those comprised primarily of plant species utilizing the C_4 photosynthetic pathway becoming increasingly dominant on the continent (Couvreur, Chatrou, Sosef, & Richardson, 2008; Edwards, Osborne, Strömberg, & Smith, 2010; Kissling et al., 2012; Maslin et al., 2014). Subsequent climatic cooling and aridification during the Pliocene and Pleistocene, 2.8–1.0 Ma, was associated with further open habitat expansion and the dominance of C_4 grasslands and savanna (deMenocal, 1995; Kissling et al., 2012). This aridification was punctuated by short moist periods that could have facilitated temporary forest re-expansion (Maslin et al., 2014; Trauth, Maslin, Deino, & Strecker, 2005). Regardless, since the Cretaceous, the widespread forest/woodland lost most of its extent, with open habitats becoming dominant in the landscape (Kissling et al., 2012; Morley, 2007).

The prominent expansion of open habitats in sub-Saharan Africa is thought to have played a key role in the evolution of open habitat fauna. Multiple hypotheses have been invoked to explain this faunal evolution in open habitats (Potts, 1998; Vrba, 1985, 1992), and collectively these have been termed "habitat-specific hypotheses" (deMenocal, 2004; Potts, 1998). The paradigm essentially points to ecological speciation, where diversification is driven by directional selection in differing environments (e.g. Rundle & Nosil, 2005; Schluter, 2009). Here, we adopt the term "habitat-dependent" evolution to specifically refer to ecological diversification of lineages inhabiting novel habitats due to reorganization of habitat types on the African continent.

The mammalian fossil record provides considerable evidence for habitat-dependent evolution in sub-Saharan Africa. In particular, the expansion of C_4 grassland during the Plio-Pleistocene appears to have played a role (Hewitt, 2004) as the first appearance of many arid adapted species across a range of taxa coincides with this period (Bobe & Behrensmeyer, 2004; Bobe, Behrensmeyer, & Chapman, 2002; Bowie & Fjeldså, 2008; Vrba, 1992; Wesselman, 1985). Phylogenetic studies also support this hypothesis, with a number of forest-dependent taxa showing strong signatures of allopatric speciation corresponding to fragmentation of forests (Barej, Penner, Schmitz, & Rödel, 2015; Bowie, Fjeldsa, Hackett, Bates, & Crowe, 2006; Demos, Kerbis Peterhans, Agwanda, & Hickerson, 2014; Lawson, 2010; Menegon et al., 2014; Tolley et al., 2008), whereas recent radiations appear to correspond with occupation of more open habitats (Bowie & Fjeldså, 2008; Demos et al., 2014; Tolley, Burger, Turner, & Matthee, 2006; Tolley, Townsend, & Vences, 2013). These patterns are clearly taxon dependent, presumably because of the idiosyncratic life-history characteristics and dispersal ability of the taxa. In general however, highly vagile species are either generalists, or can disperse across unsuitable habitat (Fuchs et al., 2013; Oatley, Voelker, Crowe, & Bowie, 2012), which facilitates gene flow resulting in low genetic structure. In contrast, most forest-dependent species will find the open habitat a formidable barrier and require either forest reconnection or habitat corridors to maintain population connectivity and gene flow (Barej et al., 2015; Bittencourt-Silva et al., 2016; Bowie et al., 2006; Measey & Tolley, 2011). Given taxon idiosyncrasies, a universal model for the evolution of fauna on the continent is not plausible. However, a paradigm that incorporates the reduction in forest/woodland as an important driver of biogeographical patterns is tenable and can incorporate the idiosyncratic nature of species.

Squamate reptiles are a taxonomic group that is both widespread and highly diverse within sub-Saharan Africa, where diversification of forest-/woodland-dependent taxa has been influenced by habitat shifts. For example several clades of squamates that currently occupy open habitats diversified within the Miocene (e.g. chameleons and snakes; Barlow et al., 2013; Pook, Joger, Stümpel, & Wüster, 2009; Tolley et al., 2013; Wüster et al., 2007). Furthermore, ancient forest lineages in the southern African chameleon genus *Bradypodion* gave rise to open-habitat species following the onset of open habitat expansion in the Pliocene (Edwards, Vanhooydonck, Herrel, Measey, & Tolley, 2012; da Silva, Herrel, Measey, Vanhooydonck, & Tolley, 2014; da Silva & Tolley, 2017; Measey, Hopkins, & Tolley, 2009; Tolley et al., 2008), suggesting that shifts to open habitats beginning in the Miocene may have

been widespread on the landscape and across multiple taxonomic groups.

The African viper genus *Bitis* provides an opportunity to test the habitat-dependent hypothesis of ecological diversification. Commonly referred to as the African adders, *Bitis* is Africa's most taxonomically diverse and geographically widespread viperid genus, containing 18 extant species (*sensu* Branch, 1999; Gower et al., 2016; Lenk, Herrmann, Joger, & Wink, 1999; Uetz, Freed, & Hošek, 2017) and one documented extinct Pleistocene species, *Bitis olduvaiensis* (Rage, 1973). Several studies have investigated the phylogeny of *Bitis* using morphological evidence (Ashe & Marx, 1988; Groombridge, 1980; Wittenberg, Jadin, Fenwick, & Gutberlet, 2015) and immunological distances (Lenk et al., 1999). Higher level phylogenies of Viperidae and Viperinae have also included *Bitis* (Alencar et al., 2016; Herrmann & Joger, 1995, 1997; Herrmann, Joger, Lenk, & Wink, 1999; Lenk, Kalayabina, Wink, & Joger, 2001; Lenk et al., 1999; Pyron, Burbrink, & Wiens, 2013; Wüster, Peppin, Pook, & Walker, 2008; Šmíd & Tolley, 2019). The study of Lenk et al. (1999) identified four major mitochondrial clades within the genus *Bitis*, which were formally recognized as subgenera (Table 1). These are:

- *Macrocerastes*, a clade of large-bodied forest adders, which includes the Gaboon adders (*B. gabonica* and *B. rhinoceros*) and the rhinoceros viper (*B. nasicornis*).
- *Calechidna*, a clade of open habitat dwarf adders endemic to southern Africa. This clade is further divided into two subclades corresponding, respectively, to those taxa primarily associated with gravel or rocky habitats ("rupicolous", *B. atropos-cornuta* complex) and those associated with sandy substrates ("arenicolous", *B. caudalis* complex).
- *Keniabitis*, a monotypic clade representing the small-bodied Kenyan endemic *B. worthingtoni*, which occurs in montane grassland habitats along the Kenyan Rift Valley.
- *Bitis* (the type subgenus), representing the geographically widespread and large-bodied puff adder (*Bitis arietans*), which occurs across a variety of open woodland, grassland and scrubland habitats throughout sub-Saharan Africa, southern Arabia and Morocco.

Although the evolutionary relationships within *Bitis* are relatively well understood, several important questions remain. The

TABLE 1 Taxonomy of *Bitis* and habitat preference for each species

Subgenus	Species		Habitat
Macrocerastes	*B. gabonica*	East African Gaboon adder	Tropical and montane forest
	B. rhinoceros	West African Gaboon adder	
	B. nasicornis	Rhinoceros viper	
	B. parviocula	Ethiopian mountain adder	
	B. harenna[a]	Bale Mountains adder	
Calechidna	*B. albanica*	Albany adder	Lowland and montane rocky or gravely grassland, karroid and Sclerophyllous scrub
	B. armata	Southern adder	
	B. atropos	Berg adder	
	B. cornuta	Many-horned adder	
	B. heraldica[a]	Angolan adder	
	B. inornata	Plain mountain adder	
	B. rubida	Red adder	
	B. xeropaga	Desert mountain adder	
	B. caudalis Lineage 1	Horned adder	Sandy savanna and karroid scrub and alluvial soils
	B. caudalis Lineage 2		
	B. peringueyi	Peringuey's adder	Namib sand sea
	B. schneideri	Namaqua dwarf adder	Coastal sand dunes
Bitis (type subgenus)	*B. arietans* complex	Puff adder	Open savanna, grassland and karroid scrub
			Absent from forest and desert
Keniabitis	*B. worthingtoni*	Kenya horned viper	Montane grassland and scrub

[a]Subgeneric assignment not confirmed by genetic analysis.

relationship between the subgenera lacks resolution, and the phylogenetic positions of *B. (K.) worthingtoni* and *B. (B.) arietans* were equivocal in previous analyses due to a lack of statistical support at basal nodes (Lenk et al., 1999; Pyron et al., 2013; Wüster et al., 2008). In addition, several poorly known species have not been included in any molecular phylogeny to date (*B. harenna*, *B. albanica*, *B. heraldica* and *B. inornata*), and most studies of *Bitis* have utilized single individuals to represent species, precluding any assessment of levels of intraspecific genetic diversity or the testing of species monophyly (but see Barlow et al., 2013).

In this study, we examine evolutionary relationships within *Bitis* to investigate whether a habitat-dependent hypothesis of diversification applies to this genus. We used a time-calibrated multilocus phylogeny, including 16 of the 18 currently recognized *Bitis* species, to explore patterns and timing of diversification among the subgeneric clades. In particular, we expected that *Bitis* lineages occupying open habitats (subgenera: *Calechidna*, *Keniabitis* and *Bitis*) diverged either in response to the initial but gradual aridification of Africa (Eocene/Oligocene) or later, during the rapid mid-Miocene expansion of open habitats. If so, the origin of the genus should reflect the geographical region where the forest/woodland contraction was maximal during those time periods (either North Africa or the Southern and Zambezian regions). We carried out ancestral character state reconstruction of the broad habitat categories (forest/woodland mosaic and open-habitat), to understand if the timing of diversification corresponded to major habitat shifts on the continent, which could support habitat-dependent diversification. Furthermore, an ancestral area reconstruction allowed us to assess whether the geographical origin of key clades fits well with habitat-dependent diversification. We also included multiple representatives of species to investigate the outstanding taxonomic issues, specifically subgeneric and species monophyly and the possibility of cryptic speciation.

2 | MATERIALS AND METHODS

Tissues (scale clips, blood, shed skins, dermal tissue or liver) were sampled from all currently recognized *Bitis* species except the poorly known Angolan species *B. heraldica* and the recently described *B. harenna*. All individuals were released after sampling or retained alive by their owners. Multiple representatives of each sampled species were included except for *B. inornata* and *B. rhinoceros*, for which it was only possible to sample a single individual. Sequences from additional representatives of the Viperidae were also generated or downloaded from GenBank for use as outgroup taxa and to facilitate the dating analysis. Outgroup taxa included one to three individuals from six other genera (from Africa and Eurasia) in the subfamily Viperinae, resulting in a dataset of 77 individuals for four genes. Of these, sequences of one to three genes from 15 individuals were available on GenBank. Details of samples, vouchers and GenBank accession numbers are given in Table S1.

We generated sequence data from two mitochondrial and two unlinked nuclear markers. The mitochondrial data consisted of partial sequences of the 16s ribosomal RNA (16S) and NADH dehydrogenase subunit 2 (ND2) genes. The nuclear markers were exonic sequences of the prolactin receptor (PRLR) and ubinuclein 1 (UBN1) genes. Total DNA was extracted from tissue samples using a Qiagen DNeasy™ Tissue Kit (cat. no. 69506) following the manufacturer's instructions. Genetic markers were PCR amplified using the following primers. 16S: L2510 (5′-CGCCTGTTTATCAAAAACAT-3′) and H3080 (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi, Martin,Romano,Stice,&Grabowski,1991);ND2:L4437b(5′-CAGCTAAAAAGCTATCGGGCCCATAC-3′) (Kumazawa, Ota, Nishida, & Ozawa, 1996) and tRNA-trpR (5′-GGCTTTGAAGGCTMCTAGTTT-3′) (Ashton & de Queiroz, 2001); PRLR: PRLR-f1 (5′-GACARYGARGACCAGCAACTRATGCC-3′) and PRLR-r3 (5′-GACYTTGTGRACTTCYACRTAATCCAT-3′) (Townsend, Alegre, Kelley, Wiens, & Reeder, 2008); UBN1: BaUBN_F (5′-CCTCTGGTTACTCAGCAGCA-3′) and BaUBN_R (5′-ATTGGCCACTCCTTGTGTTC-3′). PCRs comprised 9.6 μl ABgene ReddyMix™ PCR Master Mix (cat. no. AB-0575/LD/A), 0.27 μM of each primer and 5–10 ng of template DNA, giving a final reaction volume of 11 μl. The thermocycling regimes involved an initial denaturation at 94°C for 2 min; 30–40 cycles of: 30 s denaturation at 94°C, 30 s (16s, ND2) or 60 s (PRLR, UBN1) annealing at 50°C (16s), 52°C (PRLR), 55°C (ND2), or 60°C (UBN1), and 45 s (16S, PRLR, UBN1) or 90 s (ND2) extension at 72°C; and a final extension for 5 min at 72°C. PCR products were cleaned using the enzymes exonuclease 1 and thermo-sensitive alkaline phosphatase, and direct sequencing carried out by Macrogen Inc. (dna.macrogen.com) using forward PCR primers (16s, some PRLR) or both forward and reverse PCR primers (ND2, UBN1, some PRLR).

Sequences were proof-read and aligned using the software CodonCode Aligner 3.5.6 (www.codoncode.com). Only clean sequences were retained, and we re-sequenced any sequence with questionable stretches. Protein-coding gene sequences were translated to check that no frameshift mutations or stop codons were present. Alignment was ambiguous for some sections of the 16S alignment so these regions were excluded from analyses. UBN1 contained a "TCC" tri-nucleotide repeat section with several heterozygous indels necessitating the exclusion of 30 bp.

Heterozygous positions were identified in nuclear sequence chromatograms by a combination of visual inspection for double peaks and typically low quality Phred scores (Ewing & Green, 1998) for the bases surrounding a heterozygous position. Individual allele sequences were estimated from the diploid nuclear sequences using phase (Stephens & Scheet, 2005; Stephens, Smith, & Donnelly, 2001) in DnaSP 5 (Librado & Rozas, 2009), using default settings. To verify the reliability of the phase, analysis we computed maximum likelihood (ML) trees under the GTRCAT model in RAxML 7.2.8 (Stamatakis, 2006) for both the unphased and phased alignments, with clade support assessed using 100 bootstrap replicates and specifying the *Causus* sequences as outgroup. For each nuclear gene, both phased and unphased alignments produced highly congruent topologies with broadly comparable bootstrap values for all nodes above the species level (Figures S3-6). Overall, this indicates no obvious distortion of phylogenetic signal in either dataset as a result

of the phasing procedure. The final dataset consisted of 2,415 base pairs: 16S-426 bp; ND2-1014 bp; PRLR-525 bp; UBN1-450 bp.

Species relationships were first investigated by concatenating data from all loci. A ML search was run using RAxML HPC 7.2.8 (Stamatakis, 2006) on the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010) for the 4-gene dataset. The analysis was run using both unphased and phased nuclear sequences. Each gene was partitioned separately, and the default GTR+I+G model was used with rapid bootstrapping halted automatically (Stamatakis, Hoover, & Rougemont, 2008). This analysis was run three times to ensure that independent ML searches produced the same topologies. We considered nodes with a bootstrap value of >70% as supported in this analysis.

The *Bitis* species tree was then inferred using a multispecies coalescent (MSC) model using *BEAST (Heled & Drummond, 2010), implemented in BEAST 1.7.4 (Drummond, Suchard, Xie, & Rambaut, 2012). *BEAST co-estimates individual gene trees and the species tree within which they evolved, using a fully Bayesian framework accounting for incomplete lineage sorting. We assigned individuals to species according to current taxonomy (Lenk et al., 1999) except in the case of *B. caudalis*, which preliminary analysis found to comprise two polyphyletic mitochondrial lineages (see Results). Individuals corresponding to these mitochondrial lineages were therefore assigned as separate taxa (*B. caudalis* L1 and L2). Including outgroup taxa, the resulting species tree contained 24 species/taxa, sampling 77 individuals, and was inferred from three independent gene trees: mitochondrial (estimated from concatenated 16s and ND2 sequences), PRLR and UBN1.

We estimated timing of divergence among *Bitis* species by calibrating the MSC species tree analysis based on fossil evidence from the related Eurasian viperine clade (represented by *Vipera berus*, *Daboia siamensis* and *Montivipera xanthina*), which the fossil record shows to have existed at least 20 Ma (Szyndlar & Rage, 1999). On the basis of the assumption that the most recent common ancestor (MRCA) of this clade is unlikely to have occurred considerably earlier than this, we constrained the monophyly of this clade and applied a lognormal prior to the age of the MRCA with a 20 Ma offset, mean of zero and standard deviation of 1.0, and upper limit of 40 Ma. Head, Mahlow, and Müller (2016) argued that while fossil vertebrae of the "*aspis* complex" of Szyndlar and Rage (1999) can be assigned to that lineage, other viperine vertebrae would be difficult to assign to any particular group of viperines, or even to distinguish from crotaline remains. They therefore suggested that this calibration point can only be used to date the divergence of viperines and crotalines. However, if the "*aspis* complex" fossils of Szyndlar and Rage (1999) can indeed be assigned to the genus *Vipera* based on apomorphies, then it logically follows that they can and should be used to calibrate the divergence of that genus from its sister group, most likely *Daboia* (Alencar et al., 2016; Pyron et al., 2013; Wüster et al., 2008), not the older split between viperines and crotalines. Given the relative scarcity of early Miocene/Oligocene viperid fossils, we prefer a less narrowly constrained upper age limit for this calibration point than suggested by Head, Mahlow, and Müller (2016).

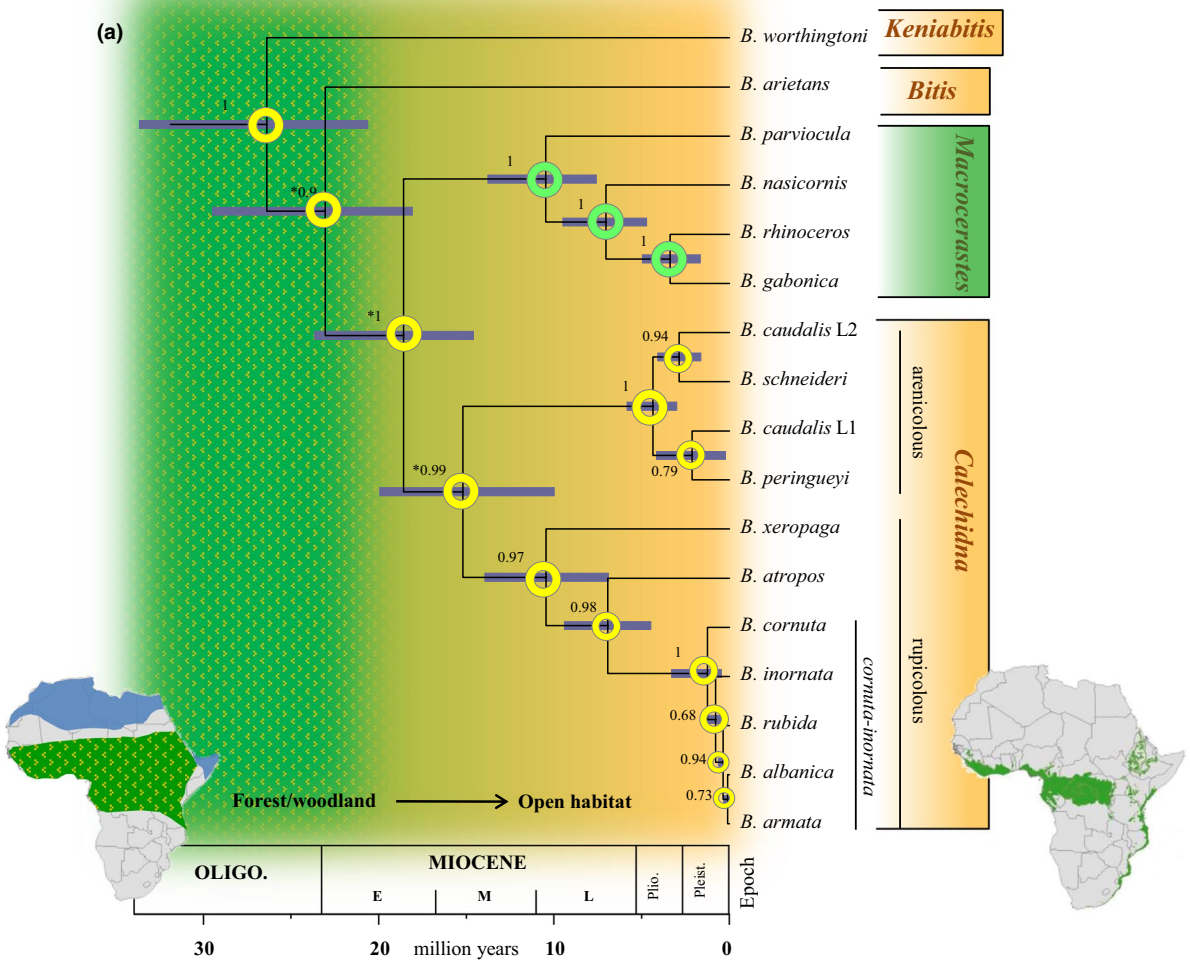
Separate, unlinked nucleotide substitution models were specified for each gene, selected from those available in BEAUTI under the Bayesian information criterion (BIC) in MEGA5 (Tamura et al., 2011). Uncorrelated, lognormal relaxed clock models were specified for each gene. A Yule speciation prior with piecewise linear population size model and constant root was specified for the species tree. The final analysis was carried out on Bioportal (www.bioportal.uio.no), and involved three independent runs of 5×10^8 generations that sampled the Markov chain Monte Carlo every 50,000 generations. The first 10% of samples from each run was removed as burn in. Convergence and adequate sampling (effective sample sizes >200) of all parameters was verified in TRACER 1.5 (Rambaut & Drummond, 2007). The maximum clade credibility tree was selected from the combined posterior sample of 27,000 species trees and annotated with posterior clade probabilities and node heights equal to the median value from the posterior sample using TREEANNOTATOR. We consider posterior probabilities ≥0.90 as providing moderate clade support, and those ≥0.95 as providing strong support.

We also examined the individual gene trees resulting from the *BEAST analysis, which are estimated independently of the species designations used to constrain the species tree. We checked whether current species designations correspond with monophyletic clades in the gene trees, and also looked for the existence of divergent genetic lineages within currently described species that may indicate the presence of monophyletic species complexes.

As the time taken for nuclear markers to reach reciprocal monophyly is expected to exceed that of mitochondrial markers due to an expected fourfold reduction in effective population size of the latter, we also investigated whether currently recognized species possess unique nuclear alleles. The presence of unique alleles provides evidence of lineage isolation because shared alleles are expected to be lost over time due to genetic drift, before reciprocal monophyly has been achieved. Shared alleles, in contrast, could indicate allele sharing between groups due to ongoing gene flow, or alternatively a relatively recent speciation event. The ability to detect shared alleles is governed by sample sizes, which are relatively small for the majority of species studied here. Nuclear allele sharing can thus only be seen as an additional line of evidence for lineage isolation, rather than as providing conclusive support.

As an independent indicator of relationships among subgenera, we included an additional nuclear marker, the anonymous nuclear marker Ba34 (Barlow, Grail, de Bruyn, & Wüster, 2012). Ba34 sequences were not available for all species, precluding their use in the species-level *BEAST analysis. However, all four subgenera, including both sand- and rock-dwelling *Calechidna* clades, are represented by published sequences (Barlow et al., 2012). These were phased (as described previously) and analysed using *BEAST, assigning sequences to one of the five major *Bitis* clades. Relaxed clock models were used for data partitions and the HKY substitution model specified for Ba34. Other aspects of the analysis were as described previously.

Ancestral character state estimation for habitat was carried out using the APE 3 and PHYTOOLS packages in R (Paradis, 2012; Popescu, Huber, & Paradis, 2012; Revell, 2012). Each taxon was


(a)
B. worthingtoni
B. arietans
B. parviocula
B. nasicornis
B. rhinoceros
B. gabonica
B. caudalis L2
B. schneideri
B. caudalis L1
B. peringueyi
B. xeropaga
B. atropos
B. cornuta
B. inornata
B. rubida
B. albanica
B. armata
Keniabitis
Bitis
Macrocerastes
Calechidna
arenicolous
rupicolous
cornuta-inornata
1
*0.9
*1
*0.99
0.94
0.79
0.97
0.98
0.68
0.73
Forest/woodland
Open habitat
OLIGO.
MIOCENE
E
M
L
Plio.
Pleist.
Epoch
30
20
million years
10
0

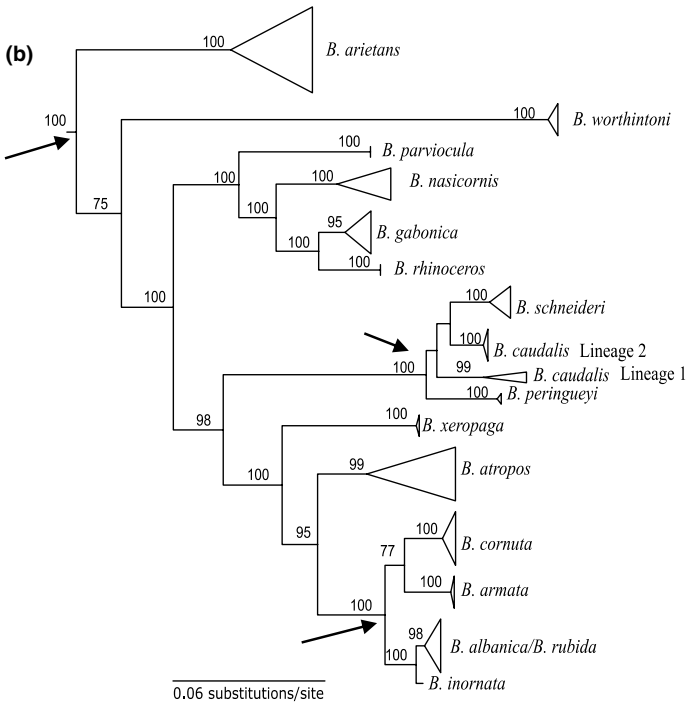

(b)
B. arietans
B. worthintoni
B. parviocula
B. nasicornis
B. gabonica
B. rhinoceros
B. schneideri
B. caudalis Lineage 2
B. caudalis Lineage 1
B. peringueyi
B. xeropaga
B. atropos
B. cornuta
B. armata
B. albanica/B. rubida
B. inornata
0.06 substitutions/site

FIGURE 1 (a) *Bitis* MSC species tree. Nodes are centred on the median age from the posterior sample, and the 95% CIs indicated by the blue bars. Node support values are Bayesian posterior clade probabilities. Support values are from the three locus analysis (those preceded by asterisks were supported in the four locus analysis). The major subgeneric *Bitis* clades are indicated to the right of the figure and are coloured according to habitat preference. The general shift from forest (green) to open (yellow) habitats in the mid-Miocene is indicated, with inset maps showing rough extent of forest/woodland mosaic (stippled green) in the Oligocene and at present (blue indicates areas inundated by sea). The ancestral character states at major nodes are shown by coloured circles. (b) Maximum likelihood bootstrap consensus tree for the concatenated four gene analysis, with terminal tips collapsed for each clade/species. Bootstrap values are given for nodes with >70% support. The topology differs from the species tree at the nodes indicated by arrows. For both figures, outgroup taxa have been removed for clarity but are shown in Supporting Information

coded as occurring in closed (forest/woodland) or open (e.g. open savanna, karroid, grassland, heathland, desert) habitat (Figure 1). Outgroup taxa were included to polarize the analysis, and were coded as belonging to open habitats (this being the dominant habitat across each outgroup genus included; Phelps, 2010). Because five Viperinae genera were missing from our analysis, we must treat the results of this analysis with caution. However, it should be noted that four of these five missing genera occur in open habitats, with only *Atheris* found in forest. A more comprehensive Viperinae phylogeny would be needed to test whether inclusion of *Atheris* and the other genera would change our results. The reconstructions were run with the 'ace' function using the equal states Markovian (Mk) model of character evolution (https://www.r-phylo.org/wiki). The ancestral habitat reconstruction analyses were also run in MESQUITE 3.6 (Maddison & Maddison, 2018) using the same character coding, a likelihood optimization, and the Mk model. Because the ML topology differed from the MSC species tree in the positon of *B. arietans* and *B. worthingtoni*, both of the ancestral habitat analyses were run on the ML tree (pruned to retain one tip per taxon as in Figure 1b) as well as on the MSC species tree.

An ancestral area reconstruction was carried out using a Dispersal-Extinction-Cladogenesis model (DEC; Ree & Smith, 2008) in RASP 4.0 beta (Yu, Harris, Blair, & He, 2015) using the ultrametric MSC species tree generated in *BEAST, and including the six outgroup genera from the Viperinae (*Causus*, *Cerastes*, *Daboia*, *Echis*, *Montivipera*, *Vipera*). The analysis was also run on the ML tree (pruned to retain one tip per taxon as in Figure 1b). The terminal taxa for *Bitis* were coded for the analysis based on their known distributions, whereas the taxa that represented the six Viperinae genera were coded according to the distribution of the entire genus (see Phelps, 2010). The following regions were used for the coding: Eurasia, North Africa (including Saharan), Sudanian, Congolian, Ethiopian, Somalian, Zambezian, Southern following the biogeographical regions from Linder et al. (2012; Figure S2 & Table S2). The DEC analysis allows for both range and dispersal constraints to be defined, so that lineage dispersal can be modelled taking into account timing of divergences and the connectivity between geographical regions (Ree, Moore, Webb, & Donoghue, 2005). Ancestral ranges were constrained to adjoining geographical regions (Table S3). Dispersal probabilities between regions were assigned at four time points (0–2, 2–11, 11–30, 30–47 Ma; Table S4) based on the potential for connectivity between regions. This was guided by present day vegetation and climate of the continent and palaeo-vegetation maps for Africa (Morley, 2007; Kissling et al., 2012; Figure S1).

3 | RESULTS

Both MSC species tree and ML analyses of the concatenated alignment supported the monophyly of *Bitis* and its subdivision into four previously recognized subgeneric clades (Figure 1, Figures S7 & S8). However, these methods supported different relationships between some major clades. The MSC species trees have *Keniabitis* (*Bitis worthingtoni*) sister to all other species of *Bitis* and showed moderate support (0.90 pp) for *B. arietans* as sister to *Calechidna+Macrocerastes*. In contrast, the ML topology for the concatenated alignment shows *B. arietans* (100% bootstrap) as sister to all other species (Figure 1b, Figure S8). The topologies from the ML and MSC analyses for the four-gene dataset also differed slightly for some clades within the *Calechidna* (Figure 1b), although the ML and mitochondrial gene tree generated in the MSC analysis were in agreement for these relationships (Figure 2).

In other respects, topologies from the two methods (MSC and ML) were in agreement, and there were no discrepancies between the unphased (Figure S8) and phased (figure not included) ML topologies. Furthermore, the *BEAST analysis supported monophyly of the four subgeneric clades for each individual gene tree (Figures S9-10), with the exception of *Calechidna*, for which monophyly was not supported in the PRLR and UBN1 trees. The position of *B. arietans* was sister to all other *Bitis* in the PRLR tree, albeit without notable support. The inclusion of sequences of the anonymous nuclear marker Ba34 provided improved resolution of relationships among the major clades (Figure S11), providing strong support for the *Calechidna+Macrocerastes+B. arietans* clade (posterior probability 0.95 compared to 0.90 in the three locus analysis).

Relationships among the four representatives of the subgenus *Macrocerastes* are well resolved in the species and ML trees, with the two Gaboon adders (*B. rhinoceros* and *B. gabonica*) sister to each other. *Bitis nasicornis* forms the sister group to this Gaboon adder clade, with *B. parviocula* in turn sister to this clade (Figure 1). Individual gene trees largely recovered identical relationships and the monophyly of all species was strongly supported with the exception of *B. nasicornis* in the UBN1 tree (Figure S10). All recognized species exhibited unique alleles with the exception of *B. rhinoceros* and *B. gabonica*, which share PRLR alleles (Figure 2b).

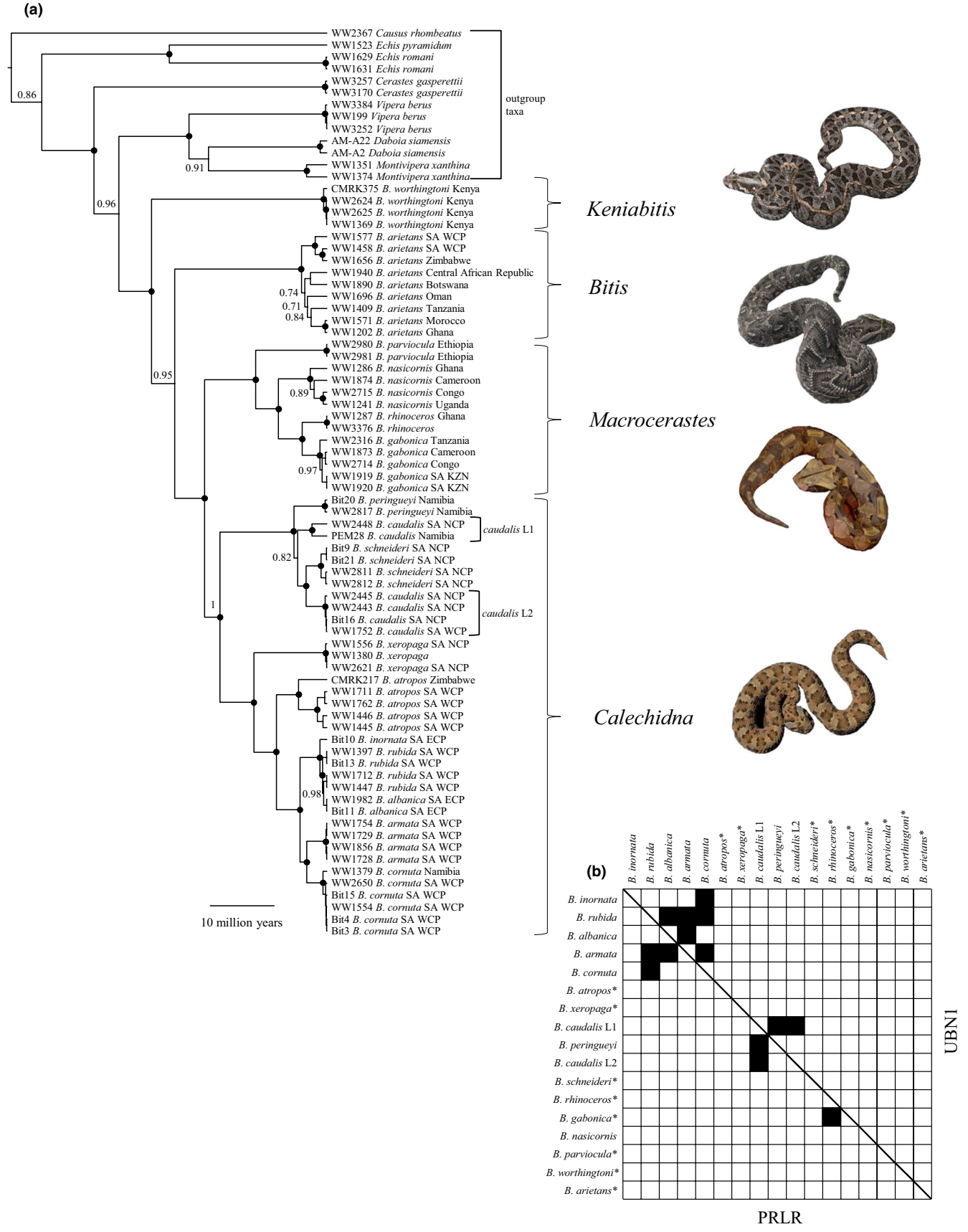

(a)
WW2367 Causus rhombeatus
WW1523 Echis pyramidum
WW1629 Echis romani
WW1631 Echis romani
WW3257 Cerastes gasperettii
WW3170 Cerastes gasperettii
WW3384 Vipera berus
WW199 Vipera berus
WW3252 Vipera berus
AM-A22 Daboia siamensis
AM-A2 Daboia siamensis
WW1351 Montivipera xanthina
WW1374 Montivipera xanthina
outgroup taxa
0.86
0.91
0.96
CMRK375 B. worthingtoni Kenya
WW2624 B. worthingtoni Kenya
WW2625 B. worthingtoni Kenya
WW1369 B. worthingtoni Kenya
Keniabitis
WW1577 B. arietans SA WCP
WW1458 B. arietans SA WCP
WW1656 B. arietans Zimbabwe
WW1940 B. arietans Central African Republic
WW1890 B. arietans Botswana
WW1696 B. arietans Oman
WW1409 B. arietans Tanzania
WW1571 B. arietans Morocco
WW1202 B. arietans Ghana
0.74
0.71
0.84
Bitis
WW2980 B. parviocula Ethiopia
WW2981 B. parviocula Ethiopia
WW1286 B. nasicornis Ghana
WW1874 B. nasicornis Cameroon
WW2715 B. nasicornis Congo
WW1241 B. nasicornis Uganda
WW1287 B. rhinoceros Ghana
WW3376 B. rhinoceros
WW2316 B. gabonica Tanzania
WW1873 B. gabonica Cameroon
WW2714 B. gabonica Congo
WW1919 B. gabonica SA KZN
WW1920 B. gabonica SA KZN
0.95
0.89
0.97
Macrocerastes
Bit20 B. peringueyi Namibia
WW2817 B. peringueyi Namibia
WW2448 B. caudalis SA NCP
PEM28 B. caudalis Namibia
caudalis L1
Bit9 B. schneideri SA NCP
Bit21 B. schneideri SA NCP
WW2811 B. schneideri SA NCP
WW2812 B. schneideri SA NCP
0.82
WW2445 B. caudalis SA NCP
WW2443 B. caudalis SA NCP
Bit16 B. caudalis SA NCP
WW1752 B. caudalis SA WCP
caudalis L2
1
WW1556 B. xeropaga SA NCP
WW1380 B. xeropaga
WW2621 B. xeropaga SA NCP
CMRK217 B. atropos Zimbabwe
WW1711 B. atropos SA WCP
WW1762 B. atropos SA WCP
WW1446 B. atropos SA WCP
WW1445 B. atropos SA WCP
Bit10 B. inornata SA ECP
WW1397 B. rubida SA WCP
Bit13 B. rubida SA WCP
WW1712 B. rubida SA WCP
WW1447 B. rubida SA WCP
WW1982 B. albanica SA ECP
Bit11 B. albanica SA ECP
0.98
WW1754 B. armata SA WCP
WW1729 B. armata SA WCP
WW1856 B. armata SA WCP
WW1728 B. armata SA WCP
WW1379 B. cornuta Namibia
WW2650 B. cornuta SA WCP
Bit15 B. cornuta SA WCP
WW1554 B. cornuta SA WCP
Bit4 B. cornuta SA WCP
Bit3 B. cornuta SA WCP
Calechidna
10 million years
(b)
B. inornata
B. rubida
B. albanica
B. armata
B. cornuta
B. atropos*
B. xeropaga*
B. caudalis L1
B. peringueyi
B. caudalis L2
B. schneideri*
B. rhinoceros*
B. gabonica*
B. nasicornis*
B. parviocula*
B. worthingtoni*
B. arietans*
UBN1
PRLR

FIGURE 2 (a) Mitochondrial gene tree estimated in the three-locus multispecies coalescent (MSC) analysis for *Bitis*. Filled circles at nodes indicate Bayesian clade support of 1.0, whereas values <1.0 are given numerically. (b) Matrix of *Bitis* species showing instances of shared alleles (filled squares) for the nuclear prolactin receptor (PRLR) (below the diagonal) and UBN1 (above the diagonal) genes. Asterisks indicate species for which monophyly was supported by posterior probabilities ≥0.9 in the nuclear gene trees estimated in the three-locus MSC analysis for PRLR (vertical list, see Figure S4 in Supporting Information) and UBN1 (horizontal list, refer to Figure S5 in Supporting Information)

Species tree and ML analyses supported the subdivision of *Calechidna* into two clades corresponding to the rupicolous and arenicolous dwarf adders. Most members in the rupicolous clade are within a recent radiation (Figure 1; *B. albanica*, *B. armata*, *B. cornuta*, *B. inornata* and *B. rubida*). *Bitis rubida* is paraphyletic with respect to *B. albanica* in the mitochondrial gene tree, and the occurrence of shared nuclear alleles is widespread among these five taxa (Figure 2b). Monophyly of the remaining species within the rupicolous clade was supported across all gene trees. Notably a single *B. atropos* individual from Zimbabwe is divergent from South African individuals in the mitochondrial and UBN1 gene trees and also possesses unique alleles for both nuclear markers (Figure 2b, Figure S10).

Within the arenicolous *Calechidna* clade, the monophyly of *B. schneideri* was strongly supported across all analyses and it does not share any nuclear alleles with other species (Figure 2b). The monophyly of *B. caudalis* was not supported in any of the analyses. Furthermore, the two polyphyletic mitochondrial lineages (*B. caudalis* L1 and L2) also failed to form a monophyletic group in the species and ML trees, with an alternative sister species relationship between *B. caudalis* L2 and *B. schneideri* being moderately supported (Figure 1). This relationship was fully supported in the mitochondrial tree, with no nuclear allele sharing (Figure 2). Further examination of the posterior sample of species trees showed that *B. caudalis* was paraphyletic in 98.9% of the posterior sample. The monophyly of *B. peringueyi* was supported in the mitochondrial and the ML trees, and this species shares nuclear alleles with *B. caudalis* L1 (Figure 2b).

The dating analysis using a single Eurasian viperine fossil calibration provided a median estimated age for the basal divergence of *Bitis*, and the origin of the *Keniabitis* lineage, of 26.4 Ma (95% credibility interval [CI] 20.7–33.7 Ma). Divergence of the *B. arietans* lineage occurred 23.5 Ma (95% CI 18.1–29.5 Ma), and the *Macrocerastes* and *Calechidna* lineages separated 18.9 Ma (95% CI 14.6–23.7 Ma). The two *Calechidna* clades are estimated to have diverged 15.2 Ma (95% CI 10.0–20.0 Ma). Ancestors of the extant species within *Macrocerastes* and *Calechidna* are estimated to have arisen within approximately the last 10.5 Ma, with the most recent speciation events occurring in the *cornuta-inornata* (rupicolous) complex, which radiated within approximately the last 0.1–1.3 Ma.

The ancestral habitat state for the genus is unambiguously open habitat for both the APE and MESQUITE analyses. In addition, the estimated marginal ancestral states at each node were unequivocal with all proportional likelihood values >0.98 (Figure 1a, Figure S12). There is a single transition to forest in the *Macrocerastes* clade, with no transitions out of that habitat. The ancestral habitat reconstructions based on the ML topology produced essentially the same support values (>0.98) for character states at each node (results not shown).

The ancestral area reconstruction with the DEC analysis suggests that *Bitis* originated in the Zambezian and Somalian/Ethiopian biogeographical regions (Figure 3, Table S5). The divergence of *B. arietans* likely occurred in the Zambezian and Southern regions, with the divergence and diversification of the *Calechidna* clade accompanied by a transition into the Southern biogeographical region. The ML topology differed from the species tree at the deepest node (placement of *B. arietans* and *B. worthingtoni*), resulting in the geographical origin of *Bitis* estimated as the Southern region with subsequent northward transition to the Zambezian region, followed later by a return transition to the Southern region (Figure S13, Table S5). None of the analyses suggested a North African nor a Eurasian origin.

4 | DISCUSSION

In Africa, groups that have undergone habitat-dependent evolution should show phylogenetic signatures that match the expansion of open habitats starting in the late Oligocene and the particularly notable habitat shifts in the Miocene. Our results show that the genus *Bitis* diverged from sister clades in the early Oligocene, and this does not seem to be in response to the reduction in forest/woodland, given that most other African viper genera are also found in open habitat. Consistent with this, our analysis shows the ancestral state for *Bitis* as open habitat. Therefore, habitat-dependent evolution does not seem to be the initial driver of diversification within the African viperines, nor did it initiate the divergence of *Bitis* from other viperines. The majority of species level diversification within *Bitis* began in the late Miocene, with noteworthy divergence events occurring more recently for the species in hyper-arid regions. We found four well-supported clades that correspond to the currently recognized subgenera, and our phylogeny shows at least one cryptic taxon within *B. caudalis* and possibly *B. atropos*.

4.1 | Is the evolution of *Bitis* habitat dependent?

We hypothesized that open habitat *Bitis* lineages (subgenera: *Calechidna*, *Keniabitis* and *Bitis*) diverged either in response to the initial but gradual aridification of Africa (Eocene/Oligocene), or later during the rapid mid-Miocene expansion of open habitats. The ancestral state for the genus is an open habitat at the basal node (median estimated age 26.2 Ma, 95% CI 20.6–33.7 Ma), with one shift to forest by *Macrocerastes* in the mid-Miocene. Given that the ancestral state is open habitat, the origin of *Bitis* does not appear to

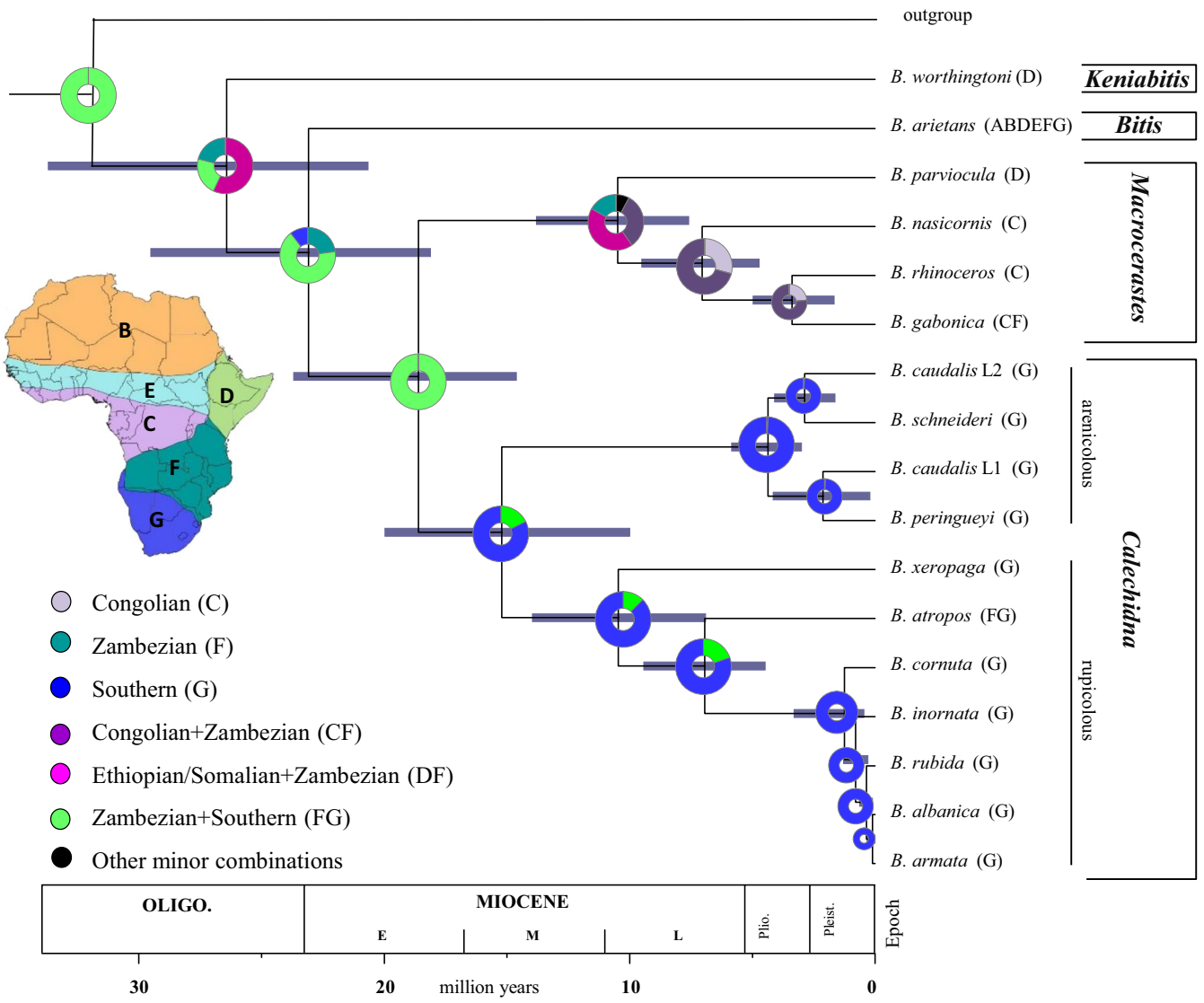


FIGURE 3 Ancestral area reconstruction for *Bitis*. Proportional likelihood values are shown for each node by coloured doughnut charts (colour codes match key). Area coding for each taxon/tip is indicated: A-Eurasia, B-North Africa, C-Congolian, D-Ethiopian/Somalian, E-Sudanian, F-Zambezian, G-Southern and corresponds to the map of biogeographical regions for Africa (inset)

be a case of habitat-dependent evolution in response to a shift from closed to open habitats, because the genus emerged at a time when open habitats already existed. Indeed, it is likely that closed or dense canopy forest and woodland formed a mosaic with open habitats (Linder, 2017) providing ample opportunity for diversification into open vegetation. The "forest-living" ancestral condition for the entire subfamily is itself questionable, as most other viperine lineages except *Atheris* and some *Causus* inhabit primarily open formations. It is highly likely then, that Viperinae evolved in an open habitat setting in the Oligocene, with multiple shifts into forest by certain lineages (i.e. *Atheris* and subgenus *Macrocerastes*).

Although the origin of *Bitis* in the Oligocene is inconsistent with habitat-dependent evolution, within the genus there are indications of habitat-dependent diversification. Vicariance initiated by the fragmentation of forest during the late-Miocene and Pliocene may have contributed to cladogenesis within the forest-dwelling *Macrocerastes*. Furthermore, the mid-Miocene divergence of the *Calechidna* clade coincides with the intensification of the Benguela oceanic current and associated development of the arid conditions in the west, including establishment of the Namib Desert (Scott, Anderson, & Anderson, 1997; Udeze & Oboh-Ikuenobe, 2005). All four arenicolous *Calechidna* lineages occur in the west, suggesting they shifted to the arid niche as it became available. Diversification within *Calechidna* is more recent, within the last *c.* 5 Ma. This corresponds well to the late Miocene/Pliocene shift from moist woodland and forest to the present day arid open habitat conditions in Namaqualand and the Karoo (Scott et al., 1997; see Figure S14 for these localities). It is likely that an arid-living ancestral clade from the Namib region (*B. peringueyi* and *B. caudalis* L1) diversified and shifted to the more southern central Karoo (*B. caudalis* L2) and west coast Namaqualand (*B. schneideri*) as habitat became more xeric. However, throughout the Pleistocene the climate varied widely due

to glacial cycling. Indeed, the central Karoo is considered to have high "climate velocity", whereby the biome has shifted in position and extent during the Pleistocene (Tolley, Bowie, Price, Measey, & Forest, 2014). The current biomes have apparently been relatively stable in extent through the Holocene (Scott et al., 1997). Although the region has been climatically dynamic, there has been a long-term aridification trend which has undoubtedly influenced cladogenesis within the *Calechidna*. The formation of the arid west and Namib Desert has also been linked to evolutionary diversification in lizards (Edwards, Herrel, Vanhooydonck, Measey, & Tolley, 2016; Lamb & Bauer, 2003, 2006; Makokha, Bauer, Mayer, & Matthee, 2007), and this extreme environment certainly must have played a role in speciation and adaptation of arid-living fauna.

In addition to habitat factors, divergence timings within *Bitis* also correspond with geological events. Specifically, the divergence of *B. parviocula* from its sister clade coincides with the extension of the Main Ethiopian Rift which began around 11 Ma (postdating the initial rifting of the Red Sea/Gulf of Aden in the late Oligocene; Wolfenden, Ebinger, Yirgu, Deino, & Ayalew, 2004). Considering the limited distribution of *B. parviocula* along the Ethiopian Rift, this result strongly suggests a causal role for these geological processes in the origin of this species, as has been suggested for other East African squamate lineages (Matthee, Tilbury, & Townsend, 2004; Tolley et al., 2011; Wüster et al., 2007). It should be noted that genetic data for the newly described *B. harenna* is still lacking, but is essential to test this hypothesis. In contrast, however, *B. worthingtoni* currently has a limited distribution along the Kenyan Rift Valley but divergence from its sister clade considerably pre-dates the onset of rift formation and volcanism in Kenya, 16–20 Ma (Chorowicz, 2005), suggesting that these geological events were not involved in the divergence of this taxon.

We acknowledge that our dating analysis was calibrated using a single Eurasian viper fossil, so our interpretations regarding timing of events should be treated with some caution. However, other molecular phylogenies that include vipers also place the divergence of *Bitis* from other vipers within the Oligocene (ranging between 35–40 Ma; Alencar et al., 2016; Wüster et al., 2008), corresponding with our own analysis that suggests a divergence around 31.9 Ma (95% CI 26–40 Ma). Inclusion of additional calibration points may refine the diversification dates within *Bitis*, but it is unlikely that the dating would shift so substantially as to alter our main interpretations.

The geographical origin of *Bitis* unfortunately remains elusive, in part due to the differing topologies for the species tree and the ML tree at the deepest node. The species tree analysis showed a Zambezian+Ethiopian/Somalian ancestral area, whereas the ML topology suggests a southern African origin. The analysis would likely be improved with the addition of missing genera (*Atheris*, *Eristicophis*, *Macrovipera*, *Montatheris*, *Proatheris*, *Pseudocerastes*) and species (*B. heraldica*, *B. harenna*). The Zambezian and North African regions experienced substantial reduction in forest (opening of habitat) during the Oligocene (Morley, 2007). Both analyses are in agreement that the genus did not originate in North Africa, but rather in the south/eastern region of the continent, with the Zambezian region playing an important role. Therefore, we suggest that the opening of habitat in the Zambezian region initiated the diversification of this genus. It also appears that the common ancestor for the crown groups occurred in the Zambezian region (*c.* 20–25 Ma), and then split into a southern African clade (*Calechidna*) and a more widespread clade centred in the eastern-central portion of the continent (*Macrocerastes*).

4.2 | Phylogeny and systematics of *Bitis*

Our results provide new information on the phylogeny and systematics of *Bitis*. A key question which has remained equivocal despite numerous phylogenetic studies is relationships among the *Bitis* subgenera, specifically the relative positions of *Keniabitis* and the *B. arietans* lineage (Alencar et al., 2016; Lenk et al., 1999; Wüster et al., 2008). Through MSC analysis of mitochondrial and three nuclear loci we were able to resolve this relationship with high posterior support, placing *B. arietans* as sister to *Macrocerastes* and *Calechidna*, with *Keniabitis* in turn sister to this clade. Achieving this robust phylogenetic hypothesis for *Bitis* subgenera will benefit future studies on the evolution and diversification of this group.

Furthermore, we suggest that current taxonomy may not fully capture species diversity within the subgenus *Calechidna*. The four samples of *B. caudalis* analysed comprise two divergent and polyphyletic mitochondrial lineages. Multispecies coalescent analysis of these lineages suggests that *B. caudalis* L2 and *B. schneideri* (both from southwestern South Africa) share a recent common ancestry, whereas *B. caudalis* L1 and *B. peringueyi* (both from western Namibia) (Figure S15) share a recent common ancestry. The ML analysis, however, differed for these relationships although each of these clades was still supported as distinct. *Bitis caudalis* is widespread across south-western Africa, occurring from southern Angola southwards to the Western Cape Province of South Africa, and eastwards to southern Zimbabwe. Because our sampling was limited, we cannot make firm conclusions regarding these relationships. Indeed, a comprehensive phylogeographical analysis of this widespread taxon is a priority for future studies on *Bitis*, particularly as the two analyses showed slightly different relationships between the clades.

Further indication of potentially cryptic species diversity was found among *B. atropos* populations. Specifically, the Zimbabwean *B. atropos* possessed unique alleles for two nuclear markers (Figure 2b), and exhibited significant levels of mitochondrial divergence from conspecific samples (all from the Western Cape, South Africa), comparable with divergences of other interspecific rather than intraspecific relationships within *Calechidna* (Figure 2a). *Bitis atropos* has a fragmented distribution with populations occurring along the Cape Fold Mountains in the Western and Eastern Cape Provinces of South Africa, and additional allopatric populations in the KwaZulu-Natal and Mpumalanga provinces of South Africa, and in Zimbabwe. It was hypothesized that these isolated populations represent a assemblage of sibling species (Branch, 1999). It was later shown that the *B. atropos* "complex" comprises a suite of cryptic species that apparently originated in stepwise fashion from north to south, associated with isolation of montane grassland habitats of the Great Escarpment (Kelly, Branch, Villet, & Barker, 2011). Together with our

results, this highlights *B. atropos* as an important focus for future research efforts.

The *cornuta-inornata* complex comprises five morphologically and ecologically differentiated species (Branch, 1999), which our molecular dating analysis shows to have radiated much more recently than other *Bitis* clades (within the last *c*. 1.2 Ma). Analysis of mitochondrial sequences and the ML analysis recovered *B. albanica* and *B. rubida* as polyphyletic, and these together showed little differentiation from *B. inornata*. Sharing of nuclear alleles was also evident among these three taxa as well as among the other species in the complex, *B. armata* and *B. cornuta*. These genetic patterns are consistent with a recent radiation of these species, and any taxonomic interpretations based on our limited sampling would be premature. The relationships between these taxa might become better understood with denser sampling of individuals and additional genetic loci.

Above the species-level, previous discussions of *Bitis* systematics have considered their higher level taxonomy, specifically whether the four subgeneric clades may warrant elevation to genus level (Herrmann & Joger, 1997; Lenk et al., 1999). Changes in nomenclature are justified in cases where current taxonomy does not adequately portray evolutionary relationships, but this must be balanced against the potential negative impacts of taxonomic changes on the wider scientific community. Given the strong support for monophyly of the genus *Bitis* as currently defined, we share the view of Wüster et al. (2008) that splitting of this historically stable group would only serve to confuse the nomenclature and hinder information retrieval without significantly enhancing our understanding of the evolutionary history of the genus. The continued recognition of the *Bitis* subgenera, however, does provide an effective way of highlighting the major evolutionary and ecological divisions within the genus whilst avoiding any potentially negative effects of generic reassignment. Overall, this results in a more information-rich classification (Wallach, Wüster, & Broadley, 2009).

5 | CONCLUSION

Our analysis was limited to a dichotomy of open/closed habitats, yet the vegetation of Africa was surely more complex through space and time. Therefore, we are limited to interpretations relating only to broad scale patterns; yet diversification within *Bitis*, and indeed within viperines, could easily have been driven by nuances rather than the generalities that are characterize our study. Until such time that the complexities of African palaeo-vegetation are revealed, broad patterns over large time scales will characterise our best knowledge. Overall, we show that the diversification of *Bitis* likely began in open habitats in the late Oligocene/early Miocene, prior to the major expansion of such habitats in the mid-Miocene. This contrasts strongly with open habitat mammalian lineages which are shown by the fossil record to have diversified much later, following the expansion of C_4 grasslands in the late Pliocene and Pleistocene (Bobe & Behrensmeyer, 2004; Bobe et al., 2002; Vrba, 1992; Wesselman, 1985). Overall, our results highlight the need for taxonomic breadth in achieving a holistic understanding of faunal evolution in Africa, as well as for fine-scale analyses that aim to incorporate subtleties of vegetation and climatic dynamics.

ACKNOWLEDGEMENTS

This work was funded by a Natural Environment Research Council studentship and Systematics Research Fund small grant to A.B. For samples and for helping with sampling, we thank Mohammed Almutairi, Joe Beraducci, Donald G. Broadley, Marco Buegel, Andre Coetzer, Albert Coritz, Mark Craddock, Yvan Doljanski, Johannes Els, Robert A. Harrison, Kate Jackson, Niels Jacobson, Moshe Kahn, Thea Litschka-Koen, Anita Malhotra, Bryan Maritz, Tomáš Mazuch, Martti Niskanen, Arno Naude, Heidi Pfeifer, Franck Principaud, Paul Rowley, Wolfgang Schneyer, Zoran Tadić, Zoltan Takacs, David Theakston, Harold van der Ploeg, Mark Vandewalle, Craig van Rensburg, Jon Warner and Cathy Wüster.

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REFERENCES

Alencar, L. R. V., Quental, T. B., Grazziotin, F. G., Alfaro, M. L., Martins, M., Venzon, M., & Zaher, H. (2016). Diversification in vipers: Phylogenetic relationships, time of divergence and shifts in speciation rates. *Molecular Phylogenetics and Evolution*, *105*, 50–62. https://doi.org/10.1016/j.ympev.2016.07.029

Ashe, J. S., & Marx, H. (1988). Phylogeny of the viperine snakes (Viperidae): part II. Cladistic analysis and major lineages. *Fieldiana Zoology*, *52*, 1–23.

Ashton, K. G., & de Queiroz, A. (2001). Molecular systematics of the western rattlesnake, *Crotalus viridis* (Viperidae), with comments on the utility of the D-loop in phylogenetic studies of snakes. *Molecular Phylogenetics and Evolution*, *21*, 176–189. https://doi.org/10.1006/mpev.2001.1013

Barej, M. F., Penner, J., Schmitz, A., & Rödel, M. (2015). Multiple genetic lineages challenge the monospecific status of the West African endemic frog family Odontobatrachidae. *BMC Evolutionary Biology*, *15*, 6.

Barlow, A., Baker, K., Hendry, C. R., Peppin, L., Phelps, T., Tolley, K. A., … Wüster, W. (2013). Phylogeography of the widespread African puff adder (*Bitis arietans*) reveals multiple Pleistocene refugia in southern Africa. *Molecular Ecology*, *22*, 1134–1157. https://doi.org/10.1111/mec.12157

Barlow, A., Grail, W., de Bruyn, M., & Wüster, W. (2012). Anonymous nuclear markers for the African adders (Serpentes: Viperidae: Bitis). *Conservation Genetics Resources*, *4*, 967–969.

Bittencourt-Silva, G. B., Conradie, W., Siu-Ting, K., Tolley, K. A., Channing, A., Cunningham, M., & Loader, S. P. (2016). The phylogenetic position and diversity of the enigmatic mongrel frog *Nothophryne* Poynton, 1963 (Amphibia, Anura). *Molecular Phylogenetics and Evolution*, *99*, 89–102. https://doi.org/10.1016/j.ympev.2016.03.021

Bobe, R., & Behrensmeyer, A. K. (2004). The expansion of grassland ecosystems in Africa in relation to mammalian evolution and the origin of the genus *Homo*. *Palaeogeography, Palaeoclimatology and Palaeoecology*, *207*, 399–420. https://doi.org/10.1016/j.palaeo.2003.09.033

Bobe, R., Behrensmeyer, K., & Chapman, R. E. (2002). Faunal change, environmental variability and late Pliocene hominin evolution. *Journal of Human Evolution*, *42*, 475–497. https://doi.org/10.1006/jhev.2001.0535

Bowie, R. C. K., & Fjeldså, J. (2008). New perspectives on the origin and diversification of Africa's forest avifauna. *African Journal of Ecology*, *46*, 235–247.

Bowie, R. C. K., Fjeldsa, J., Hackett, S. J., Bates, J. M., & Crowe, T. M. (2006). Coalescent models reveal the relative roles of ancestral polymorphism, vicariance, and dispersal in shaping phylogeographical structure of an African montane forest robin. *Molecular Phylogenetics and Evolution*, *38*, 171–188. https://doi.org/10.1016/j.ympev.2005.06.001

Branch, W. R. (1999). Dwarf adders of the *Bitis cornuta-inornata* complex (Serpentes: Viperidae) in southern Africa. *Kaupia*, *8*, 39–63.

Chorowicz, J. (2005). The East African rift system. *Journal of African Earth Sciences*, *43*, 379–410. https://doi.org/10.1016/j.jafrearsci.2005.07.019

Coetzee, J. A. (1993). African flora since the terminal Jurassic. In P. Goldblatt (Ed.), *Biological relationships between Africa and South America* (pp. 37–61). New Haven, CT: Yale University Press. https://doi.org/10.2307/j.ctt22726mc

Couvreur, T. L., Chatrou, L. W., Sosef, M. S., & Richardson, J. E. (2008). Molecular phylogenetics reveal multiple tertiary vicariance origins of the African rain forest trees. *BMC Biology*, *6*, 54.

da Silva, J. M., Herrel, A., Measey, G. J., Vanhooydonck, B., & Tolley, K. A. (2014). Linking microhabitat structure, morphology and locomotor performance traits in a recent radiation of dwarf chameleons. *Functional Ecology*, *28*, 702–713. https://doi.org/10.1111/1365-2435.12210

da Silva, J. M., & Tolley, K. A. (2017). Diversification through ecological opportunity in dwarf chameleons. *Journal of Biogeography*, *44*, 834–847. https://doi.org/10.1111/jbi.12966

deMenocal, P. B. (1995). Plio-Pleistocene African climate. *Science*, *270*, 53–59. https://doi.org/10.1126/science.270.5233.53

deMenocal, P. B. (2004). African climate change and faunal evolution during the Pliocene-Pleistocene. *Earth and Planetary Science Letters*, *220*, 3–24. https://doi.org/10.1016/S0012-821X(04)00003-2

Demos, T. C., Kerbis Peterhans, J. C., Agwanda, B., & Hickerson, M. J. (2014). Uncovering cryptic diversity and refugial persistence among small mammal lineages across the Eastern Afromontane biodiversity hotspot. *Molecular Phylogenetics and Evolution*, *71*, 41–54. https://doi.org/10.1016/j.ympev.2013.10.014

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, *29*, 1969–1973. https://doi.org/10.1093/molbev/mss075

Edwards, S., Herrel, A., Vanhooydonck, B., Measey, G. J., & Tolley, K. A. (2016). Diving in head first: Morphology and performance is linked to predator escape strategy in desert lizards (*Meroles*, Lacertidae, Squamata). *Biological Journal of the Linnean Society*, *119*(4), 919–931. https://doi.org/10.1111/bij.12856

Edwards, E. J., Osborne, C. P., Strömberg, C. A. E., & Smith, S. A. & C4 Grasses Consortium (2010). The origins of C4 grasslands: Integrating evolutionary and ecosystem science. *Science*, *328*, 587–591. https://doi.org/10.1126/science.1177216

Edwards, S., Vanhooydonck, B., Herrel, A., Measey, G. J., & Tolley, K. A. (2012). Convergent evolution associated with habitat decouples phenotype from phylogeny in a clade of lizards. *PLoS ONE*, *7*, e51636. https://doi.org/10.1371/journal.pone.0051636

Ewing, B., & Green, P. (1998). Base-calling of automated sequencer traces using Phred. II. Error probabilities. *Genome Research*, *8*, 186–194. https://doi.org/10.1101/gr.8.3.186

Fuchs, J., Parra, J. L., Goodman, S. M., Raherilalao, M. J., Vanderwal, J., & Bowie, R. C. K. (2013). Extending ecological niche models to the past 120 000 years corroborates the lack of strong phylogeographic structure in the Crested Drongo (*Dicrurus forficatus forficatus*) on Madagascar. *Biological Journal of the Linnean Society*, *108*, 658–676. https://doi.org/10.1111/j.1095-8312.2012.02022.x

Gower, D. J., Wade, E. O. Z., Spawls, S., Böhme, W., Buechley, E. R., Sykes, D., & Colston, T. J. (2016). A new large species of *Bitis* Gray, 1842 (Serpentes: Viperidae) from the Bale Mountains of Ethiopia. *Zootaxa*, *4093*, 41–63. https://doi.org/10.11646/zootaxa.4093.1.3

Groombridge, B. C. (1980). A phyletic analysis of viperine snakes. PhD thesis, City of London Polytechnic, London.

Head, J. J., Mahlow, K., & Müller, J. (2016). Fossil calibration dates for molecular phylogenetic analysis of snakes 2: Caenophidia, Colubroidea, Elapoidea, Colubridae. *Palaeontologia Electronica*, *19*, 1–21.

Heled, J., & Drummond, A. J. (2010). Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, *27*, 570–580. https://doi.org/10.1093/molbev/msp274

Herrmann, H.-W., & Joger, U. (1995). Molecular data on the phylogeny of African viperines—preliminary results. *Scientia Herpetologica*, *1995*, 92–97.

Herrmann, H.-W., & Joger, U. (1997). Evolution of viperine snakes. In R. S. Thorpe, W. Wüster, & A. Malhotra (Eds.), *Venomous snakes: Ecology, evolution and snakebite* (pp. 43–61). Symposia of the Zoological Society of London, No. 70. Oxford, UK: Clarendon Press.

Herrmann, H.-W., Joger, U., Lenk, P., & Wink, M. (1999). Morphological and molecular phylogenies of viperines: Conflicting evidence? *Kaupia*, *8*, 21–30.

Hewitt, G. M. (2004). The structure of biodiversity – insights from molecular phylogeography. *Frontiers in Zoology*, *1*(1), 4. https://doi.org/10.1186/1742-9994-1-4

Kelly, C. M. R., Branch, W. R., Villet, M. H., & Barker, N. P. (2011). Adders and afromontane phylogeography: The Great Escarpment and Cape Fold Mountains as a speciation hot-bed for dwarf *Bitis* (Serpentes: Viperidae). In M. H. Villet & K. L. Crous (Eds.), *Proceedings of the Congresses of the Southern African Society for Systematic Biology* (pp. 52). Southern African Society for Systematic Biology, Grahamstown 19–21 January 2011.

Kissling, W. D., Eiserhardt, W. L., Baker, W. J., Borchsenius, F., Couvreur, T. L. P., Balslev, H., & Svenning, J.-C. (2012). Cenozoic imprints on the phylogenetic structure of palm species assemblages worldwide. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 7379–7384. https://doi.org/10.1073/pnas.1120467109

Kumazawa, Y., Ota, H., Nishida, M., & Ozawa, T. (1996). Gene rearrangements in snake mitochondrial genomes: Highly concerted evolution of control-region-like sequences duplicated and inserted into a tRNA gene cluster. *Molecular Biology and Evolution*, *13*, 1242–1254. https://doi.org/10.1093/oxfordjournals.molbev.a025690

Lamb, T., & Bauer, A. M. (2003). *Meroles* revisited: Complementary systematic inference from additional mitochondrial genes and complete taxon sampling of southern Africa's desert lizards. *Molecular Phylogenetics and Evolution*, *29*, 360–364. https://doi.org/10.1016/S1055-7903(03)00137-4

Lamb, T., & Bauer, A. M. (2006). Footprints in the sand: Independent reduction of subdigital lamellae in the Namib-Kalahari burrowing geckos. *Proceedings of the Royal Society of London, Series B.*, *273*, 855–864. https://doi.org/10.1098/rspb.2005.3390

Lawson, L. P. (2010). The discordance of diversification: Evolution in the tropical-montane frogs of the Eastern Arc Mountains of Tanzania. *Molecular Ecology*, *19*, 4046–4060. https://doi.org/10.1111/j.1365-294X.2010.04788.x

Lenk, P., Herrmann, H.-W., Joger, U., & Wink, M. (1999). Phylogeny and taxonomic subdivision of *Bitis* (Reptilia: Viperidae) based on molecular evidence. *Kaupia*, *8*, 31–38.

Lenk, P., Kalayabina, S., Wink, M., & Joger, U. (2001). Evolutionary relationships among the true vipers (Reptilia: Viperidae) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, *19*, 94–104. https://doi.org/10.1006/mpev.2001.0912

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Linder, H. P. (2017). East African Cenozoic vegetation history. *Evolutionary Anthropology: Issues, News, and Reviews*, *26*(6), 300–312. https://doi.org/10.1002/evan.21570

Linder, H. P., de Klerk, H. M., Born, J., Burgess, N. D., Fjeldså, J., & Rahbek, C. (2012). The partitioning of Africa: Statistically defined biogeographical regions in sub-Saharan Africa. *Journal of Biogeography*, *39*(7), 1189–1205. https://doi.org/10.1111/j.1365-2699.2012.02728.x

Maddison, W. P., & Maddison, D. R. (2018). Mesquite: A modular system for evolutionary analysis. Version 3.40. Retrieved from http://mesquiteproject.org.

Makokha, J. S., Bauer, A. M., Mayer, W., & Matthee, C. A. (2007). Nuclear and mtDNA-based phylogeny of southern African sand lizards, *Pedioplanis* (Sauria: Lacertidae). *Molecular Phylogenetics and Evolution*, *44*, 622–633. https://doi.org/10.1016/j.ympev.2007.04.021

Maslin, M. A., Brierley, C. M., Milner, A. M., Shultz, S., Trauth, M. H., & Wilson, K. E. (2014). East African climate pulses and early human evolution. *Quaternary Science Review*, *101*, 1–17. https://doi.org/10.1016/j.quascirev.2014.06.012

Matthee, C. A., Tilbury, C. R., & Townsend, T. (2004). A phylogenetic review of the African leaf chameleons: Genus *Rhampholeon* (Chameleonidae): The role of vicariance and climate change in speciation. *Proceedings of the Royal Society of London, Series B*, *271*, 1967–1975. https://doi.org/10.1098/rspb.2004.2806

Measey, G. J., Hopkins, K., & Tolley, K. A. (2009). Morphology, ornaments and performance in two chameleon ecomorphs: Is the casque bigger than the bite? *Zoology*, *112*, 217–226. https://doi.org/10.1016/j.zool.2008.09.005

Measey, G. J., & Tolley, K. A. (2011). Sequential fragmentation of Pleistocene forests in an East Africa biodiversity hotspot: Chameleons as a model to track forest history. *PLoS ONE*, *6*, e26606. https://doi.org/10.1371/journal.pone.0026606

Menegon, M., Loader, S. P., Marsden, S. J., Branch, W. R., Davenport, T. R. B., & Ursenbacher, S. (2014). The genus *Atheris* (Serpentes: Viperidae) in East Africa: Phylogeny and the role of rifting and climate in shaping the current pattern of species diversity. *Molecular Phylogenetics and Evolution*, *79*, 12–22. https://doi.org/10.1016/j.ympev.2014.06.007

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, pp. 1–8. 14 November 2010, New Orleans.

Morley, R. J. (2007). Cretaceous and Tertiary climate change and the past distribution of megathermal rainforests. In J. Flenley & M. Bush (Eds.), *Tropical rainforest responses to climatic change* (1st ed.) (pp. 1–34). Berlin, Heidelberg, Germany: Springer.

Oatley, G., Voelker, G., Crowe, T. M., & Bowie, R. C. K. (2012). A multilocus phylogeny reveals a complex pattern of diversification related to climate and habitat heterogeneity in southern African white-eyes. *Molecular Phylogenetics and Evolution*, *64*, 633–644. https://doi.org/10.1016/j.ympev.2012.05.022

Palumbi, S. R., Martin, A. E., Romano, W. O., Stice, L., & Grabowski, G. (1991). *The simple fool's guide to PCR*. Ver. 2.0. Honolulu, HI: University of Hawaii.

Paradis, E. (2012). *Analysis of phylogenetics and evolution with R* (2nd ed.). New York, NY: Springer. https://doi.org/10.1007/978-1-4614-1743-9

Phelps, T. (2010). *Old world vipers, a natural history of the Azemiopinae and Viperinae*. Frankfurt, Germany: Edition Chimaira.

Pook, C. E., Joger, U., Stümpel, N., & Wüster, W. (2009). When continents collide: Phylogeny, historical biogeography and systematics of the medically important viper genus *Echis* (Squamata: Serpentes: Viperidae). *Molecular Phylogenetics and Evolution*, *53*, 792–807. https://doi.org/10.1016/j.ympev.2009.08.002

Popescu, A.-A., Huber, K. T., & Paradis, E. (2012). APE 3.0: New tools for distance based phylogenetics and evolutionary analysis in R. *Bioinformatics*, *28*, 1536–1537. https://doi.org/10.1093/bioinformatics/bts184

Potts, R. (1998). Environmental hypotheses of hominin evolution. *Yearbook of Physical Anthropology*, *41*, 93–136. https://doi.org/10.1002/(ISSN)1096-8644

Pyron, R. A., Burbrink, F. T., & Wiens, J. J. (2013). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology*, *13*(1), 93. https://doi.org/10.1186/1471-2148-13-93

Rage, J. C. (1973). Fossil snakes from Olduvai, Tanzania. In L. S. B. Leakey, R. J. G. Savage, & S. C. Coryndon (Eds.), *Fossil vertebrates of Africa*, vol. 3 (pp. 1–6). London, UK: Academic Press.

Rambaut, A., & Drummond, A. J. (2007). Tracer v1.5. Retrieved from http://beast.bio.ed.ac.uk/Tracer.

Ree, R. H., Moore, B. R., Webb, C. O., & Donoghue, M. J. (2005). A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution*, *59*, 2299–2311.

Ree, R. H., & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, *57*(1), 4–14. https://doi.org/10.1080/10635150701883881

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, *8*(3), 336–352. https://doi.org/10.1111/j.1461-0248.2004.00715.x

Schluter, D. (2009). Evidence for ecological speciation and its alternative. *Science*, *323*(5915), 737–741. https://doi.org/10.1126/science.1160006

Scott, L., Anderson, H. M., & Anderson, J. M. (1997). Vegetation history. In R. M. Cowling, D. M. Richardson, & S. M. Pierce (Eds.), *Vegetation of Southern Africa* (pp. 62–69). Cambridge, UK: Cambridge University Press.

Šmíd, J., & Tolley, K. A. (2019). Calibrating the tree of vipers under the fossilized birth-death model. Scientific Reports, https://doi.org/10.1038/s41598-019-41290-2.

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*(21), 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, *57*(5), 758–771. https://doi.org/10.1080/10635150802429642

Stephens, M., & Scheet, P. (2005). Accounting for decay of linkage disequilibrium in haplotype inference and missing data imputation. *The American Journal of Human Genetics*, *76*, 449–462. https://doi.org/10.1086/428594

Stephens, M., Smith, N. J., & Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *The American Journal of Human Genetics*, *68*, 978–989. https://doi.org/10.1086/319501

Szyndlar, Z., & Rage, J.-C. (1999). Oldest fossil vipers (Serpentes: Viperidae) from the Old World. *Kaupia*, *8*, 9–20.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony

methods. *Molecular Biology and Evolution*, *28*, 2731–2739. https://doi.org/10.1093/molbev/msr121

Tolley, K. A., Bowie, R. C. K., Price, B. W., Measey, G. J., & Forest, F. (2014). The shifting landscape of genes since the Pliocene: Terrestrial phylogeography in the Greater Cape Floristic Region. In N. Allsopp, J. F. Colville, & T. Verboom (Eds.), *Ecology and evolution of fynbos understanding megadiversity* (pp. 142–163). Oxford, UK: Oxford University Press.

Tolley, K. A., Burger, M., Turner, A. A., & Matthee, C. A. (2006). Biogeographic patterns and phylogeography of dwarf chameleons (*Bradypodion*) in an African biodiversity hotspot. *Molecular Ecology*, *15*, 781–793. https://doi.org/10.1111/j.1365-294X.2006.02836.x

Tolley, K. A., Chase, B. M., & Forest, F. (2008). Speciation and radiations track climate transitions since the Miocene climatic optimum: A case study of southern African chameleons. *Journal of Biogeography*, *35*, 1402–1414. https://doi.org/10.1111/j.1365-2699.2008.01889.x

Tolley, K. A., Tilbury, C. R., Measey, G. J., Menegon, M., Branch, W. R., & Matthee, C. A. (2011). Ancient forest fragmentation or recent radiation? Testing refugial speciation models in chameleons within an African biodiversity hotspot. *Journal of Biogeography*, *38*, 1748–1760. https://doi.org/10.1111/j.1365-2699.2011.02529.x

Tolley, K. A., Townsend, T. M., & Vences, M. (2013). Large-scale phylogeny of chameleons suggests African origins and Eocene diversification. *Proceedings of the Royal Society of London, Series B*, *280*(1759), 20130184. https://doi.org/10.1098/rspb.2013.0184

Townsend, T. M., Alegre, R. E., Kelley, S. T., Wiens, J. J., & Reeder, T. W. (2008). Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: An example from squamate reptiles. *Molecular Phylogenetics and Evolution*, *47*, 129–142. https://doi.org/10.1016/j.ympev.2008.01.008

Trauth, M. H., Maslin, M. A., Deino, A., & Strecker, M. R. (2005). Late Cenozoic moisture history of East Africa. *Science*, *309*, 2051–2053. https://doi.org/10.1126/science.1112964

Udeze, C. H., & Oboh-Ikuenobe, F. E. (2005). Neogene palaeoceanographic and palaeoclimatic events inferred from palynological data: Cape basin of South Africa, OPD Leg 175. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *219*, 199–223. https://doi.org/10.1016/j.palaeo.2004.12.026

Uetz, P., Freed, P., & Hošek, J. (eds.) (2017). The reptile database. Retrieved from http://www.reptile-database.org.

Vrba, E. (1985). Environment and evolution: Alternative causes of the temporal distribution of evolutionary events. *South African Journal of Science*, *81*, 229–236.

Vrba, E. S. (1992). Mammals as a key to evolutionary theory. *Journal of Mammalogy*, *73*, 1–28. https://doi.org/10.2307/1381862

Wallach, V., Wüster, W., & Broadley, D. G. (2009). In praise of subgenera: Taxonomic status of the genus *Naja* Laurenti (Serpentes: Elapidae). *Zootaxa*, *2236*, 26–36.

Wesselman, H. B. (1985). Fossil micromammals as indicators of climatic change about 2.4 Myr ago in the Omo Valley, Ethiopia. *South African Journal of Science*, *81*, 260–261.

Wittenberg, R. D., Jadin, R. C., Fenwick, A. M., & Gutberlet, R. L. (2015). Recovering the evolutionary history of Africa's most diverse viper genus: Morphological and molecular phylogeny of Bitis (Reptilia: Squamata: Viperidae). *Organisms Diversity & Evolution*, *15*, 115–125. https://doi.org/10.1007/s13127-014-0185-3

Wolfenden, E., Ebinger, C., Yirgu, G., Deino, A., & Ayalew, D. (2004). Evolution of the northern Main Ethiopian Rift: Birth of a triple junction. *Earth and Planetary Science Letters*, *224*, 213–228. https://doi.org/10.1016/j.epsl.2004.04.022

Wüster, W., Crookes, S., Ineich, I., Mané, Y., Pook, C. E., Trape, J.-F., & Broadley, D. G. (2007). The phylogeny of cobras inferred from mitochondrial DNA sequences: Evolution of venom spitting and the phylogeography of the African spitting cobras (Serpentes: Elapidae: *Naja nigricollis* complex). *Molecular Phylogenetics and Evolution*, *45*, 437–453. https://doi.org/10.1016/j.ympev.2007.07.021

Wüster, W., Peppin, L., Pook, C. E., & Walker, D. (2008). A nesting of vipers: Phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). *Molecular Phylogenetics and Evolution*, *49*, 445–459. https://doi.org/10.1016/j.ympev.2008.08.019

Yu, Y., Harris, A. J., Blair, C., & He, X. (2015). RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Molecular Phylogenetics and Evolution*, *87*, 46–49. https://doi.org/10.1016/j.ympev.2015.03.008

BIOSKETCHES

Axel Barlow is interested in studying the evolutionary history of populations using DNA sequence data. His work encompasses a range of vertebrate taxa across a variety of geographical regions and temporal scales. He is also interested in the development of new laboratory and analytical approaches that can be applied to evolutionary questions.

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Krystal A. Tolley is interested in understanding the historical processes that generate patterns of diversity in African reptiles using biogeographical and phylogenetic approaches.

Author contributions: AB and WW funded and designed the project. AB and CMRK carried out laboratory work. AB, WW and KAT analysed the data, interpreted the results and wrote the manuscript. All authors contributed to sampling and to the manuscript text.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Barlow A, Wüster W, Kelly CMR, Branch WR, Phelps T, Tolley KA. Ancient habitat shifts and organismal diversification are decoupled in the African viper genus *Bitis* (Serpentes: Viperidae). *J Biogeogr.* 2019;00:1–15. https://doi.org/10.1111/jbi.13578