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Phylogeny and species delimitation in an iconic snake genus: the African mambas (Serpentes: Elapidae: *Dendroaspis*)

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ABSTRACT

The African mambas (*Dendroaspis*) comprise an iconic genus of four large-bodied, highly venomous elapid snakes: the black mamba (*D. polylepis*) from open formations across sub-Saharan Africa is comprised of two allopatric populations, and three species of green mamba (*D. angusticeps*, *D. jamesoni*, and *D. viridis*) from tropical and sub-tropical forests. *Dendroaspis angusticeps* occurs in multiple isolated forest patches, and the presence of cryptic species within *D. angusticeps* has been suggested. The striking coloration of the three green species, a trait unique among elapids, suggests their monophyly to the exclusion of *D. polylepis*. We generated a dated, multilocus phylogeny of the mambas and assessed species boundaries. Species distribution modelling (SDM) was used to assess the past and present potential connectivity between allopatric populations of *D. angusticeps* and *D. polylepis*. The phylogeny suggests that diversification of the crown clade began *c*. 6 Mya and, contrary to previous suggestions, we found no convincing signal of species-level diversification within *D. polylepis* or *D. angusticeps*. The hypothesis of green mamba monophyly was rejected, with *D. angusticeps* being sister to *D. polylepis*. The SDMs suggested that allopatric populations of *D. polylepis* and *D. angusticeps* were historically connected, and that their vicariance is recent.

Keywords: Africa; biogeography; snakes; reptiles; species; taxonomy

INTRODUCTION

Knowledge of geographic distributions and richness of reptiles in Africa has grown steadily, particularly in recent decades (e.g. Uetz *et al.* 2025). In part, this is due to significant efforts in terms of surveys and species discoveries in areas that had received little attention until recently (e.g. Branch *et al.* 2005, Ceríaco *et al.* 2016, 2018, Conradie et al. 2012, 2016a, b, 2021, Portik *et al.* 2016, Pietersen *et al.* 2017, Farooq *et al.* 2022, Lobón-Rovira *et al.* 2022, Telford *et al.* 2022, Tolley *et al.* 2023b). As a result, the recognized species richness of African reptiles continues to climb on a par with other areas of the globe, with over 1600 species described from continental Africa (see: Uetz *et al.* 2025). Despite this, significant gaps in knowledge still exist, even for large-bodied, widespread species. For example, several species of large African cobras (Wüster and Broadley 2007, Trape *et al.* 2009, Ceríaco *et al.* 2017, Wüster *et al.* 2018, Collet and Trape 2020, Major *et al.* 2023), large-bodied water snakes (Chaney *et al.* 2024), a large viper (Gower *et al.* 2016), and large, conspicuous, rear-fanged colubrids (Greenbaum *et al.* 2021) have been described only recently. Furthermore, long-standing taxonomic uncertainty within African rock pythons (*Python sebae* and *P. natalensis*; Broadley 1984, 1999, Spawls *et al.* 2018) has only now been unambiguously resolved (Tolley and Alexander 2025), despite these two species being the largest and most

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recognizable snakes on the continent. Challenges to closing the knowledge gap centre around logistical constraints for accessing remote areas and political instability causing issues of personal safety. We also highlight the constraints on sampling imposed by the different, often intractable, legal requirements for carrying out research across a very wide range of countries (e.g. Alexander *et al.* 2021). As a result, large expanses of the continent remain under-sampled (Tolley *et al.* 2016).

The African mambas (genus *Dendroaspis*) are an iconic group of highly venomous elapid snakes. Despite their notoriety, the composition of the genus has never been critically assessed in a framework that incorporates modern species concepts and analytical approaches. Four species have been conventionally recognized for the last ~80 years (see: FitzSimons 1962, Spawls *et al.* 2018, Chippaux and Jackson 2019): the largely terrestrial black mamba (*D. polylepis*), occurring in the savannas of sub-Saharan Africa; the eastern green mamba (*D. angusticeps*), from the coastal forests along the eastern margin of Africa, extending inland to eastern Zimbabwe and Malawi; the western green mamba (*D. viridis*), from the Upper Guinea forests of West Africa; and Jameson's mamba (*D. jamesoni*), from central African forests. Broadley and Blaylock (2013) recognized the northern populations of *D. angusticeps* as a separate species, *D. intermedius* Günther, 1865, but without stating any reasons. Strong evidence to support its distinction is lacking and the validity of *D. intermedius* has not been commonly accepted (but see: Broadley and Blaylock 2013, Wallach *et al.* 2014, Wilkey and Terrell 2019). *Dendroaspis intermedius* was described from the 'Zambesi River' probably in Mozambique (Günther 1865), and it was suggested that populations from inland areas of Zimbabwe, as well as coastal Mozambique, refer to *D. intermedius* (Broadley and Blaylock 2013). Ceríaco *et al.* (2025) identified historical records of green mambas from the island of São Tomé as being closest to *D. viridis* and suggested that the island population could conceivably represent an additional endemic species. However, in the absence of extant specimens, they refrained from a formal taxonomic decision. At the subspecies-level, the only widely recognized taxon is *Dendroaspis jamesoni kaimosae* Loveridge, 1936 from East Africa (western Kenya, Uganda, eastern Democratic Republic of the Congo, and neighbouring areas). Despite some coloration and scalation differences (Loveridge 1936), there is little genetic differentiation between the subspecies and the nominate form from Central Africa based on mitochondrial genes (Ainsworth *et al.* 2018), and the geographic boundaries between the two are poorly defined.

The three recognized species with green body coloration (*D. angusticeps*, *D. jamesoni*, and *D. viridis*) are allopatric (Spawls *et al.* 2018, Chippaux and Jackson 2019), and although their ranges overlap with that of *D. polylepis*, they are not usually syntopic. *Dendroaspis angusticeps*, *D. jamesoni*, and *D. viridis* are arboreal, occurring in forest habitats. In particular, *D. angusticeps* occurs within naturally fragmented forest patches along the southern and eastern coasts of Africa, with multiple inland populations in eastern Zimbabwe, Malawi, and parts of Tanzania that may be isolated from the coastal populations. In contrast, the black mamba (*D. polylepis*) differs profoundly from the other three mambas in its ecology (Loveridge 1950, FitzSimons 1962, Branch *et al.* 1995, Luiselli *et al.* 2000). It is much larger in body size (i.e. reported up to 4.5 m as opposed to 1.5–2.0 m; Spawls *et al.* 2018) and is primarily terrestrial but also spends time in burrows and in trees (Loveridge 1950, FitzSimons 1962, Shine and Spawls 2020). It is very widespread across the savanna biome, ranging from South Africa to West Africa, apparently occurring as two allopatric populations (Fig. 1).

Because near-obligate arboreality and the bright green coloration of the three green *Dendroaspis* species are unique in the family Elapidae, we hypothesized that they could be a monophyletic sister-clade to *D. polylepis*. However, non-monophyly of the three green species would open the way for comparative studies of adaptations associated with the independent gain or secondary loss of arboreality in these snakes. Overall, a fully sampled phylogeny would enhance the usefulness of mambas as model organisms for studies of adaptations of venom, e.g. for subduing prey or possibly for enhancing digestion (Kochva *et al.* 1983, Daltry *et al.* 1996, Barlow *et al.* 2009, Bottrall *et al.* 2010, Ainsworth *et al.* 2018) and would ultimately have utility in pharmacological research (e.g. Bradley 2000, Diochot *et al.* 2012, Laustsen *et al.* 2015, Oliveira *et al.* 2022).

Despite their obvious importance, the phylogenetic relationships among the four mamba species are poorly known, and there has been no attempt to assess phylogeographic structure within them, leaving the question of the validity of *D. intermedius* and *D. jamesoni kaimosae* unresolved. Furthermore, multiple studies of phylogenetic relationships within the Elapidae, or among snakes or squamates as a whole, suggest that *Dendroaspis* is monophyletic but have failed to yield a robust and consistent estimate of the sister-group relationships of *Dendroaspis*, although several have recovered a close relationship with *Bungarus*, *Elapsoidea*, *Hemibungarus*, and especially *Ophiophagus* (Slowinski and Keogh 2000, Castoe *et al.* 2007, Kelly *et al.* 2009, Pyron *et al.* 2011, 2013, Lee *et al.* 2016). However, none of these studies included more than two species of *Dendroaspis*, limiting their utility and preventing adaptive hypotheses from being tested. The only published phylogenetic analysis of the entire genus (Ainsworth *et al.* 2018) used two mitochondrial genes of single individuals of each recognized species, as well as *D. jamesoni kaimosae*, precluding any phylogeographic or species delimitation analysis. To address this knowledge gap, we generated a comprehensively sampled phylogeny of the genus *Dendroaspis* with broad geographic sampling for each species to assess the species limits within the genus and to place the genus within the Elapidae. We apply a general lineage species concept, whereby species are considered to be separately evolving metapopulations (de Queiroz 1998, 2007), that can be diagnosed by integrating different lines of evidence, such as geographic isolation, ecology, morphology, genetics/clade monophyly, and/or reproductive isolation (e.g. Paterson 1980, de Queiroz 1998, 2007, Padial *et al.* 2010, Cicero *et al.* 2021).

Given that the distribution of *D. angusticeps* consists of allopatric populations (Fig. 1), we assessed whether any of these populations, and in particular the inland population, could be supported as distinct species, thereby perhaps validating '*D. intermedius*'. Furthermore, given that the West African population of *D. polylepis* is possibly isolated from the main distribution area in East and southern Africa (Trape *et al.* 2005, Spawls *et al.* 2018), and there are some differences in scalation and possibly coloration (Trape *et al.* 2005; Fig. 2), we surmised that the West African population may be divergent. We also tested the hypothesis that the three

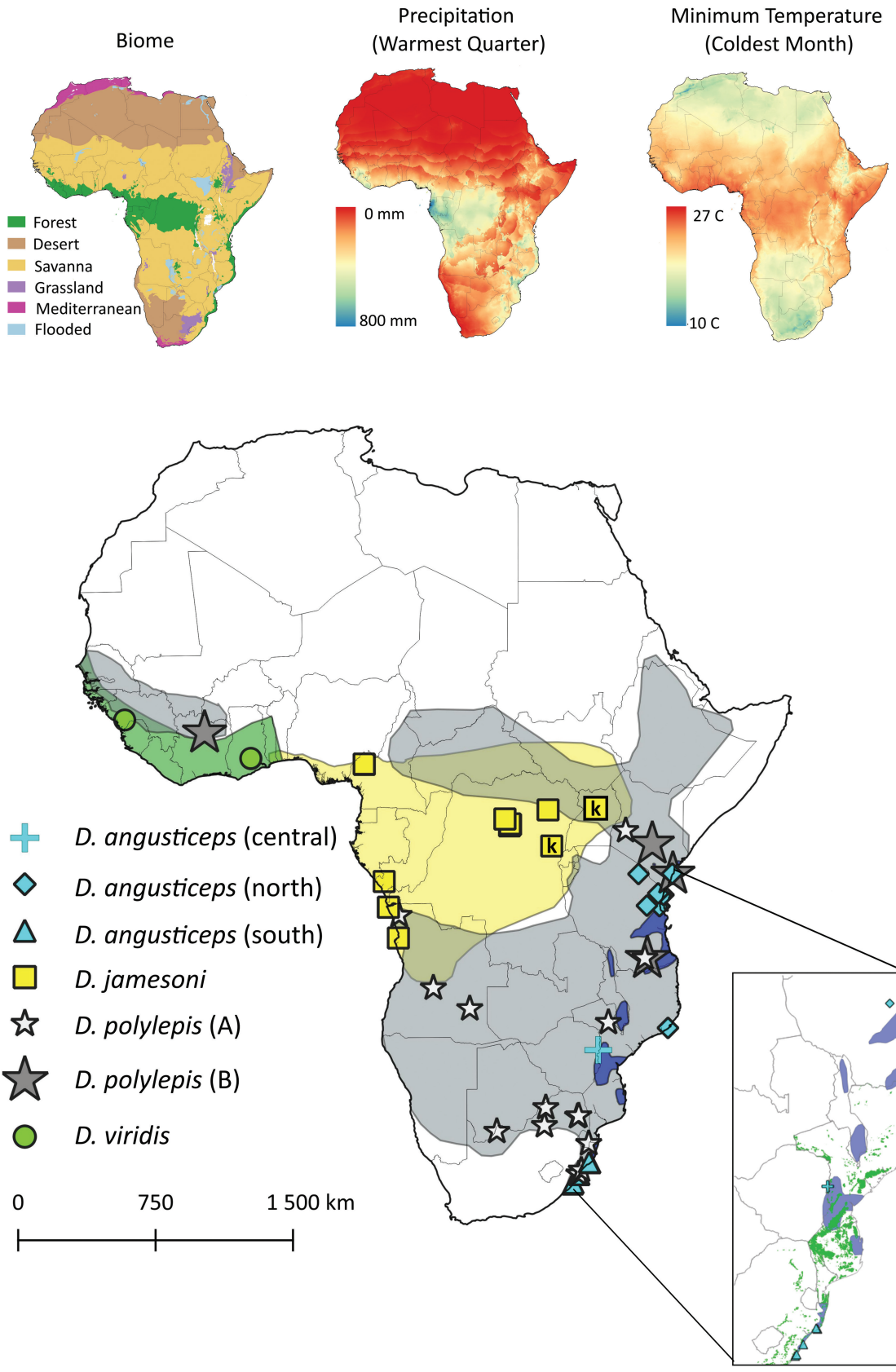

Figure 1. Top: mapped environmental features potentially important for limiting the ranges of mambas. Bottom: the inferred distribution for each species and the sampling localities for the phylogenetic analyses. Samples of *D. jamesoni kaimosae* are marked with a 'k'. Clades recovered of *D. angusticeps* and *D. polylepis* (Fig. 3) are shown with separate symbols. Distribution polygons based on IUCN Red List distribution polygons (https://www.iucnredlist.org/), FitzSimons (1962), Marques *et al.* (2018), Spawls *et al.* (2018), and Chippaux and Jackson (2019). Inset: polygons show the inferred IUCN distribution for *D. angusticeps*, with green polygons showing forest patches.

Figure 2. Examples of *Dendroaspis polylepis* from (A) south-eastern Africa (South Africa) and (B) western Africa (Guinea). Photo credits: W. Wuster (A) and J.-F. Trape (B).

arboreal, green coloured species may have a common ancestor, to the exclusion of *D. polylepis*. Finally, we assessed the placement of *Dendroaspis* within Elapidae given that to date, the sister-genus to *Dendroaspis* has not been corroborated through broad sampling. To test these hypotheses, we used several approaches to generate multilocus phylogenies. This was complemented by species distribution modelling to assess potential connectivity of discontinuously distributed species with naturally fragmented populations and back-casting the models to the Late Pleistocene to provide insight into potential connectivity in the past.

MATERIALS AND METHODS

Phylogenetics

We sourced tissue samples (scale clippings, blood, shed skins, dermal tissue, or liver) from 70 individuals from all four species of mambas from the South African National Wildlife Biobank (Pretoria), as well as from specimens field-collected by other researchers or maintained in research collections, zoos, or by private herpetoculturists, to provide broad geographic sampling (Fig. 1; Supporting Information, Tables S1–S3). Of particular interest, we include a newly sequenced individual of *D. jamesoni kaimosae* from the Democratic Republic of the Congo, plus samples of *D. angusticeps* from an inland area that might be representative of '*D. intermedius*' *sensu* Broadley and Blaylock (2013; e.g. Mozambique). Total genomic DNA was extracted using either a salt-extraction protocol (Aljanabi and Martinez 1997), or using a Qiagen DNeasy™ Tissue Kit (catalogue no. 69506), following the manufacturer's instructions, except for blood samples, where phospate buffered saline was not used and 200 µL of blood was added to 20 µL proteinase K. For 54 individuals, fragments of the mitochondrial genes NADH dehydrogenase subunit 4 (*ND4*, 808 bp) and cytochrome *b* (*Cyt-b*, 1018 bp), and two single-copy nuclear genes, the prolactin receptor gene (*PRLR*, 526 bp) and ubinuclein 1 (*UBN1*, 482 bp), were sequenced (Townsend *et al.* 2008); Table 1; Supporting Information, Tables S1–S4) to construct a four-gene dataset. In addition, neurotrophin 3 (*NTF3*, 657 bp), human *c-mos* proto-oncogene (*c-mos*, 538 bp), and the mitochondrial 16S ribosomal (16S, 469 bp including indels) genes were sequenced for an additional subset of individuals (Table 1; Supporting Information, Tables S1–S4). Polymerase chain reactions (PCR) were run under standard conditions, varying the concentrations of reagents and the reaction cycling according to the quality of the samples. Sanger sequencing was carried out at Macrogen (Amsterdam, Netherlands) using the same forward primers used in PCRs for mitochondrial fragments and for both forward and reverse primers for nuclear loci. Sequences from five *Dendroaspis*, as well as the outgroup taxa and extended dataset of elapid snakes for the dating analyses, were downloaded from GenBank (Table 1; Supporting Information, Tables S1–S3).

Sequence trace files were checked and aligned using GENEIOUS v.11 (http://www.geneious.com). All coding genes were translated to proteins to check that no frameshift mutations or unexpected stop codons were present. Heterozygous positions in diploid nuclear loci were identified by a combination of visual inspection for double peaks and low-quality Phred scores (Ewing *et al.* 1998), and were coded as ambiguous, using standard IUPAC codes. Corresponding allele sequences were estimated using PHASE v.2.1.1 (Stephens *et al.* 2001, Stephens and Scheet 2005) after preparation of the sequence data using SEQPHASE (Flot 2010).

The evolutionary models that best fit each gene were estimated in jModelTest v.1.1 (Posada 2008) using the Akaike information criterion (AIC) test, and these results were incorporated into the relevant analyses (*Cyt-b*: GTR+G, *ND4*: HKY+G, *PRLR*: GTR+G, *UBN1*: GTR). Maximum likelihood (ML) analyses were run using RAxML (Stamatakis 2014) for different datasets: (i) a four-gene dataset (*Cyt-b*, *ND4*, *PRLR*, and *UBN1*) as these genes have the most complete taxon sampling; (ii) a six-gene dataset (*Cyt-b*, *ND4*, *16S*, *PRLR*, *UBN1*, and *c-mos*) with fewer individuals; (iii) a mitochondrial-only dataset (*Cyt-b* and *ND4*; Table 1; Supporting Information, Table S1). The datasets were partitioned by gene, with the model available in RAxML (GTR+I+G) applied for each partition. The analyses were run for 1000 bootstrap replicates, and node support of ≥70% was considered well-supported.

A gene tree and a species tree were both estimated using the four-gene dataset using BEAST v.2.6 (Bayesian evolutionary analysis sampling trees), with xml files created in BEAUTi v.2 (Bouckaert *et al.* 2019) and the *BEAST template applied for the species tree. These analyses were run at the Cyberinfrastructure for Phylogenetic Research (CIPRES; Miller *et al.* 2010). In both cases, the genes were partitioned and the evolutionary models unlinked. A relaxed-clock was applied with the mitochondrial genes linked and the Yule speciation model was used with all genes linked for the trees, and the evolutionary rates along branches followed an uncorrelated log-normal distribution. The analyses were both run for 100 million generations, saving trees every 1000 generations to produce a set of 100 000 trees followed by a 50% burn-in. The log files were checked in TRACER v.1.7 (Rambaut *et al.* 2018) to examine tree likelihood and parameter estimates for evidence mixing and convergence, which was evaluated by the effective sample size (ESS) being >200 (post burn-in). TreeAnnotator v.2.1.2 (Drummond and Rambaut 2007) was used to produce ultrametric maximum clade credibility trees for the post burn-in trees, setting the posterior probability limit to ≥0.5. Nodes with posterior probabilities ≥0.95 were considered supported.

A dated phylogeny was constructed using BEAST v.2.6 with xml files created in BEAUTi v.2 for the four-gene dataset (*Cyt-b*, *ND4*, *PRLR*, and *UBN1*) and were run at the CIPRES platform (Miller *et al.* 2010). Because there are no relevant calibration points within *Dendroaspis*, a higher level phylogeny, including other elapid representatives (Supporting Information, Tables S1, S3) with nodes that could incorporate calibration points, was generated. This dataset ($N = 46$) used a reduced *Dendroaspis* sampling, retaining a few representatives of each species and included several representatives of closely related genera (*Aspidelaps*, *Bungarus*, *Hemachatus*, *Hemibungarus*, *Naja*, *Ophiophagus*, *Pseudohaje*, and *Walterinnesia*) and *Pseudechis and Oxyuranus* as outgroup taxa. Calibration points and priors (Supporting Information, Table S5) were based on those used in a previously dated elapid phylogeny (Kazandjian *et al.* 2021) and fossil ages (Head *et al.* 2016). The analysis was run with two different sets of calibration priors. First, by incorporating a single fossil prior (Head *et al.* 2016), constraining the age of the node uniting *Naja*, *Hemachatus*, and *Pseudohaje* at 17 Mya as the youngest possible age for this node (Kazandjian *et al.* 2021). Second, the same fossil prior was used plus four secondary priors based on the dating from Kazandjian *et al.* (2021), incorporating normal priors for key nodes (Supporting Information, Table S5). The dated BEAST analyses were run with separate partitions for each gene for the substitution model estimation, with the two mitochondrial genes linked for the relaxed log-normal clock model and all genes linked for the phylogenetic tree estimation using a Yule model. The HKY model was applied for each partition with gamma shape parameter and proportion of

Table 1. Individuals included in the maximum likelihood and BEAST four-gene phylogenetic analyses for *Dendroaspis*, with GenBank accession numbers for each gene and locality information. In cases where samples have matching vouchers, the museum codes are provided: CAS, California Academy of Sciences; PEM, Port Elizabeth Museum. Additional information on these samples (locality, analysis type, and additional genes sequenced) and for samples in the extended datasets are in the Supporting Information (Tables S1–S3). New sequences generated for this study are prefixed by 'PV'.

Genus	species	Sample Number	*Cyt-b*	*ND4*	*PRLR*	*UBN1*
Dendroaspis	*angusticeps*	4059	PV573655	PV573882	PV573814	PV573765
Dendroaspis	*angusticeps*	4060	PV573656	PV573883	PV573815	PV573766
Dendroaspis	*angusticeps*	4061	PV573657	PV573884	PV573816	PV573767
Dendroaspis	*angusticeps*	4062	PV573658	PV573885	PV573817	PV573768
Dendroaspis	*angusticeps*	1408	PV573649	PV573872	PV573808	
Dendroaspis	*angusticeps*	1413	PV573650	PV573873	PV573809	PV573761
Dendroaspis	*angusticeps*	1414	PV573651	PV573874	PV573810	
Dendroaspis	*angusticeps*	1423	PV573652	PV573878	PV573811	PV573762
Dendroaspis	*angusticeps*	1424	PV573653	PV573879	PV573812	PV573763
Dendroaspis	*angusticeps*	1425	PV573654	PV573880	PV573813	PV573764
Dendroaspis	*angusticeps*	192 (PEM R15610)	PV573644	PV573854	PV573805	PV573758
Dendroaspis	*angusticeps*	MTSN 8206	PV573659	PV573894		PV573769
Dendroaspis	*angusticeps*	TGET1-29	PV573661	PV573897	PV573820	PV573772
Dendroaspis	*angusticeps*	1322	PV573646	PV573867	PV573806	PV573759
Dendroaspis	*angusticeps*	1327	PV573647	PV573868	PV573807	PV573760
Dendroaspis	*angusticeps*	TGET1-21	PV573660	PV573895	PV573818	PV573770
Dendroaspis	*angusticeps*	TGET1-27		PV573896	PV573819	PV573771
Dendroaspis	*angusticeps*	TGET2-49	PV573662	PV573898	PV573821	
Dendroaspis	*jamesoni*	1072	PV573679	PV573865	PV573826	PV573777
Dendroaspis	*jamesoni*	1073	PV573680	PV573866	PV573827	PV573778
Dendroaspis	*jamesoni*	2971	PV573663	PV573881	PV573828	PV573779
Dendroaspis	*jamesoni*	CRT3674	PV573667	PV573886	PV573829	PV573780
Dendroaspis	*jamesoni*	CRT3691	PV573668	PV573887	PV573830	PV573781
Dendroaspis	*jamesoni*	CRT3884	PV573669	PV573888	PV573831	PV573782
Dendroaspis	*jamesoni*	CRT3920	PV573670	PV573889	PV573832	PV573783
Dendroaspis	*jamesoni*	CRT4003	PV573671	PV573890	PV573833	PV573784
Dendroaspis	*jamesoni*	CRT4055	PV573672	PV573891	PV573834	PV573785
Dendroaspis	*jamesoni*	CRT4197	PV573673	PV573892	PV573835	PV573786
Dendroaspis	*jamesoni*	CRT4253	PV573674	PV573893	PV573836	PV573787
Dendroaspis	*jamesoni*	13-118	PV573666	PV573901	PV573824	PV573775
Dendroaspis	*jamesoni*	13-76	PV573664	PV573899	PV573822	PV573773
Dendroaspis	*jamesoni*	13-80	PV573665	PV573900	PV573823	PV573774
Dendroaspis	*jamesoni*	MBUR 02936	PV573676	PV573902	PV573837	PV573788
Dendroaspis	*jamesoni*	PM068	PV573675	PV573913	PV573838	PV573789
Dendroaspis	*jamesoni kaimosae*	895	PV573677	PV573855	PV573825	PV573776
Dendroaspis	*polylepis*	1333	PV573685	PV573869	PV573840	PV573791
Dendroaspis	*polylepis*	1334	PV573686	PV573870	PV573841	PV573792
Dendroaspis	*polylepis*	10-43	PV573690	PV573903	PV573839	PV573790
Dendroaspis	*polylepis*	AJC564	PV573691	PV573904	PV573844	PV573795
Dendroaspis	*polylepis*	EI_0128		PV573905	PV573845	PV573796
Dendroaspis	*polylepis*	S924	PV573692	PV573906	PV573846	PV573797
Dendroaspis	*polylepis*	TGET1-4		PV573907		
Dendroaspis	*polylepis*	TGET1-16	PV573694	PV573908	PV573847	PV573798
Dendroaspis	*polylepis*	TGET1-22	PV573695	PV573909	PV573848	PV573799
Dendroaspis	*polylepis*	TGET1-23	PV573696	PV573910	PV573849	PV573800
Dendroaspis	*polylepis*	TGET1-25	PV573698	PV573912		PV573801
Dendroaspis	*polylepis*	TM17-AT193 (PEM R24277)	PV573700			
Dendroaspis	*polylepis*	1417	PV573687	PV573875	PV573842	PV573793

Table 1. Continued

Genus	species	Sample Number	*Cyt-b*	*ND4*	*PRLR*	*UBN1*
Dendroaspis	*polylepis*	1421	PV573689	PV573877	PV573843	PV573794
Dendroaspis	*polylepis*	IRD 302.CI	PV573699	PV573914	PV573853	
Dendroaspis	*polylepis*	TGET1-24	PV573697	PV573911		
Dendroaspis	*viridis*	899	PV573701	PV573856	PV573850	PV573802
Dendroaspis	*viridis*	1069	PV573702	PV573863	PV573851	PV573803
Dendroaspis	*viridis*	1070	PV573703	PV573864	PV573852	PV573804
Ophiophagus	*hannah*	2923 (CAS 206601)	MT346770	MT346943	MT347350	MT347572
Oxyuranus	*scutellatus*	1274 (mt genes 1199)	MT346947	MT346774	MT347355	MT347576

invariable sites using values estimated in bModelTest (Bouckaert and Drummond 2017). Three independent runs of 100 million generations of the MCMC were run, sampling the MCMC every 5000 generations. The effective sample sizes of the parameters were checked in TRACER v.1.7.1 (Rambaut *et al.* 2018) for values exceeding 200, after which TreeAnnotator (Drummond and Rambaut 2007) was used to apply a 50% burn-in for each of the runs.

In lieu of separate nuclear gene trees, sharing of alleles and reticulation among species for nuclear genes was explored through median-joining networks. Networks were constructed for each nuclear gene in PopART (population analysis with reticulate trees) v.1.7 (Leigh and Bryant 2015). All available sequences were included from the phased datasets to construct the networks (i.e. two alleles per individual).

To assess species boundaries, we used the reversible-jump Markov chain Monte Carlo (rjMCMC) method of Yang and Rannala (2010), implemented through a multispecies coalescent method using the software BPP (Bayesian phylogenetics and phylogeography) v.3.1 (Rannala and Yang 2003, Yang and Rannala 2014), which incorporates species tree uncertainty. This method accommodates the species phylogeny, as well as lineage sorting due to ancestral polymorphism while using a nearest-neighbour interchange (NNI) to alter the species tree. Using a dataset of three nuclear genes (*PRLR*, *UBN1*, and *NTF3*) and 52 individuals in total (Table 1; Supporting Information, Tables S1–S3), we varied values of model parameters theta (θ) and tau (τ_0) to simulate the effects of different hypothesized ancestral population sizes and ages of nodes on inferred species limits (Leaché and Fujita 2010). We considered four different combinations of priors, each being assigned a gamma $G(\alpha, \beta)$ distribution to simulate extremes of small or large ancestral population size and shallow or deep divergences. Large and small ancestral population size estimates were modelled as $\theta \sim G(1, 10)$ and $\theta \sim G(2, 2000)$ and deep and shallow divergences as $\tau_0 \sim G(1, 10)$ and $\tau_0 \sim G(2, 2000)$, respectively (Leaché and Fujita 2010). Each analysis was run twice with different seed numbers to confirm consistency between runs. We used the four-gene phylogeny as a guide tree, and tested six candidate species based on mitochondrial, nuclear, and morphological data: the described species *D. jamesoni*, *D. viridis*, and *D. polylepis*, and the three major mitochondrial clades identified within *D. angusticeps*. In addition, the analysis was run to test the hypothesis that *D. polylepis* contains more than one species. Priors assuming large ancestral population sizes and shallow divergences are likely to be more conservative in terms of numbers of species estimated. This applies particularly since most taxa in the genus *Dendroaspis* occupy large areas and there is no specific reason for assuming a small founder stock.

In addition to the tree-based method, distance-based approaches were used to examine the range of divergence among species or clades. Pairwise sequence divergences were estimated for the genes with the best taxon sampling (*Cyt-b*, *ND4*, *PRLR*, and *UBN1*) and these were used to generate frequency distributions of intra- and interspecific sequence divergences using SpeciesIdentifier v.1.8 (Meier *et al.* 2006). With this approach, intra- and interspecific divergence values should not overlap given that genetic diversity should be much lower within species as compared to between species. This approach can provide additional support for species delimitation (Lefébure *et al.* 2006). In addition, sequence divergences between *Dendroaspis* species (or clades) were estimated using uncorrected net *p*-distances separately for each gene using MEGA v.11 (Tamura *et al.* 2021). This allowed for a comparison of the interspecific sequence divergence values between species in the genus as compared to the frequency distributions generated by SpeciesIdentifier.

Species distribution modelling

Locality points for *D. polylepis* and *D. angusticeps* were retrieved from McBride *et al.* (2023) and Tolley *et al.* (2023a), and supplemented with points published in publicly accessible databases as of November of 2024 (https://www.gbif.org/, 2024; https://www.inaturalist.org/ 2024), end of 2020 (https://vertnet.org/), and from direct observations gleaned from the literature (see Supporting Information, Table S6). Maximum entropy modelling in MaxEnt v.3.4.4 is robust to small locality samples and presence-only data but performs best when localities are roughly evenly distributed across the whole of the target species range (Phillips *et al.* 2004, Phillips and Dudík 2008). We, therefore, performed two rounds of quality control on our full locality listings, one using embedded data on locality precision to remove highly uncertain points and another using ArcGIS Pro 3.3 (https://www.esri.com, 2024) and the SDM Toolbox Pro (Brown 2014) in which we inspected locality distributions to identify any apparent encounter and/or recording bias. *Dendroaspis angusticeps* showed no evidence of sampling bias and was rarefied to a minimum distance of 1 km to avoid double-counting the same localities. *Dendroaspis polylepis*, however, had been very densely sampled in South Africa as that region has received comparatively high survey effort and has the

highest record density in Africa for reptiles (Tolley *et al.* 2016). A preliminary model run in MaxEnt 3.4.4 (Phillips *et al.* 2004) showed a tendency to predict highly suitable habitat only in this densely-sampled region and to underpredict suitability elsewhere. We, therefore, rarefied the South African localities for *D. polylepis* with a minimum distance of 50 km, and the remainder of the distribution with the same 1 km minimum used for *D. angusticeps*. Our final locality samples numbered 195 points for *D. angusticeps* and 243 for *D. polylepis.*

High-resolution (30 arc-second) maps of 19 bioclimatic variables usually considered important for influencing animal species distributions were downloaded from the WorldClim online database for each time-period modelled (Otto-Bliesner *et al.* 2006, Fick and Hijmans 2017). For the Mid-Holocene and last glacial maximum (LGM) periods we used data from three global climate models, the CCSM4, MIROC-ESM, and MPI-ESM-P models, while the last interglacial (LIG) data were generated using the NCAR climate model (Otto-Bliesner *et al.* 2006). Layers were trimmed using Cliptools in ArcGIS Pro (https://www.esri.com, 2024) and the resulting maps of continental Africa were tested for spatial autocorrelation using the SDM Toolbox Pro (Brown 2014). Variables were selected for inclusion based on their utility in previous models of African snakes as described in (McBride *et al.* 2023), and in the end we selected the same subset of 11 variables: mean diurnal range, maximum temperature of the warmest month, minimum temperature of the coldest month, temperature annual range, mean temperatures of the wettest and driest quarters, precipitation of the wettest and driest months, precipitation seasonality, and the precipitation of the warmest and coldest quarters.

All models were constructed in MaxEnt 3.4.4 (Phillips *et al.* 2004) using five-fold replication and settings matching those used by McBride *et al.* (2023). Models with an Area Under the Receiving Operator Curve score of >0.8 were classed as having good performance and scores >0.9 were considered excellent (Fielding and Bell 1997). The accuracy of present-day models was also confirmed by comparing the resulting maps of habitat suitability to the species interpreted distributions according to the literature and the IUCN Red List (Branch *et al.* 2014b, Wagner *et al.* 2021). Model projections of suitable habitats in the present day and in different past periods were then re-imported into ArcGIS Pro for exploration. Where multiple global climate models had been used (Mid-Holocene and LGM), exploration included checking these against one another to identify patterns of variability and calculating the mean habitat suitability maps for the period using the Raster Calculator function. The Raster Calculator was also used with conditional (IF/THEN) clauses to create maps of areas of highly suitable habitat that persisted across all four modelled time periods and in different combinations of them to identify refugia that persist over time. Highly suitable habitat was defined as including any scoring >0.5 and might represent refugia or core habitats for the species.

RESULTS

Phylogenetics

The phylogenetic analyses showed four well-supported clades of *Dendroaspis* corresponding to each of the described species (Fig. 3; Supporting Information, Figs S1–S3), and this result was consistently recovered for each of the datasets (four-gene, six-gene, and mitochondrial only) and analyses (Supporting Information, Figs S1–S3). *Dendroaspis polylepis* and *D. angusticeps* were recovered as sister-species, as were *D. jamesoni* and *D. viridis*. With our additional sample of *D. jamesoni kaimosae* from the Democratic Republic of the Congo (DRC), we found no notable differentiation between samples identified as *D. j. kaimosae* (from Uganda and DRC) and *D. j. jamesoni* (Supporting Information, Fig. S2), but rather, that the 'subspecies' is polyphyletic. There were two shallowly divergent clades within *D. polylepis* (Fig. 3; Supporting Information, Figs S1–S3) that overlap geographically in East Africa. The sample from Ivory Coast was not notably distinctive from other *D. polylepis* and was in a clade with samples from East Africa. The species tree reflected the same structure as the gene trees (Supporting Information, Fig. S4) supporting the presence of four species in the genus.

Within species, *D. angusticeps* showed notable diversity, with three consistently recovered clades that do not overlap geographically (Fig. 1): a southern clade from South Africa, a northern clade from the Zambesi Delta north to the Kenya coast, and a central (inland) clade here recovered solely from eastern Zimbabwe. The northern and southern clades typically grouped as sister-taxa to the exclusion of the central (inland) clade, although none of the analyses showed strong support for that node.

The dated phylogeny showed the same topology and node support as the uncalibrated phylogenies. In addition, node divergence times were similar between the dated phylogenies regardless of the priors used (Table 2). The results suggest that the African genus *Dendroaspis* and the Asian *Ophiophagus* diverged around 22 Mya in the Early Miocene, although this sister-group relationship did not have node support (Fig. 4; Table 2). The dated phylogeny also recovered the four *Dendroaspis* species as well-supported (Fig. 4), with species-level lineage diversification beginning at the Miocene–Pliocene transition (*c.* 6 Mya). Divergence between *D. angusticeps* and *D. polylepis* occurred approximately 5 Mya, and between *D. jamesoni* and *D. viridis* approximately 4 Mya. The three clades within *D. angusticeps* are inferred to have diverged within the Pleistocene (<2 Mya), and the shallow divergence within *D. polylepis* within the last 1 Mya, as recently as *c.* 220 000 Kya (Table 2, node 9).

The networks revealed some sharing of alleles between species, particularly for alleles occurring at high frequency in the dataset (Fig. 5). The species sharing alleles differed for each gene: *NTF3—D. jamesoni* and *D. viridis*, *PRLR—D. jamesoni* and *D. angusticeps*, *UBN1—D. polylepis*, *D. angusticeps*, and *D. jamesoni*. There is evidence of allele sharing between *D. angusticeps* and *D. polylepis* for all genes. *Dendroaspis jamesoni* showed notably high allele diversity as compared to the other species.

The BPP species delimitation analyses showed the currently recognized species as supported, regardless of parameters or priors used (Table 3). Among the three clades of *D. angusticeps*, the northern population (from central Mozambique northwards to Kenya) was supported as a separate taxon with high probability. However, the status of the southern and central populations was strongly dependent on priors, varying widely between runs and not showing any overwhelmingly high probabilities under any scenario. Similarly, the hypothesis that *D. polylepis*

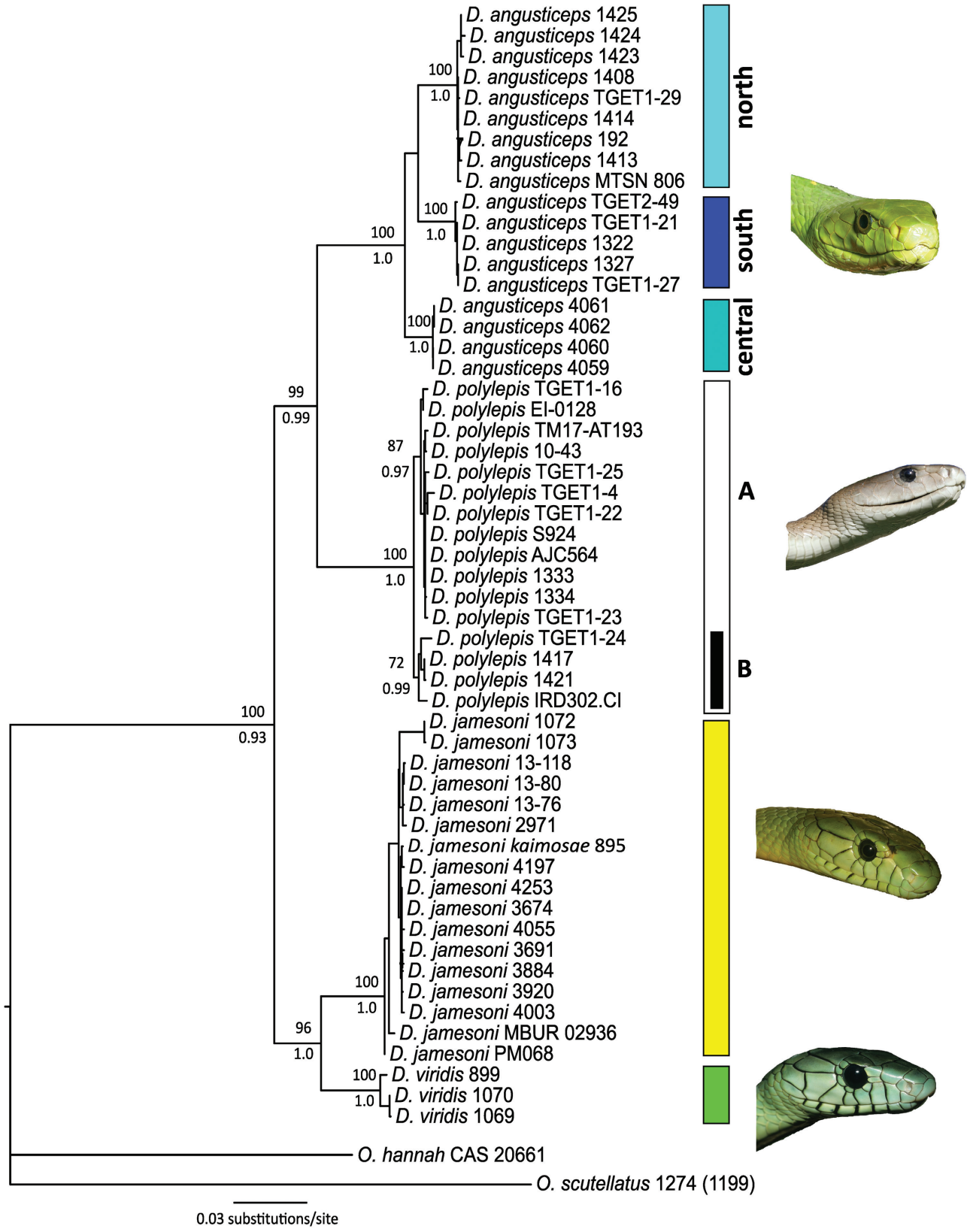

Figure 3. Maximum likelihood phylogeny based on four genes for *Dendroaspis* with bootstrap values above (≥70%) and posterior probabilities (≥0.95) from the Bayesian analysis in BEAST below. Nodes with lower values are not labelled. Clades within species are demarcated with bars.

might consist of two taxa did not show consistent results and varied according to priors used.

The histogram of pairwise sequence divergences showed a gap, or transition, between the inter- and intraspecific *p*-distances for each of the genes (Fig. 6). The pairwise divergences estimated between species and clades (Table 4) indicated that the *p*-distances between the two clades of *D. polylepis* clearly fell within the intraspecific values for the mitochondrial

Table 2. Dates estimated (Mya) for internal nodes in the dated phylogeny from the BEAST analysis using five calibration priors and one calibration prior, with 95% highest posterior density values for each node. The nodes used as calibration priors are indicated by an asterisk (five priors). Double asterisk indicates the node that was used as a single calibration prior (one prior) based on fossil information and was run as a separate analysis.

Node	1 prior	95% HPD	5 priors	95% HPD
1	38.03	28.27–50.53	39.65	30.47–48.91
2*	29.14	22.57–36.36	31.31	27.82–34.59
3	26.82	21.54–33.58	28.59	24.49–32.60
4	23.06	19.21–28.71	24.26	20.46–28.55
5	21.33	17.47–27.03	22.42	18.47–26.78
6	5.88	3.62–8.67	6.14	3.85–8.86
7	4.21	2.12–6.88	4.40	2.16–6.71
8	4.74	2.7–7.32	5.02	2.91–7.41
9	0.99	0.22–2.25	1.06	0.29–2.33
10	1.88	0.78–3.38	1.94	0.88–3.49
11	1.22	0.47–2.42	1.31	0.52–2.39
12	20.02	17.53–23.87	20.55	17.87–23.86
13	18.58	17.12–21.41	18.91	17.29–21.56
14**	17.01	17.00–17.08	17.01	17.00–17.03
15	13.52	10.53–15.93	13.81	11.97–15.82
16*	11.43	8.72–14.24	11.82	10.63–13.06
17*	10.21	7.37–12.92	10.67	9.35–12.07
18*	10.30	7.58–13.28	10.76	9.30–12.08
19	4.01	1.98–6.55	4.37	2.11–6.86
20	4.46	2.12–7.09	4.84	2.40–7.47
21	6.35	3.47–9.43	6.35	3.69–9.28
22	6.58	3.58–9.77	7.12	4.27–9.96

genes, whereas the *p*-distances estimated among the three *D. angusticeps* clades were at the lower end of the interspecific range. The nuclear genes examined, however, did not show a clear distinction between inter- and intraspecific levels of diversity.

Species distribution modelling

Our present-day species distribution models performed well for both species. The model's average AUC (area under the receiver operating curve) scores were excellent at 0.987 for *D. angusticeps* and 0.907 for *D. polylepis*, and mapped outputs predicted the target species known distributions well [see Fig. 7 and cf. Fig. 1 or published maps, e.g. those given by Wagner *et al.* (2021), and on VertNet (https://vertnet.org/ (2024)]. The standard deviation of the differences between replicates was low in both cases (0.002 for *D. angusticeps* and 0.010 for *D. polylepis*). Precipitation of the warmest quarter was the most important variable in both species models, making a 40.7% contribution to the *D. angusticeps* model and a 54.5% contribution to that for *D. polylepis* (see Table 5). Other variables making a clear percentage contribution or having high permutation importance differed between our models for *D. angusticeps* and *D. polylepis*, suggesting some differences in the ecological niches of these species. The *D. angusticeps* model relied additionally upon precipitation of the driest month and was sensitive to permutation in temperature annual range and the mean temperature of the wettest quarter, while the *D. polylepis* model was secondarily reliant on precipitation of the coldest quarter and sensitive to permutation in mean temperature of the wettest and driest quarters. A full listing of percentage contributions and permutation importances for all included bioclimatic variables is given in Table 5.

Our projection models for the Mid-Holocene, Last Interglacial (LIG), and Last Glacial Maximum (LGM) periods also showed differences in the patterns of expansion and contraction these species experienced under changing climatic regimes (Fig. 7). Specifically, *D. angusticeps* was projected to have had a fragmented distribution throughout the Holocene and to have had greater connectivity between the different parts of its range during wetter periods (the LIG and LGM; Fig. 7). *Dendroaspis polylepis*, in contrast, was predicted to have experienced more limited connectivity between populations in the present and LGM than in the warmer Mid-Holocene and LIG, when it may have been more widespread across central Africa (Fig. 7). This reinforces the finding from our present-day model variable contributions that these two taxa are sensitive to changes in different ecological and environmental variables, and suggests that they would have had shifting historical distributions over the last 140 Kya or more. *Dendroaspis angusticeps* and *D. polylepis* would also have used different parts of their ranges as refugia, with *D. polylepis* consistently occupying areas of southern Africa and Ethiopia, and *D. angusticeps*' core habitat persisting along parts of the coast of South Africa and Mozambique and in eastern Zimbabwe (Fig. 8).

DISCUSSION

Although *Dendroaspis* has been included in higher level phylogenies with limited taxon sampling, its placement within

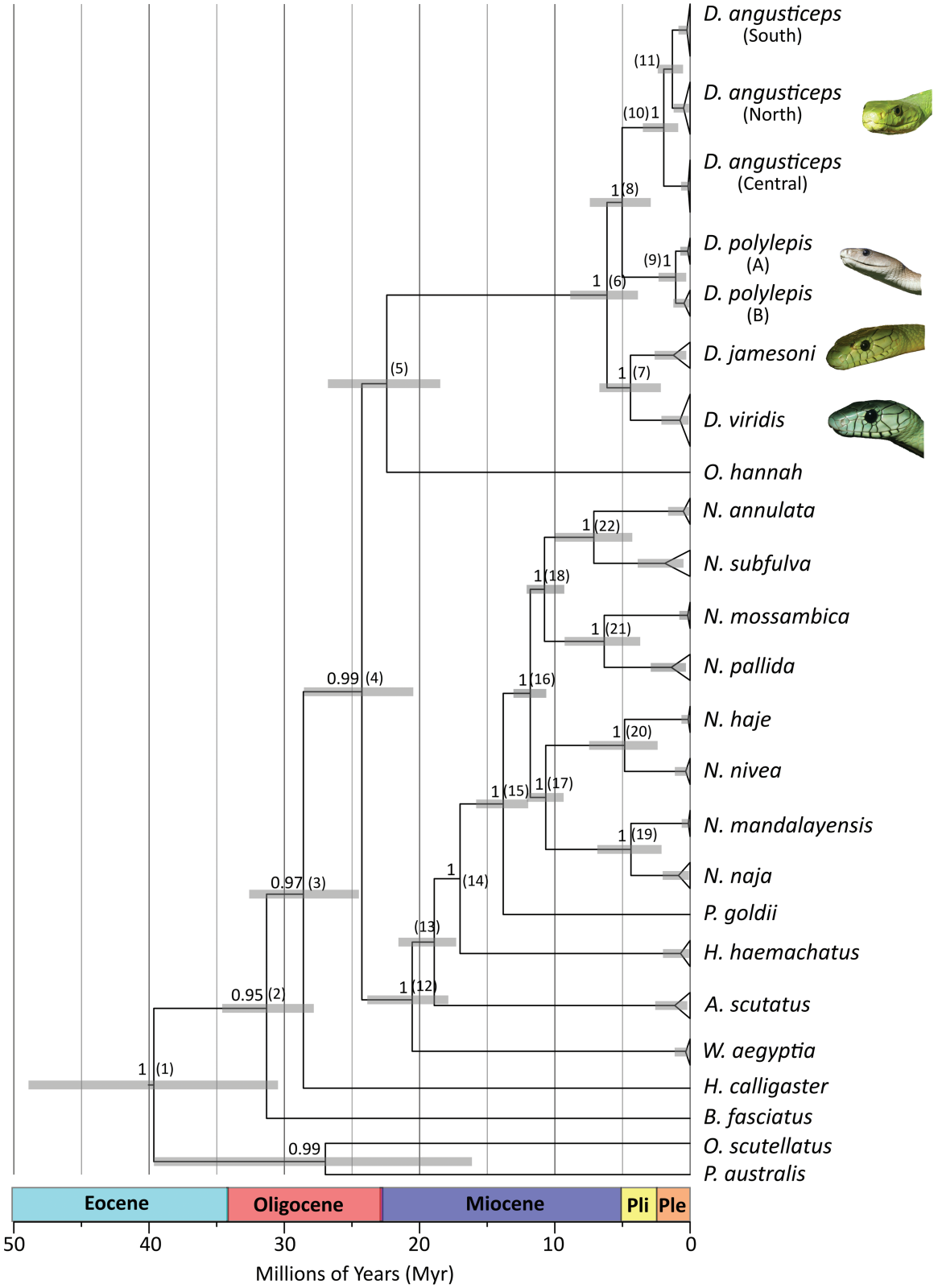

Figure 4. Dated phylogeny based on five node calibration priors (Supporting Information, Table S5). The 95% highest posterior densities are shown at each node by the grey bars and the posterior probabilities for node support are indicated for all internal nodes. Numbers in brackets refer to node numbers in Table 2. Tips have been collapsed for each species (Supporting Information, Tables S1–S3 for samples included). Geological epochs are shown by the timescale at the bottom of the figure. Photo credits: Wolfgang Wüster.

Elapidae has been unresolved (Slowinski and Keogh 2000, Castoe *et al.* 2007, Kelly *et al.* 2009, Pyron *et al.* 2011, 2013) and remains so despite our improved taxon sampling. Our phylogenetic analyses of elapid snakes placed the mambas (*Dendroaspis*) as sister-taxon to the genus *Ophiophagus* from Asia, as found previously in some studies (Kelly *et al.* 2009, Pyron *et al.* 2014, Lee

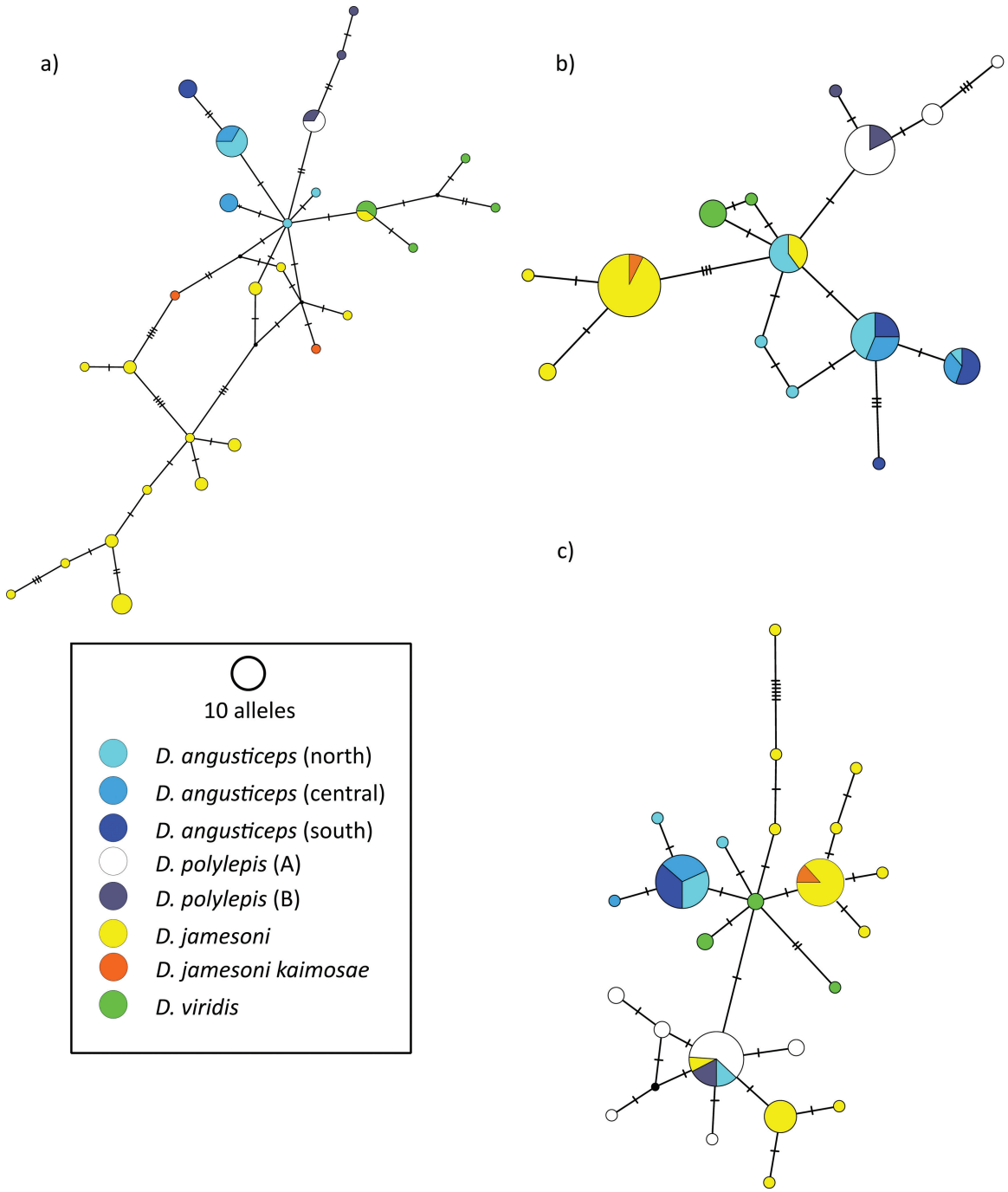

Figure 5. Networks of alleles for the nuclear genes sequenced for *Dendroaspis* based on phased sequences (A) *NTF3*, (B) *PRLR*, and (C) *UBN1*. Alleles are coded according to the proportion of individuals per species having that allele and the size of the circles represent the frequency of that allele in the dataset. Clades of *D. angusticeps* and *D. polylepis* and the samples identified as *D. jamesoni kaimosae* are colour coded separately. The hatch marks indicate the number of mutations along each branch.

et al. 2016, Zheng and Wiens 2016), but contrary to alternative placements with *Dendroaspis* as sister to the larger cobra clade (Zaher *et al.* 2019, Kazandjian *et al.* 2021). However, the relevant nodes have received limited support across all studies, suggesting near-simultaneous divergence amongst these genera, possibly coupled with incomplete lineage sorting. Resolving this polytomy will require more extensive data, and probably can be informed by next-generation sequencing approaches (e.g. SNPs, sequence capture, or whole-genome sequencing). Despite this, other generic-level nodes did receive strong support in our phylogeny, with the African genera *Aspidelaps*, *Hemachatus*, *Pseudohaje*, and *Walterinnesia* supported as sister-taxa to *Naja* (occurring in Africa and Asia). The current phylogeny, as well as other dated phylogenies, places the divergence between these taxa and *Ophiophagus*/*Dendroaspis* at the Late Oligocene/Early Miocene. Prior to that time, Africa was isolated from Europe and Asia by the Tethys Sea but with the sea narrowing in the Late Oligocene, Africa and Asia came in contact (Hamon *et al.*

Table 3. Mean posterior probabilities and ranges for candidate species of *Dendroaspis* estimated using the multispecies coalescent method. The range of values for parameters (theta and tau) are given for each different priors model. The probability values show the means and the range of probability values over multiple runs in support of different combinations as separate. Probability values are provided for the best estimate of the number of species in the genus. High probabilities indicate greater confidence in support of species status.

	Multispecies coalescent–priors models							
	Large ancestral population, deep divergences		**Small ancestral population, shallow divergences**		**Small ancestral population, deep divergences**		**Large ancestral population, shallow divergences**	
Theta	**1, 10**		**2, 2000**		**2, 2000**		**1, 10**	
Tau	**1, 10**		**2, 2000**		**1, 10**		**2, 2000**	
	Probability		**Probability**		**Probability**		**Probability**	
Dendroaspis	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
angusticeps north	1	1	1	1	1	1	1	1
angusticeps south	0.471	0.111–0.790	0.969	0.964–0.975	0.975	0.971–0.980	0.183	0.109–0.220
angusticeps central	0.471	0.111–0.790	0.969	0.964–0.975	0.975	0.971–0.980	0.183	0.109–0.220
angusticeps south + central	0.529	0.210–0.889	0.031	0.025–0.036	0.025	0.020–0.029	0.817	0.780–0.891
angusticeps north + south	0	0	0	0	0	0	0	0
angusticeps north + central	0	0	0	0	0	0	0	0
angusticeps all	0	0	0	0	0	0	0	0
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
jamesoni	1	1	1	1	1	1	1	1
polylepis (A)	0.606	0.545–0.685	0.882	0.796–0.957	0.824	0.771–0.913	0.617	0.556–0.648
polylepis (B)	0.606	0.545–0.685	0.882	0.796–0.957	0.824	0.771–0.913	0.617	0.556–0.648
polylepis all	0.394	0.315–0.455	0.118	0.043–0.204	0.176	0.087–0.229	0.383	0.352–0.444
viridis	1	1	1	1	1	1	1	1
number of species	**Probability**		**Probability**		**Probability**		**Probability**	
4 species	0	0	0	0	0	0	0	0
5 species	0.233	0.072–0.385	0.003	0–0.006	0.004	0.003–0.007	0.332	0.295–0.373
6 species	0.458	0.382–0.532	0.144	0.068–0.227	0.192	0.106–0.236	0.537	0.478–0.589
7 species	0.309	0.083–0.546	0.853	0.768–0.932	0.804	0.757–0.891	0.131	0.084-0.161

2013). It is possible that a common ancestor of *Dendroaspis* and other elapids was able to disperse between regions, as has been proposed for other taxon groups (Vermeij 1991, Godinot *et al.* 2003, Seiffert 2012), including reptiles e.g. vipers—Pook *et al.* (2009); *Varanus*—Portik and Papenfuss (2012); and *Python*—Tolley and Alexander (2025).

Notably, with the exception of *Naja*, most of the elapid genera are species-poor. While this may be an artefact due to generic-level over-splitting, it is also plausible that lineage extinctions in the Miocene could have played a role, resulting in reduced species richness. The Miocene Epoch is generally characterized by a cooling climate with a concomitant increase in open and arid environments in Africa, as compared to the warmer and more mesic forested environments of the Oligocene (Zachos *et al.* 2001, Couvreur *et al.* 2021, Steinthorsdottir *et al.* 2021). Miocene cooling has been generally construed as a significant driver of genus- and species-level diversification for African herpetofauna as a result of the expanding savanna and grasslands habitats (e.g. Barlow *et al.* 2019, Engelbrecht *et al.* 2019, Zhao *et al.* 2020, 2023). In contrast, however, are the significant ecological changes on the global landscape due to forest contraction during the Miocene (Steinthorsdottir *et al.* 2021) that would have brought about extreme range reduction of forest taxa (Couvreur *et al.* 2021).

It has been suggested that ancestral taxa occurred in widespread forest prior to the Miocene, and with forest contraction, these populations underwent vicariance, resulting in allopatric speciation (e.g. Branch *et al.* 2014a, Hughes *et al.* 2018). However, in cases where forests contracted completely, this would have brought about extinction of populations unable to persist in open habitats (e.g. Tolley *et al.* 2008, 2011, Ceccarelli *et al.* 2014, Dagallier *et al.* 2020). Assuming that the ancestral taxa of African elapids were forest-living, the pattern observed in the phylogeny suggests that many forest-living lineages may have declined to extinction, with the remaining few lineages having successfully transitioned to open grasslands and savanna (e.g. *Hemachatus* and *Aspidelaps*). Notably within *Dendroaspis*, three of four species occur in the remaining African forest habitats, with *D. angusticeps* limited to very small forest fragments that potentially consist of isolated populations. In contrast, *D. polylepis*

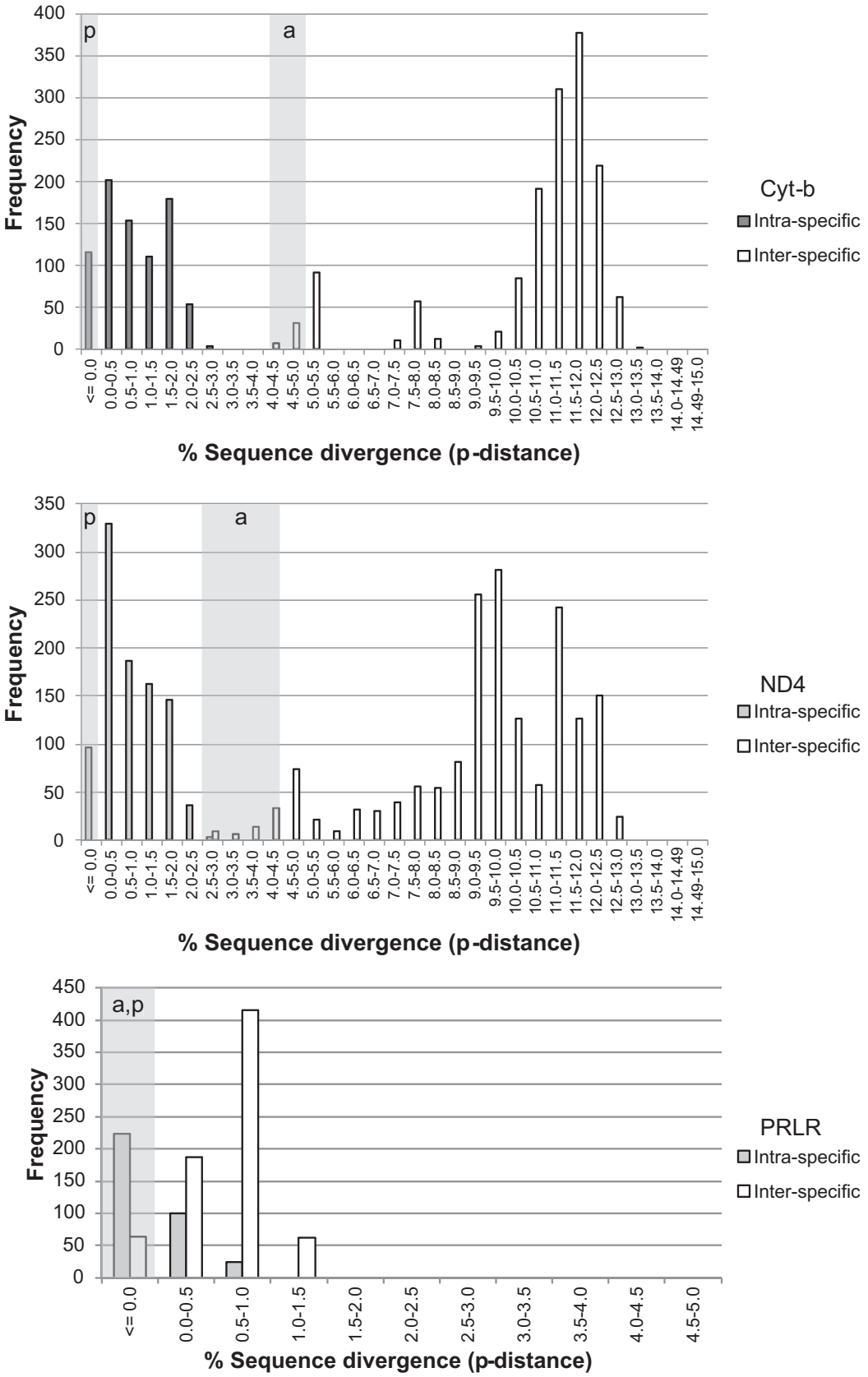

Figure 6. Frequency distributions of pairwise sequence divergence values for *Dendroaspis* for three genes (*Cyt-b*, *ND4*, and *PRLR*). Interspecific distances are shown by the white bars, and intraspecific distances are shown by dark grey bars. Grey shading shows the range of divergence values relating to the three *D. angusticeps* ('a') and two *D. polylepis* ('p') clades. (Axes are to different scales).

Table 4. Uncorrected net *p*-distances among (lower matrix) and within (diagonal) clades/species of *Dendroaspis* for the four genes used in the phylogenetic analyses. The genes are denoted for each matrix. Clades of *D. angusticeps* and *D. polylepis* have been estimated separately.

	Cyt-b	***D. angusticeps* (north)**	***D. angusticeps* (central)**	***D. angusticeps* (south)**	***D. jamesoni***	***D. polylepis* (A)**	***D. polylepis* (B)**	***D. viridis***
1	*D. angusticeps* (north)	0.0120						
2	*D. angusticeps* (central)	0.0371	0.0070					
3	*D. angusticeps* (south)	0.0422	0.0411	0.0020				
4	*D. jamesoni*	0.1109	0.1056	0.1107	0.0090			
5	*D. polylepis* (A)	0.1093	0.1037	0.1023	0.1175	0.0100		
6	*D. polylepis* (B)	0.1090	0.1037	0.1056	0.1195	0.0055	0.0090	
7	*D. viridis*	0.1072	0.0982	0.1072	0.0673	0.1128	0.1180	0.0080
	ND4	***D. angusticeps* (north)**	***D. angusticeps* (central)**	***D. angusticeps* (south)**	***D. jamesoni***	***D. polylepis* (A)**	***D. polylepis* (B)**	***D. viridis***
1	*D. angusticeps* (north)	0.0100						
2	*D. angusticeps* (central)	0.0309	0.0050					
3	*D. angusticeps* (south)	0.0413	0.0445	0.0010				
4	*D. jamesoni*	0.1103	0.1087	0.1066	0.0100			
5	*D. polylepis* (A)	0.0913	0.0805	0.0820	0.0868	0.0190		
6	*D. polylepis* (B)	0.1042	0.0928	0.0887	0.0924	0.0047	0.0060	
7	*D. viridis*	0.1189	0.1136	0.1198	0.0647	0.0948	0.1001	0.0040
	PRLR	***D. angusticeps* (north)**	***D. angusticeps* (central)**	***D. angusticeps* (south)**	***D. jamesoni***	***D. polylepis* (A)**	***D. polylepis* (B)**	***D. viridis***
1	*D. angusticeps* (north)	0.0008						
2	*D. angusticeps* (central)	0.0007	0.0000					
3	*D. angusticeps* (south)	0.0005	0.0000	0.0008				
4	*D. jamesoni*	0.0068	0.0090	0.0090	0.0012			
5	*D. polylepis* (A)	0.0037	0.0059	0.0061	0.0079	0.0004		
6	*D. polylepis* (B)	0.0039	0.0061	0.0060	0.0072	0.0000	0.0000	
7	*D. viridis*	0.0033	0.0056	0.0053	0.0061	0.0037	0.0032	0.0000
	UBN1	***D. angusticeps* (north)**	***D. angusticeps* (central)**	***D. angusticeps* (south)**	***D. jamesoni***	***D. polylepis* (A)**	***D. polylepis* (B)**	***D. viridis***
1	*D. angusticeps* (north)	0.0017						
2	*D. angusticeps* (central)	0.0000	0.0015					
3	*D. angusticeps* (south)	0.0004	0.0000	0.0000				
4	*D. jamesoni*	0.0016	0.0021	0.0031	0.0012			
5	*D. polylepis* (A)	0.0020	0.0026	0.0040	0.0014	0.0000		
6	*D. polylepis* (B)	0.0024	0.0031	0.0047	0.0017	0.0000	0.0000	
7	*D. viridis*	0.0008	0.0011	0.0019	0.0007	0.0012	0.0021	0.0014

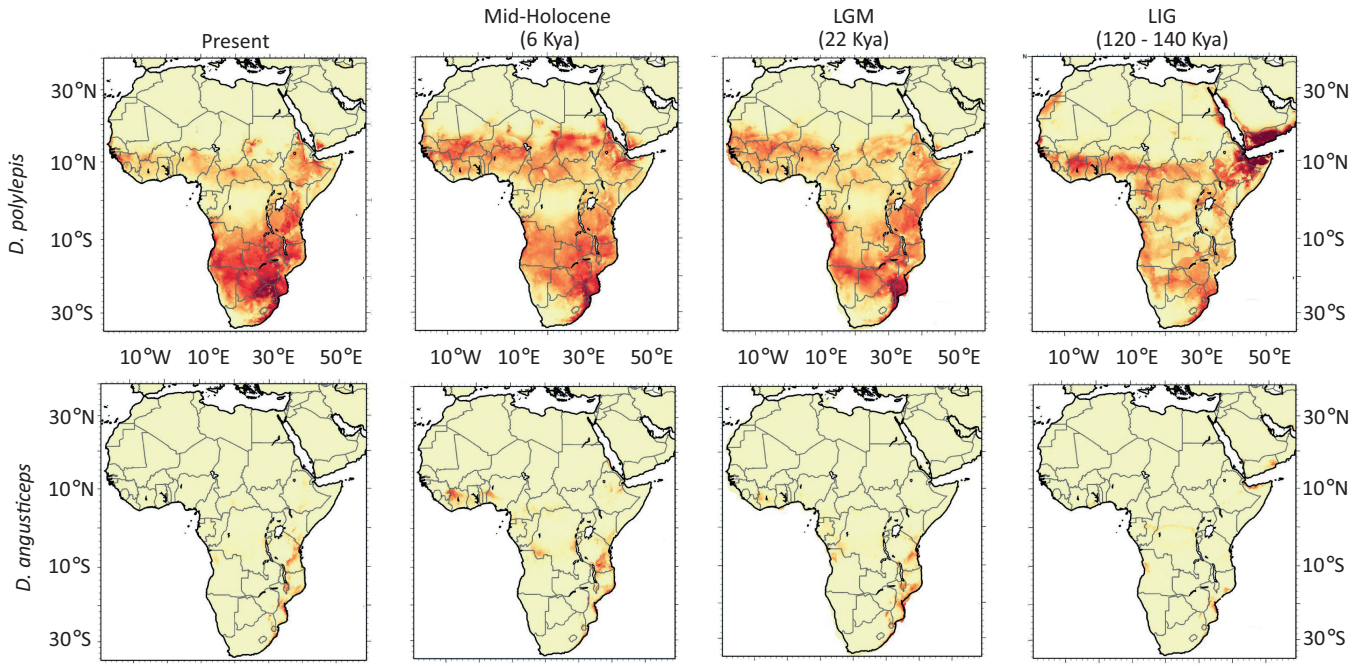

Figure 7. Probability of occurrence maps for *D. angusticeps* and *D. polylepis* based on the predicted habitat suitability outputs from Maxent models. Predicted occurrence is mapped for present-day, Mid-Holocene (6 Kya), Last Glacial Maximum (22 Kya), and the Last Interglacial (120 –140 Kya). Darkest shade indicates areas with highest probabilities of suitability.

occurs in savanna, and is widespread across Africa, presumably having adapted to this novel habitat.

Within the mambas, our multilocus analysis mirrors the interspecific relationships identified by Ainsworth *et al.* (2018)—there is a strongly supported sister-group relationship between *D. viridis* and D. *jamesoni*, both forest species of Central and West Africa, and an equally strongly supported sister-group relationship between the narrowly distributed forest species, *D. angusticeps*, and the very widespread savanna species, *D. polylepis*. Thus, the hypothesis of monophyly for the highly arboreal green species of mambas can be considered falsified. Notwithstanding the extensive geographic sampling, from South Africa to Ivory Coast, genetic structure is minimal within *D. polylepis*, the black mamba. In contrast, the eastern green mamba, *D. angusticeps*, is clearly separated into three clades, but these diverged only recently—less than 2 Mya. These are the oldest intraspecific divergences in *Dendroaspis*, with all four mamba species being relatively young.

Despite the very widespread geographic distribution of *D. polylepis* (Fig. 1; estimated 9.8 million km^2), sampling across most of its range showed very little genetic diversity. Although there are two clades, the divergence between and within them was very low, estimated to have begun <1 Mya, suggesting that genetic diversity is at the population level. In addition, samples from both West and East Africa are in the same clade, despite a minimum distance of 4700 km between these samples and spanning the observed 'gap' in distribution in Central Africa. The relatively low present-day climatic suitability (Fig. 7) may explain the apparent rarity of the species in Central and West Africa, with the distribution gap being real. However, sampling across that region is known to be sparse (Tolley *et al.* 2016) and the gap is possibly due to lack of data. If the present-day gap is real, then the most plausible scenario is that the gap has only recently formed, and the lack of genetic structure reflects past connectivity. Indeed, the SDMs for *D. polylepis* show fragmented, but ample suitable climatic space throughout the Holocene particularly during warmer phases (LIG, Mid-Holocene; Bova *et al.* 2021). In addition, the green Sahara environment and the African humid period between about 11 000 to 5000 years ago (Chandan and Peltier 2020, Pausata *et al.* 2020) could have provided ample habitat that promoted past connectivity. At present, the northern extent of *D. polylepis* is essentially limited by the Sahel, but during the greening of the Sahara, initiated by a strengthening of the north African summer monsoon or by increased precipitation access the Mediterranean (Tierney *et al.* 2017, Pausata *et al.* 2020, Cheddadi *et al.* 2021), the savanna biome pushed significantly northwards into what is now the Sahara desert (Watrin *et al.* 2009). Given the black mamba's propensity to occupy the open savanna environment, its distribution would very probably have included areas that are now extremely arid. Unfortunately, we were unable to sample material from the Horn of Africa, from where *Dendraspis antinorii* was described by Peters (1873). The taxon has been considered synonymous with *D. polylepis* in the recent literature but may warrant further investigation.

The notable genetic diversity within *D. angusticeps* is reflected by its fragmented current-day distribution in forest patches with long-term fragmentation further emphasized by the SDM results. Overall, we suggest there has been minimal connectivity between northern and southern parts of the distribution, each area forming a separate refuge throughout the Holocene. While connectivity between fragments within each area is likely (Fig. 7), both the genetic results and the modelling support historical vicariance between those regions. Despite our fairly good

Table 5. Variable contributions and permutation importances for each bioclimatic variable used to generate present-day species distribution models for (top) *D. angusticeps* (mean AUC = 0.987 and standard deviation = 0.002) and (bottom) *D. polylepis* (mean AUC = 0.907 and standard deviation = 0.010).

***Dendroaspis angusticeps* (AUC = 0.987, standard deviation = 0.987)**		
Bioclimatic variable	**Percentage contribution**	**Permutation importance**
Precipitation of Warmest Quarter	40.7	1.6
Precipitation of Driest Month	16.1	6.7
Temperature Annual Range (Max Temperature of Warmest Month—Min Temperature of Coldest Month)	15	41.5
Min Temperature of Coldest Month	12.3	12.3
Mean Diurnal Range (Mean of monthly (max temp—min temp))	8.8	9.3
Precipitation of Coldest Quarter	4	4.1
Mean Temperature of Wettest Quarter	1.8	18.1
Precipitation of Wettest Month	0.7	4.5
Precipitation Seasonality (Coefficient of Variation)	0.3	0.5
Mean Temperature of Driest Quarter	0.2	1
Max Temperature of Warmest Month	0.1	0.3
***Dendroaspis polylepis* (AUC = 0.907, standard deviation = 0.010)**		
Bioclimatic variable	**Percentage contribution**	**Permutation importance**
Precipitation of Warmest Quarter	54.5	10.9
Precipitation of Coldest Quarter	12.4	1
Max Temperature of Warmest Month	7.5	9.9
Precipitation Seasonality (Coefficient of Variation)	5.7	0.9
Min Temperature of Coldest Month	5.4	6.5
Mean Temperature of Driest Quarter	4.8	22.3
Mean Temperature of Wettest Quarter	3.6	22.7
Temperature Annual Range (Max Temperature of Warmest Month—Min Temperature of Coldest Month)	3.1	17.9
Precipitation of Wettest Month	2.2	7.2
Mean Diurnal Range (Mean of monthly (max temp—min temp))	0.4	0.6
Precipitation of Driest Month	0.4	0.2

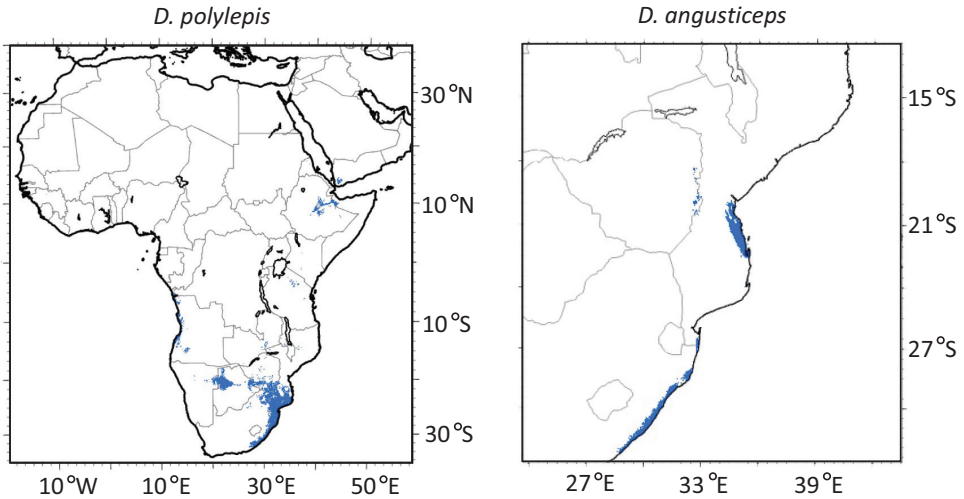

Figure 8. Overlap (shaded pixels) in the occurrence of highy suitable habitats (scoring >0.5) for four time points present-day, Mid-Holocene (6 Kya), Last Glacial Maximum (22 Kya), and the Last Interglacial (120–140 Kya) for *D. angusticeps* and *D. polylepis*.

sampling, there are many further forest patches in both regions within which this species is presumed to occur, and we, therefore, are unlikely to have captured the species' full range of genetic diversity. Indeed, the central clade has only been documented from Zimbabwe, yet the same mitochondrial haplotype clade has been recorded in captive specimens presumed to have originated from unspecified locations in Tanzania (e.g. Ainsworth *et al.* 2018). In this context, the affinities of the various isolated (unsampled) populations in Malawi and south-western Tanzania (including a recent sighting from Mt. Rungwe reported in the citizen science platform iNaturalist—https://www.inaturalist.org/observations/193120821) warrant further investigation.

The presence of three, rather than two, major clades of *D. angusticeps* is inconsistent with the two-species concept for eastern green mambas *sensu* Broadley and Blaylock (2013). Instead, the phylogeny shows support for three clades—southern (South Africa), central (inland Zimbabwe), and northern (East Africa). The northern and southern clades typically grouped as sister-clades, to the exclusion of the central (inland) clade, although none of the analyses showed strong support for this node. In contrast, our species delimitation analyses showed some limited evidence for the northern clade as a separate species. However, the nature of the contact zones between the southern, central, and northern clades in Mozambique remain unresolved. The connections between the Zimbabwean and coastal Mozambique populations, as well as the distribution gaps in central Mozambique mapped in the literature (Spawls and Branch 2020), require confirmation. Although our species delimitation analyses suggested the northern clade may be distinctive at the species-level, we refrain from making specific taxonomic adjustments due to the lack of sampling of crucial contact zones, and due to evidence that our chosen species delimitation algorithm is prone to over-splitting in widespread species (Sukumaran and Knowles 2017). If further evidence warrants recognition of additional taxa from within what is currently *D. angusticeps*, the application of available names to the different clades is complicated by uncertainty on type locality of *D. intermedius*, stated to originate from 'the Zambesi'. This could refer to anywhere along this 2500-km long river. The northern *D. angusticeps* clade (specimen PEM R15610, from Namalope Village, Zambezi Delta, Mozambique) occurs at the mouth of the Zambezi River, whereas further upstream, the river approaches the Zimbabwean localities of the central clade, meaning that the name could be available for either the northern or the central clade. If the type locality of *D. intermedius* were to be restricted to the central clade, then the name *Dendroapsis sjöstedti* (Lönnberg, 1907), described from 'Kibonoto' (now Kibongoto) on the western side of Mount Kilimanjaro, would be available for the northern clade.

CONCLUSION

Despite their clear geographic, ecological, and/or morphological distinctiveness among elapids, mambas are a comparatively young group of species, with species-level divergence approximately 4–5 Mya. We acknowledge that our dataset includes few loci (two mitochondrial and two nuclear genes) limiting the scope of the study and the inferences made. Although we do not expect that a larger genomic dataset would provide a substantial reinterpretation of the main findings, such a dataset could improve confidence or provide re-interpretation for nodes that were unresolved, particularly where there are shallow branches, such as found for *D. angusticeps*.

Species-level divergence within *D. angusticeps* is not adequately supported given the current state of knowledge, noting that divergence between the northern and southern clades was dated to within 2 Mya. The current taxonomy thus appears to be a valid reflection of the evolutionary relationships among *Dendroaspis*. We find no convincing evidence in support of *D. intermedius* as a separate species, nor of *D. jamesoni kaimosae* as a subspecies. However, further sampling and the application of genomic approaches may yield additional insights into the status of the northern and southern clades of *D. angusticeps*. Given the young age of mamba species, the notable nuclear gene allele and mitochondrial haplotype sharing amongst species is not surprising. This essentially reflects their very recent divergence and can be interpreted as either retained ancestral alleles or porous barriers to gene flow. Indeed, recent developments in the application of next-generation genomic sequencing to systematics clearly show that species boundaries are quite permeable with notable instances of hybridization and introgression (Twyford and Ennos 2012), even among species that are considered to have diverged some millions of years in the past. While formerly considered as hampering the assessment of systematics, introgression is now considered to be an important component of adaptive evolution, providing a reservoir of genetic variants increasing resilience in species (Edelman and Mallet 2021). The allele/haplotype sharing we observed amongst species of mambas, therefore, can merely be considered a reflection of their evolutionary history (Aguillon *et al.* 2022), and that the species are considered to be separately evolving metapopulations diagnosed by integrating evidence on their geographic distributions, ecology, and morphology, as well as their genetic divergence.

SUPPLEMENTARY DATA

Supplementary data are available at *Zoological Journal of the Linnean Society* online.

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CONFLICTS OF INTEREST

None declared.

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DATA AVAILABILITY

The data underlying this article are available from GenBank Nucleotide Database as detailed in Table 1 and Supporting Information, Table S1.

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