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RESEARCH ARTICLE

Into Africa: The biogeography of the genus *Python* in Africa

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Abstract

Most of the nine genera and 38 species in the family Pythonidae occur in south-east Asia and Australasia, but the genus *Python* is distinctive in that it also occurs in sub-Saharan Africa. There is a large distribution gap for *Python* between Africa and south-east Asia, but fossil evidence suggests *Python* once occurred in this gap, as well as in Europe until the mid-Miocene. However, because all African species have not previously been included in any previously published phylogeny, their monophyly has not been established. Further, it has been suggested that the genus *Python* may have an Asian origin, and this scenario would require the African species to be monophyletic. Otherwise, multiple independent dispersals into Africa would be required to explain current day biogeographic patterns. To test these competing hypotheses, a dated phylogeny was constructed based on one nuclear and two mitochondrial genes and biogeographic scenarios evaluated using ancestral range reconstruction. In addition, fossil evidence was appraised as supporting evidence for their paleo-distribution but also to evaluate the hypothesis that large pythons (e.g., > 6 m in body length) are restricted to warmer climatic zones, and we suggest this influenced their paleo- and present-day distributions. The dated phylogeny indicates monophyly for the African species, diverging from the Asian species approximately 33 Mya. Ancestral area reconstruction supports an Asian origin for the genus, with a single dispersal event to Africa after which species diversified across the continent. We find no support for multiple dispersal events into Africa. Accounting for the distribution of fossils, it appears that *Python*, including large-bodied species, were once more widespread, but ranges contracted in response to global cooling since the mid-Miocene, and they are now excluded from temperate (i.e., Europe, Iranian plateau) as well as hyper-arid areas (i.e., Arabian, Saharan and Namib deserts).

Highlights

- The genus *Python* originated in Asia, with a single ancestral lineage entering Africa around 33 Mya where it diversified into four extant species.
- Fossil evidence reveals that *Python* was much more widespread but with mid to late Miocene global cooling, the African and south-east Asian clades became isolated geographically from each other.
- Large body size may have had an important influence on geographic range, limiting species to the warmer areas.
- Long-term global cooling has presumably driven range contractions, creating the distribution gap, and possibly causing extinctions of *Python* species.

Keywords

Africa, adaptive radiation, biogeography, Miocene cooling, Pythonidae, range limits, reptiles, snakes

Introduction

Snakes are a diverse clade of squamates that arose around 170 million years ago (Zheng and Wiens 2016; Title et al. 2024). With more than 4,000 species, snakes are adapted to aquatic, fossorial, terrestrial and arboreal habitats and occur on every continent except Antarctica. They are divided into two main Infraorders, the Scolecophidia (fossorial thread and blind snakes), and the Alethinophidia (all other snakes). The python family (Pythonidae) consists of nine genera composed of 38 extant species and several extinct species (Uetz et al. 2025) and diverged from other snake lineages

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early in the evolution of the Alethinophidia, some 60–90 Mya (Pyron et al. 2013; Zheng and Wiens 2016; Zaher et al. 2019). Extant species of Pythonidae occur across southern Asia, Australia, Oceania and a large portion of Africa (Torr 2000; O'Shea 2007; Uetz et al. 2025; Figs 1, 2), while extinct species, and locally extinct populations of extant species, formerly occurred in Europe, western Asia, north Africa and southern Africa (Römer 1870; Rage 1976; Szyndlar and Rage 2003). Amongst snakes, pythons are known for their unique morphological characteristics and ecology (O'Shea 2007; Esquerré and Keogh 2016; Leal and Cohn 2016; Esquerré et al. 2017, 2020). Furthermore, they show extreme range in body size, with species ranging in adult body length from 0.7 m to 9 m (O'Shea 2007; Esquerré et al. 2017). Their ecological and phenotypic diversity is attributed to adaptive radiations into different habitats, but they also show convergence when species occupy similar ecological niches (Esquerré and Keogh 2016; Esquerré et al. 2017, 2020).

Within the Pythonidae, the genus *Python* is the most geographically widespread, occurring in four biogeographic regions – the Oriental, Oceanian, Australian and Afrotropical, although they are absent from the island of Madagascar. There is a large gap in the occurrence of *Python* across northern Africa, the Saharo-Arabian-Levant region and Iranian plateau (Figs 1, 2). However, fossil evidence dated to the early and mid-Miocene (20–12 Mya) shows that, historically, some species of *Python* occurred in this gap as well as along the western seaboard of Namibia (Thomas 1982; Szyndlar and Rage 2003) where they currently do not occur (Fig. 2, Suppl. material 1: table S1). Together with Miocene fossils from central and south-eastern Europe (Szyndlar and Rage 2003) and Pakistan (Hoffstetter 1964; Head 2000), there is evidence that *Python* was more widespread up until the mid-Miocene and occurred across much of Eurasia (Fig. 2, Suppl. material 1: table S1). Notably, there are no known python fossils from Madagascar, although fossil evidence in general from that island is rare (Krause et al. 2006).

Python females actively incubate and protect their eggs, and it is thought that ranges are limited to regions where environmental temperatures are suitable for successful incubation (Lillywhite 1987; Alexander 2007, 2018). Of the ten *Python* species, four have giant body sizes, can exceed 5 m (*P. natalensis*, *P. sebae*, *P. molurus* and *P. bivittatus*) and are restricted to the tropics and sub-tropics, supporting the hypothesis that large pythons are restricted to warmer climates (Ivanov and Böhme 2011). It is therefore plausible that long-term environmental cooling initiated in the Eocene, intensifying in the Miocene (Zachos et al. 2001a, b, 2008; Westerhold et al. 2020) has played a role in range contractions, vicariance and extinctions of *Python* species and populations.

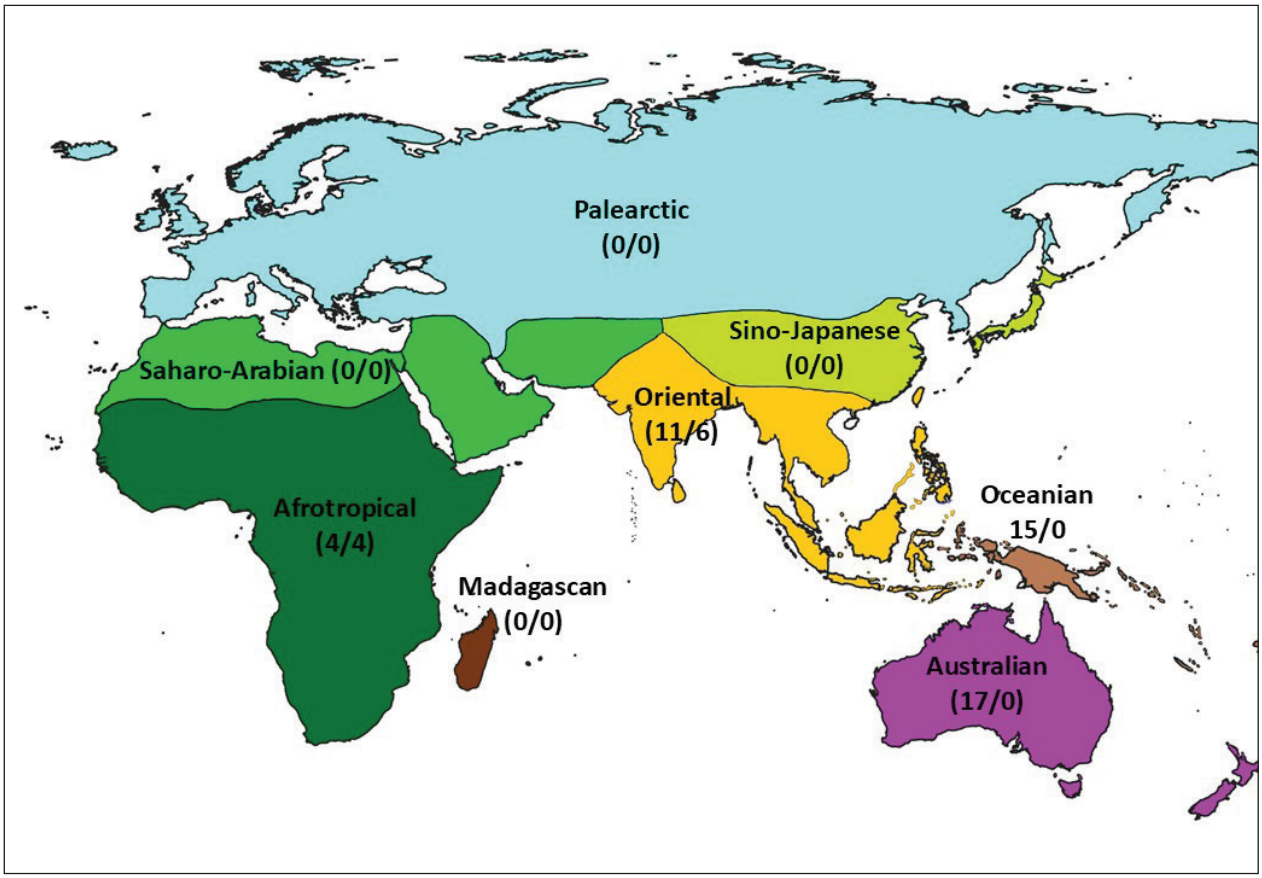

Figure 1. Biogeographic regions (from Holt et al. 2013) with the number of Pythonidae species that occur in each area. Numbers in brackets show the number of species occurring in each region for the entire family and for the genus *Python* (family Pythonidae / genus *Python*).

Most Asian *Python* species have relatively small geographic ranges, apart from the two widespread, giant-bodied species (Fig. 2), *P. bivittatus* (Burmese Python) and *P. molurus* (Indian Rock Python). On average, African *Python* species have more extensive geographic distributions than most Asian species (Fig. 2). The large bodied (maximum recorded body length ~6 m) *P. natalensis* (Southern African Python) and *P. sebae* (Northern African Python) are the most widespread African species, with *P. natalensis* occurring across most of the southern parts of Africa and *P. sebae* in East and West Africa (Broadley 1984). These two species are morphologically similar (Fig. 3) and occur primarily in mesic to arid savanna, although *P. sebae* may extend into forest in parts of its range. *Python sebae* and *P. natalensis* appear to be sympatric in parts of East Africa (Fig. 2; Broadley 1999; Spawls et al. 2018) and parapatric or sympatric in other areas (Fig. 2). Their morphological and colouration similarities led to *P. natalensis* first being classified as a subspecies of *P. sebae* (Broadley 1984), although *P. natalensis* was later elevated to a full species (Broadley 1999). In contrast, *P. anchietae* (Anchieta's Dwarf Python) and *P. regius* (Ball Python) are small- bodied (maximum body length < 1.8 m) and have smaller distributions than the large-bodied species (Fig. 2). *Python anchietae* occurs mainly in arid environments, extending into arid savanna in southern Angola (Branch 2018) and central and northern Namibia (Marques et al. 2018), while *P. regius* occurs in forest and mesic savanna extending from Central to West Africa (Spawls et al. 2018).

The present-day disjunct distribution of the genus, as well as their absence from Madagascar, is intriguing in terms of biogeography. Indeed, several competing hypotheses regarding the origin of the genus *Python* and family Pythonidae have been proposed, but these hypotheses have not been tested by means of comprehensive datasets, and none have addressed the significant gap in their distribution, nor their absence from Madagascar. For example, Reynolds et al. (2014) concluded that *Python regius* (from Africa) is sister to all other *Python* species (African and Asian species), suggesting a possible African origin for the Pythonidae. In a subsequent study, Esquerré et al. (2020), also recorded a basal divergence of *P. regius* from the rest of the genus dated to approximately 32 Mya, but it should be noted that *P. regius* was the only African species included in that phylogeny. An Asian origin for the entire family Pythonidae with subsequent dispersal into Africa and southwards into Australasia has been suggested based on the high species richness and morphological diversity in Austral-Asia (Branch and Erasmus 1984; Esquerré et al. 2020). This has been supported by a recent phylogenomic analysis for the family (Esquerré et al. 2022), although this phylogeny included only single individuals of three African species. We tested these prevailing hypotheses by generating a near-complete, dated phylogeny and historical biogeographical assessment of *Python* in Africa, with complete sampling for African species. This allowed us to specifically test for monophyly of the African

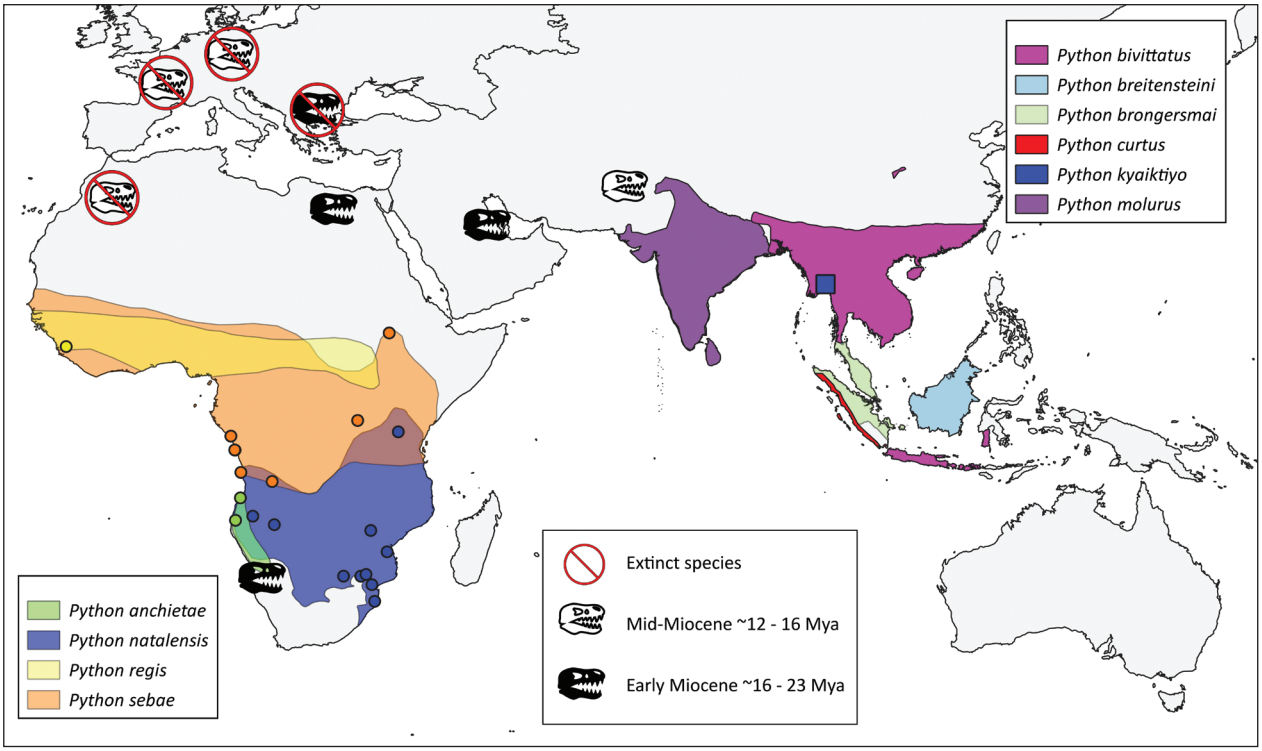

Figure 2. Current day distribution of the ten species in the genus *Python* and the locations of python fossils. Fossils are denoted for the early Miocene (black skulls) and the mid-Miocene (white skulls), with extinct species indicated by the red interdictory circles. All other fossils represent extant species, or those unidentified at the species level. DNA samples of African species included in the phylogenetic analyses (Table 1) are shown by coloured circles. For samples that lacked coordinates, the centre point of the country of collection (or other locality descriptor) was used for mapping. Pet trade samples were not mapped. Distribution polygons from https://www.iucnredlist.org.

Figure 3. Comparison between *Python sebae* (top) and *Python natalensis* (bottom) for colouration and scalation.

species, the validity of *P. natalensis* as a full species, the geographic origin of the genus, and to explore possible scenarios as to their absence from Madagascar.

Materials and methods

Thirty *Python* samples were included in the ingroup dataset, representing eight of the 10 species in the genus (Table 1; no comparative sequence data available on GenBank for *P. breitensteini* from Borneo or *P. kyaiktiyo* from Myanmar). Twenty of these were sequenced for this study, with the remainder of the data downloaded from GenBank (Table 1). In addition, sequence data from five individuals representing four outgroup species from other genera in Pythonidae were also downloaded from GenBank (Table 1). Some samples were composed of chimeric sequences, whereby gene sequences from different individuals were concatenated, given that a full complement of genes was not available for all individuals of a species. However, this affected mainly the outgroup with only a few *Python* species being chimeric (Table 1). Using the BLAST tool to verify GenBank data (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and by checking linked museum records online, some publicly available *Python* sequences appear to be incorrectly annotated and were therefore not included (GenBank Accessions: MK194022, EF545065, EF545064).

To generate the new sequences, total genomic DNA was extracted from tissue samples preserved in 99% ethanol, using a salt extraction protocol (Aljanabi and Martinez 1997). Gene fragments were amplified with polymerase chain reaction (PCR): mitochondrial ribosomal large subunit (*16S* 493 bp), cytochrome-b (*Cyt-b* 1,110 bp), and nuclear (nDNA) gene oocyte maturation factor Mos (*c-mos* 509 bp) using primers *16S*: 16Sa/16Sb (Palumbi et al. 2002); *Cyt-b*: L14910/H16064 (Burbrink et al. 2000); *c-mos*: FUF/FUR (Gamble et al. 2008) or S77/S78 (Lawson et al. 2005). Amplification took place using a 25 µl reaction mixture consisting of 2.5 µl reaction buffer, 2.5 mM $MgCl_2$, 2 µM of each primer, 0.2 mM DNTP solution, 0.5 U/µl Taq Polymerase (SuperTherm), and 25–50 ng/µl of DNA template. The thermal cycling consisted of initial denaturation for 4 min at 95 °C, followed by 35 cycles of denaturation (94 °C, 45 s), annealing (50–60 °C, 30 s), and extension (72 °C, 1 min), with a final extension at 72 °C for 10 min. PCR products were visualised through electrophoresis on a 0.8% agarose gel and Sanger sequencing was carried out at Macrogen (Amsterdam, Netherlands) using the forward primers for each gene. Sequences were aligned in Geneious v.11 (https://www.geneious.com) using MUSCLE alignment, and the coding genes were checked for the presence of stop codons or frame shifts in the codon sequences.

Phylogenetic estimation and dating

All phylogenetic analyses were run at the Cyberinfrastructure for Phylogenetic Research (CIPRES; Miller et al. 2010). Bayesian inference and maximum likelihood (ML) analyses were run on a combined evidence dataset of 2,118 characters and a total of 35 taxa, including outgroup taxa (analysis details provided in Suppl. material 1: supplementary methods). Thereafter, a multi-species coalescent approach was used to generate gene trees and a species tree using the *BEAST package in BEAST v2 with xml files created in BEAUTi v2 (Bouckaert et al.

Table 1. Individuals included in the phylogenetic analyses for *Python*, with identification numbers, GenBank accession numbers for *16S*, *Cyt-b* and *c-mos* genes, and locality information. Chimeric sequences were used for some individuals (denoted with an x). Samples from the area of sympatry or parapatry between *P. natalensis* and *P. sebae* are denoted with an asterisk in the ID number column. All new GenBank accessions are within the numbering series prefixed by "PV". Dashes indicate data not available.

Genus and species	chimeric	*ID number*	*16S*	*Cyt-b*	*c-mos*	Latitude, Longitude	Country	Locality	phylogeny	networks
Python anchietae		CAS 263501/ AMB.10656	PV364097	-	-	-16.20, 12.40	Angola	Namibe Province, Omahua	x	
Python anchietae		MVZ 232856	-	KF811118	KF811103	-	Pet trade	-	x	
Python anchietae		P9-182	PV364096	PV389862	PV389843	-12.99, 13.10	Angola	Dombe Grande	x	
Python bivittatus		1	KF293729	KF293729	-	-	China	Hainan Island	x	
Python bivittatus	x	2	KF010492	KF010492	AF435016	-	-	-	x	
Python brongersmai		ABTC24797	EF545066	EF545107	-	-	-	-	x	
Python curtus	x	UMFS 11257	AF215277	KF811119	KF811104	-	Indonesia	-	x	
Python molurus	x	-	-	AY099983	AY099968	-	-	-	x	
Python natalensis		BHLP017	PV364098	PV389863	PV389844	-24.20, 30.35	South Africa	Limpopo Province, Lekgalameetse	x	x
Python natalensis		BHLP061	PV364099	PV389864	PV389845	-24.20, 27.88	South Africa	Limpopo Province, near Vaalwater	x	x
Python natalensis		EI_0140	PV364100	PV389865	PV389846	-17.66, 31.78	Mozambique	Italthai	x	x
Python natalensis		F465	PV364101	PV389866	PV389847	-20.71, 34.12	Mozambique	Gorongosa Province	x	x
Python natalensis		HB109	PV364102	PV389867	PV389848	-27.80, 32.33	South Africa	KwaZulu-Natal Province, Phinda Nature Reserve	x	x
Python natalensis		HB111*	PV364103	PV389868	PV389849	-3.54, 35.72	Tanzania	Lake Manjara	x	x
Python natalensis		HB503	PV364104	PV389869	PV389850	-25.42, 31.94	South Africa	Mpumalanga Province, Crocodile Bridge	x	x
Python natalensis	x	MBUR 00795 (cmos: MBUR 00819)	PV364105	PV389870	PV389851	-23.95, 31.10	South Africa	Limpopo Province, Phalaborwa (MBUR 00819: -24.05, 30.99)	x	x
Python natalensis		NB0794 / REPT_0162	PV364106	PV389871	PV389852	-15.61, 14.88	Angola	Bicuar National Park	x	x
Python natalensis		WC12-A052	PV364107	PV389872	PV389853	-16.83, 17.96	Angola	Cuanado Cubango, near Savate village	x	x
Python regius		CHS268	MK194023	MK201373	-	-	-	-	x	
Python regius		-	AB177878	AB177878	-	-	-	-	x	
Python regius		HB481	PV364108	PV389873	PV389854	-	Pet trade	-	x	
Python regius		SL 92	PV364109	PV389874	PV389855	-	Sierra Leone	unknown	x	
Python sebae		ID#10-51	PV364110	PV389875	-	-	Angola	Zaire Province, Soyo	x	
Python sebae		ID#12-35	PV364111	PV389876	PV389856	-	Angola	Zaire Province, Soyo	x	x
Python sebae		KB-02	PV364112	PV389877	PV389857	-	Rwanda	unknown	x	x
Python sebae		MBUR 03034	PV364114	PV389879	PV389859	-4.15, 11.74	Republic of Congo	Kouilou	x	X (*c-mos*)
Python sebae		MBUR 08506	PV364113	PV389878	PV389858	10.62, 34.42	Ethiopia	Benishangul-Gumuz, near Kutaworke	x	x
Python sebae		NB0747 / P8-010*	PV364115	PV389880	PV389860	-9.34, 13.17	Angola	Kwanza River floodplain	x	x
Python sebae		NB0781 / P8-001*	PV364116	PV389881	PV389861	-10.65, 17.65	Angola	10 km north of Luquembo	x	x
Python sebae		UMFS 11459	-	KF811120	KF811105	-	-	-	x	x
Outgroup										
Antaresia childreni	x	-	EF545058	AY099994	AY099967	-	-	-	x	
Aspidites melanocephalus	x	-	EF545060	AMU69741	DQ465557	-	-	-	x	
Liasis mackloitsavuensis	x	-	AF544820	LMU69839	AF544726	-	-	-	x	
Apodora papuana	x	-	AF544814	LPU69843	AF544720	-	-	-	x	
Malayopython reticulatus	x	-	MH410033	MH410033	AF544675	-	-	-	x	

2019). *BEAST uses a Bayesian framework to account for incomplete lineage sorting and co-estimates individual gene trees and the species tree within which they evolved. For this analysis, each sample was assigned to a species according to current taxonomy. The *BEAST analysis was run with separate partitions for each gene for the substitution model estimation but with the two mitochondrial genes linked for the gene trees, and the clock model and all genes linked for the species tree estimation. The HKY model was applied for each partition with gamma shape

parameter and proportion of invariable sites using values estimated in *BEAST. The uncorrelated log-normal clock model was applied, the mean clock rates estimated and allowed to vary between partitions, and a Yule model of speciation was applied. The Monte Carlo-Markov chain (MCMC) was run for 1 billion generations, sampling the MCMC every 10,000 generations. The effective sample sizes of the parameters were checked in Tracer v1.7.1 (Rambaut et al. 2018) for values exceeding 200, after which TreeAnnotator (packaged with BEAST v2.6) was used to apply a 50% burn-in.

A dated phylogeny was constructed using BEAST v2.6 with xml files created in BEAUTi v2. Calibration points were based on previous dated phylogenies (Zheng and Wiens 2016; Esquerré et al. 2020) and fossil ages (see Hipsley and Müller 2014; Suppl. material 1: tables S1, S2). Fossils from the Miocene (ca. 18 Mya) identified as *P. sebae* and/or *P. natalensis* (Thomas 1982; Rage 2003) provided a conservative minimum age constraint for the most recent common ancestor (MRCA) for the stem group of the African *Python* clade (as recommended in Forest 2009). Other Miocene fossils exist of extinct (*P. maurus* Rage 1976), extant and unclassified *Python* species from southern, Central Africa, North Africa and Europe (e.g., Bailon and Rage 1994; Rage 2003; Szyndlar and Rage 2003; Rage and Bailon 2005; Georgalis et al. 2020; Suppl. material 1: table S1), but it is unknown to which phylogenetic clade these fossils should be assigned, only providing a minimum age for the stem group of the genus *Python*. Because the *P. sebae*/*natalensis* fossils already provide a constraint during approximately the same time period for the stem African clade, these additional fossils were not incorporated as calibration points (Suppl. material 1: table S1).

The dated BEAST analysis was 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 of nucleotide evolution was applied for each partition with gamma shape parameter and proportion of invariable sites using values estimated in bModeltest (Bouckaert and Drummond 2017). Three independent runs of 500 million generations of the MCMC were run sampling the MCMC every 5000 generations. The effective sample sizes of the parameters were checked in Tracer v1.7.1 (Rambaut et al. 2018) for values exceeding 200, after which TreeAnnotator (packaged with BEAST v2.6) was used to apply a 20% burn-in for each of the runs.

Networks were constructed for *Cyt-b* and for *c-mos* to examine haplotype/allele sharing between the partly sympatric, and morphologically similar species, *P. natalensis* and *P. sebae*. Other members of the ingroup were not included in the networks given that there were sequence data from few individuals for each of the other species. The datasets were trimmed to remove missing data, and several individuals were removed completely due to short or missing sequences, for a final dataset with complete coverage across all individuals (*Cyt-b*: 694 bp, n = 16; *c-mos*: 400 bp n = 17). Networks were constructed using the TCS algorithm (Clement et al. 2002) applied in PopArt v1.7 (Leigh and Bryant 2015). Heterozygous positions were identified in nuclear *c-mos* gene sequences by visual inspection for clean, double peaks in the chromatograms. For heterozygotes, allele sequences were estimated from the diploid nuclear sequences using PHASE v2.1.1 (Stephens et al. 2001). PHASE was run eight different times, using the recombination-linkage disequilibrium decay model, with different starting seeds for each run. Runs varied between 10000 and 100000 iterations for 100000 to 1 million generations, and all with a 10% burn-in. The resulting alleles were chosen based on those with the highest frequencies in the majority of the runs. For all individuals (homozygotes and heterozygotes), both alleles (n = 34) were inputted for construction of the *c-mos* network.

An ancestral area reconstruction was carried out using a Bayesian Binary MCMC (BBM) in RASP v4.0 beta (Yu et al. 2020) using the set of post burn-in ultrametric gene trees generated in BEAST and including the outgroup taxa. The coding for the terminal taxa was based on the known distributions of the species in the phylogeny: Central Africa, East Africa, southern Africa, Oriental, Oceania, and Australia (for the outgroup coding) generally following the biogeographic regions from Holt et al. 2013 (Suppl. material 1: table S3). The set of post burn-in trees from the first BEAST run was imported into RASP, from which 1000 trees were randomly sampled, and a consensus tree was generated. Given that current day distributions of some species incorporate biogeographic areas, the maximum number of areas allowed in the analysis was set to three.

Results

Both Bayesian (MrBayes and BEAST) and the likelihood analyses produced the same topology for *Python*, with well-supported nodes for each species in the genus (Suppl. material 1: fig. S1). The *BEAST species tree produced the same topology and support as the Bayesian and likelihood analyses (Suppl. material 1: fig. S2). All four African species (*P. anchietae*, *P. natalensis*, *P. regius*, *P. sebae*) were monophyletic to the exclusion of the four Asian species included in the analysis (*P. bivittatus*, *P. brongersmai*, *P. curtus*, *P. molurus*). Within the African clade, *P. regius* is sister the other three African species, having diverged earliest. This placement agrees with that of Reynolds et al. (2014) but contrasts with Pyron et al. (2013) who found *P. regius* and *P. sebae* to be within the Asian clade rendering both the African and Asian clades polyphyletic.

The dated phylogeny suggests that the genus *Python* diverged from other Pythonidae genera around 43 Mya (Fig. 4, Suppl. material 1: fig. S3, table S4). The African and Asian *Python* clades diverged approximately 33 Mya during the Late Eocene/Early Oligocene (Fig. 3). Most species level diversity in the Asian clade originated in the Late Miocene/Early Pliocene between 15 and 5 Mya (Fig. 4, Suppl. material 1: fig. S3, table S4). Two Asian species are missing from

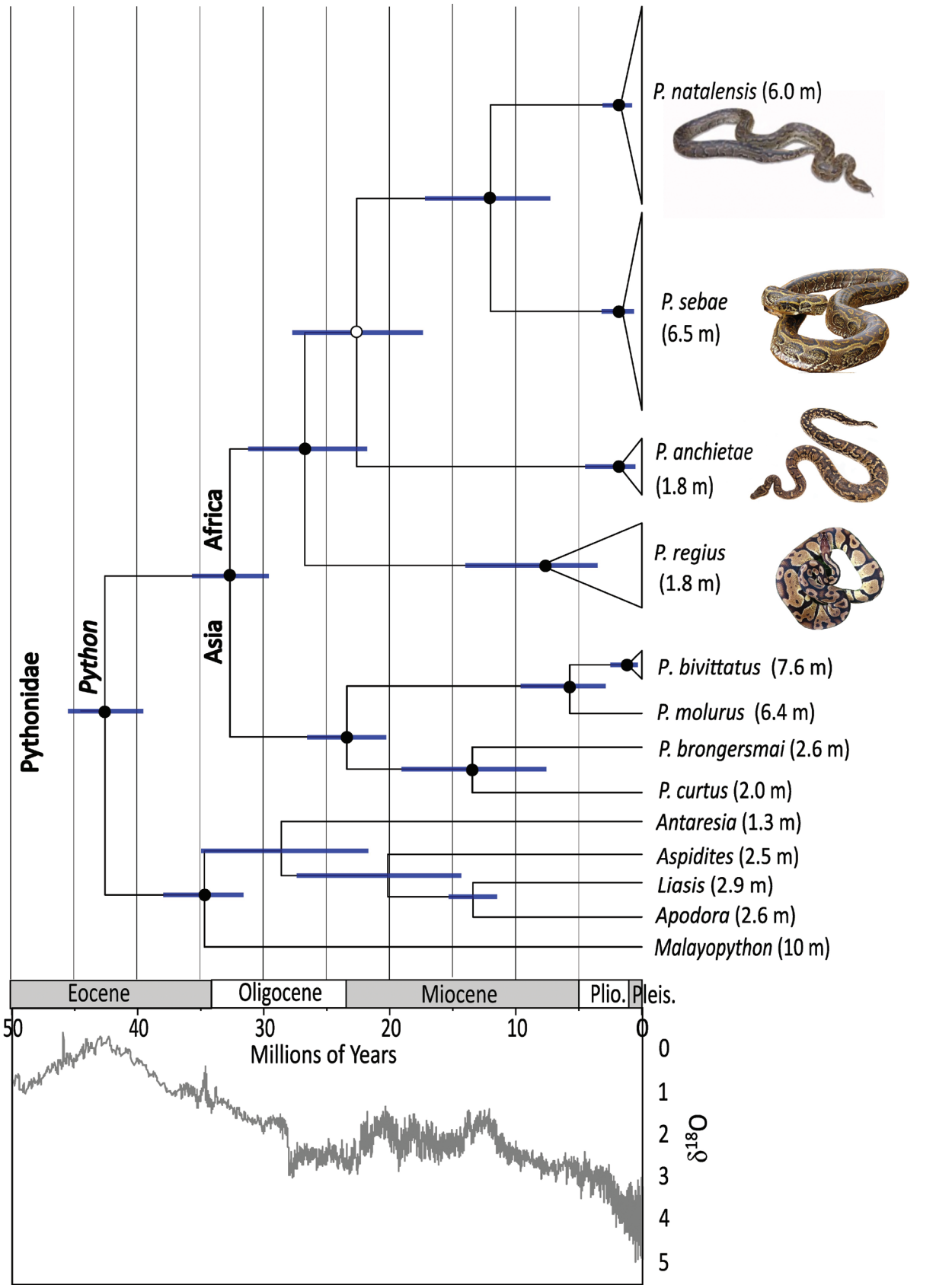

Figure 4. Dated phylogeny for Pythonidae with near-complete taxon sampling for the genus *Python*. Nodes with black dots supported by all analyses, whereas white dots indicate nodes supported by two of three analyses (Suppl. material 1: figs S1, S3). Body sizes are noted for species or genera (Suppl. material 1: table S5), and example photos of the African species are shown. Bottom: Time scale with geological Epochs labelled. Trend line shows the deep-sea oxygen isotopic ($\delta^{18}O$) record as a proxy for (global) temperature since the Eocene (graph reproduced from Zachos et al. 2001a).

our phylogeny (*P. breitensteini* and *P. kyaiktiyo*). It should be noted that *P. breitensteini* has been shown to be sister to *P. curtus* (Keogh et al. 2001), but those comparative data were unavailable from GenBank. However, we assume that *P. breitensteini – P. curtus* divergence also occurred within this same time frame and would be more recent than the split between *P. curtus* and *P. brongersmai* (i.e., less than ~13 Mya). Species level divergences within the African clade are generally older, with *P. regius* diverging around 26 Mya and *P. anchietae* at about 22 Mya (Fig. 4, Suppl. material 1: table S4). Although *P. natalensis* was formerly regarded as a subspecies of *P. sebae*, these taxa are clearly distinct sister species having diverged approximately 12 Mya. Furthermore, the networks showed clear divergence, with no haplotype or allele sharing between *P. natalensis* and *P. sebae* for either mitochondrial or nuclear genes, even for the slow-evolving *c-mos* gene, despite some individuals being sampled from the zones of sympatry (Fig. 5). We acknowledge, however, that to investigate potential gene flow leading to hybridisation or introgression between these species would require denser sampling in the presumed sympatric/parapatric zones.

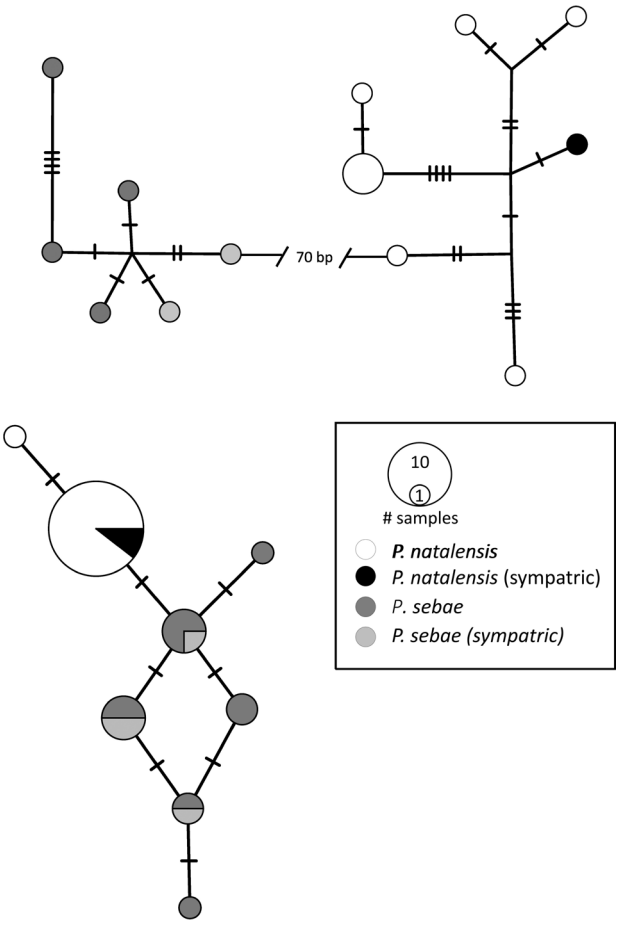

Figure 5. Network of haplotypes (Top: *Cyt-b*) and alleles (Bottom: *c-mos*) for *Python natalensis* and *Python sebae*. The size of the circles is proportional to the frequency of individuals with that haplotype/allele, and the branch lengths are proportional to the number of mutations. The branches interrupted by hatch marks are shortened, with the number of mutations along that branch indicated.

The BBM analysis showed a high probability for the Oriental biogeographic region as the ancestral area for the genus *Python* (Fig. 6). The reconstruction also suggests that *Python* colonised Africa from Asia (Oriental region) around 33 Mya given the divergence time of the African and Asian clades (Fig. 6, node 6 in Suppl. material 1: table S4). However, the presence of *Python* fossils in the Palearctic (Europe) and the Saharo-Arabian biogeographic regions from the Miocene (Fig. 2) more accurately indicates that *Python* would have arrived in Africa via those regions rather than directly from the Oriental region.

Discussion

Previous interpretations of the biogeographic history of pythons focussed on Australasian taxa, with limited taxon sampling and geographic coverage of African *Python* (Keogh et al. 2001; Reynolds et al. 2014; Esquerré et al. 2020, 2022). With improved taxonomic and geographic sampling, we show that all African species are monophyletic to the exclusion of Asian species, a finding that has not been shown previously. Furthermore, the clear divergence between *P. natalensis* and *P. sebae* dated at ca. 12 Mya, and the lack of allele or haplotypes sharing despite some individuals being sampled from zones of sympatry, provides strong support for *P. natalensis* being considered a full species as argued by Broadley (1999). In addition, our results support an Asian origin for the genus, with a single dispersal event into Africa in the early Oligocene (ca. 33 Mya). This was followed by diversification on the African continent beginning around 26 Mya, a time span that was relatively warm compared to the subsequent Miocene cooling (see Fig. 4 – bottom; Westerhold et al. 2020; Couvreur et al. 2021). Species level diversification ended in Africa around 12 Mya concomitant with the acceleration of the mid-Miocene cooling trend (Zachos et al. 2001a, b; Westerhold et al. 2020), and the fossil evidence shows an overall range contraction with some populations and species becoming extinct during that epoch (Fig. 2).

From the high-level dated phylogeny (Fig. 4), it can be inferred that the genus *Python* diverged from other genera of family Pythonidae in the late Eocene, approximately 43 Mya in Asia (see also Esquerré et al. 2020). Subsequently, the African and Asian clades of *Python* diverged (ca. 33 Mya) during the Eocene–Oligocene transition. The time frame is concurrent with the Afro-Arabian tectonic plates being partly submerged and positioned such that they were isolated from the Eurasian landmass by the Tethys Sea during the Cretaceous and into the early Cenozoic (Guiraud et al. 2005). Prior to that time, faunal or floral interchange between Afro-Arabia and Eurasia was probably rare and only possible via overseas dispersal, although this may have become more achievable by the early Oligocene as the continental plates drew closer and the marine transgressions receded (Godinot et al. 2003; Seiffert 2012; Hamon et al. 2013). Connections between the Afro-Arabian plates with Eurasia in the Oligocene presumably

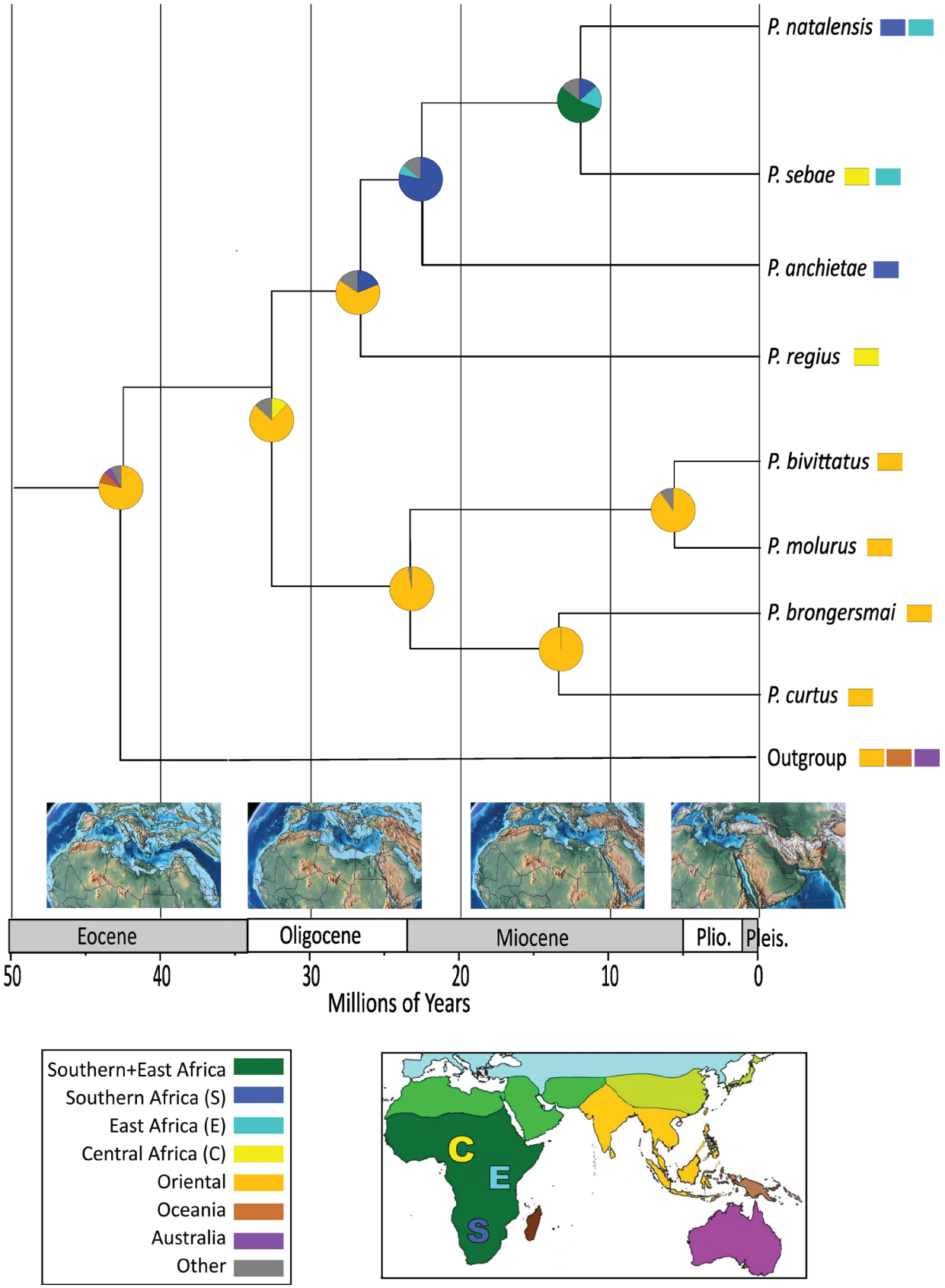

Figure 6. Probabilities of ancestral ranges at each node in the *Python* dated phylogeny, represented by pie charts that are colour-coded to the areas as indicated in the key (bottom left). Biogeographic regions (Holt et al. 2013) used to code the terminal taxa (bottom right), with Africa sub-partitioned into Central (C), East (E) and southern (S). Coding used at each terminal is shown by the coloured block(s) to the right of the species name. The relative locations of Africa, Arabia, Asia and Europe at four different time points since the Eocene are depicted (source: C.R. Scotese, PaleoMap Project 2013).

allowed for faunal and floral interchange (Vermeij 1991; e.g., vipers and cobras – Pook et al. 2009, *Varanus* – Portik and Papenfuss 2012; primates – Springer et al. 2012), providing an opportunity for at least one lineage of *Python* to enter the Afro-Arabian landmass.

Contraction of the tropics towards the equator intensified around the Eocene-Oligocene transition (Couvreur et al. 2021), approximately 33 Mya, forcing the African and Asian *Python* clades to retreat away from each other as the climate became cooler. Currently, environmental temperatures across much of the distribution gap regularly fall below 20 °C (Suppl. material 1: fig. S4), which is a critical threshold for egg incubation in *Python* (Alexander 2007) and is possibly similar for all Pythonidae. Furthermore, fossil evidence shows that the Arabian Peninsula and the Namib region were occupied by pythons during the early and mid-Miocene (Fig. 2, Suppl. material 1: table S1), prior to the Late Miocene progression toward hyper-aridity in these regions (Sepulchre et al. 2006; Saarinen et al. 2020; Siesser 2020; Couvreur et al. 2021; Doman and Early 2022), suggesting that hyper-aridity is an additional limiting factor. Overall, the present-day distribution of *Python* can be attributed to life-history traits that limit them to warm environments, ultimately forcing them to contract toward the topics and sub-tropics (Alexander 2007; Suppl. material 1: fig. S4).

The absence of *Python* on Madagascar both at present and in the fossil record is perhaps unsurprising. Given the post-Gondwanan age of Pythonidae, a Gondwanan distribution – which would include Madagascar – can be ruled out. Furthermore, most Malagasy reptile fauna colonised the island through overseas dispersal from eastern Africa during the Eocene and early Oligocene (ca. 55–35 Mya; Ali and Hedges 2023), a time period prior to the arrival of *Python* in Africa. It is presumed that paleo-currents were optimal during that period and facilitated colonisation of the island via overseas dispersal from eastern Africa (Ali and Huber 2010; Samonds et al. 2012, 2013; Tolley et al. 2013). The arrival of *Python* in northern Africa from Asia coincided with the shifting of the relative positions of Africa and Madagascar that changed the relative direction of the oceanic currents away from Madagascar (Ali and Huber 2010), making overseas dispersal to Madagascar much less likely. Thus, the arrival of *Python* in Africa after the optimal dispersal period toward Madagascar provides a reasonable explanation as to the absence of pythons on the island. Notably, this explanation is independent of climatic drivers that have been invoked to explain the other distribution gaps for *Python*.

Of the ten species in the genus *Python*, four qualify as being amongst the largest extant snakes on Earth (Murphy and Henderson 1997; Suppl. material 1: table S5), and all these large-bodied species are limited to the tropical and sub-tropical regions of Asia and Africa (O'Shea 2007). Fossil evidence also indicates that species which previously occurred in the geographic gap between current day African and Asian *Python* were large-bodied with vertebrae easily exceeding 10 mm in breadth (see Rage and Bailon 2005; Ivanov and Böhme 2011; Georgalis et al. 2020) correlating to body sizes in excess of 4 m (Rage 1976; compare to Head et al. 2009). Given their fossil record, it is likely that the ancestral lineage of *Python* that extended its range into Africa was large-bodied. Functional traits of snakes such as their elongated shape, ectothermic physiology and prey size selection, which are associated with gape-limitation, are thought to have a significant influence on the evolution of snake body size (Boback and Guyer 2003). For example, it has been suggested that prey size is an important determinant of snake body size, where availability of large prey drives the evolution toward larger body and gape size (Boback and Guyer 2003) so that large-bodied snakes can meet their energy budgets. An alternative hypothesis for the restriction of large snakes to warm areas is that large-bodied snakes warm more slowly than small-bodied snakes while basking, restricting the time spent at target body temperatures in cool environments (Bogert 1949; Alexander 2007). Shine (1994) has also proposed that selection for large body size can result from larger females due to fecundity advantages. Thus, the evolution of large body size may be linked to warm climates, abundance of mega-faunal prey which existed on both continents (Steinthorsdottir et al. 2021) and/or reproductive advantages linked to large body size.

While some areas of Africa are hyper-diverse biologically (e.g., Fjeldså and Lovett 1997; Bayliss et al. 2024), Africa stands out as having relatively low species richness compared to other tropical regions (Couvreur 2015; Raven et al. 2020; Hagen et al. 2023), consistent with the low *Python* richness in Africa. Along these lines, the adaptive diversification that occurred with the invasion of Australasia by Pythonidae (27 species from 9 genera; Esquerré et al. 2017, 2022) was far more dramatic than for Africa (4 species from one genus). The high richness for pythons in Australasia has been explained by the colonization of environmentally diverse regions with novel niches, promoting allopatric speciation (Esquerré et al. 2022), consistent with the hypothesis that speciation rates are higher outside Africa. The comparatively low speciation rates for Africa appear to be driven by the unique combination of an arid climate, reduced topographic and habitat complexity, and long-term geographic isolation through the early Cenozoic (Couvreur 2015; Raven et al. 2020; Couvreur et al. 2021; Hagen et al. 2023). These same environmental features probably also reduced opportunities for *Python* to speciate in Africa, limiting niche diversity and reducing allopatric speciation due to an arid, homogeneous habitat.

Conclusions

With improved taxon sampling compared to previous studies, we showed that the most likely geographic origin of genus *Python* is Asia, with a single dispersal event into Africa corresponding with the closing of the Tethys Sea and newly established contact of the African and Eurasian landmasses. Our interpretation, however, is based on a dataset that includes few loci (two mitochondrial and one

nuclear genes) and confidence in this hypothesis could be improved through more detailed genomic datasets and approaches. Nevertheless, our overall topology agrees with recent analyses that incorporate genomic approaches (Esquerré et al. 2020, 2022), thus, we do not expect that our current interpretation, despite being based on limited gene sampling, would notably change.

At present, *Python* species occur in mesic to humid sub-tropical or tropical regions and are excluded from temperate and arid regions in both Asia and Africa, with the exception of *P. anchietae* which has adapted to arid (but not hyper-arid) conditions. The fossil record shows that the present-day distribution gap was once occupied by pythons up until the mid-Miocene, during an era when those areas were warmer and more mesic. Thus, the present day and presumed paleo-distributions based on fossil evidence support the hypothesis that climate, particularly temperature and aridity, can be considered strong range limiters for pythons due to decreased recruitment of offspring outside warm and mesic environments.

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Author contributions

Conceptualization: KAT, GJA. Formal analysis: KAT. Funding acquisition: KAT. Investigation: KAT, GJA. Writing – original draft: KAT, GJA. Writing – review and editing: KAT, GJA.

Data accessibility statement

Genetic data are accessible on GenBank as per Table 1.

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Supplementary materials

Supplementary material 1

Supplementary tables S1–S5 and figures S1–S4 (Phylogenetic tree files (figs S1–S3) https://doi.org/10.6084/m9.figshare.28737716) (.docx)
Link: https://doi.org/10.21425/fob.18.146955.suppl1