

## APPENDIX

### Appendix I: Methods and results further details

#### *DNA Sequencing and Alignment Preparation*

To obtain sequence data, we used anchored hybrid enrichment (Lemmon et al. 2012), with modifications described in Prum et al. (2015). This process involved sonication of each extract to fragments of ~150–500 bp using a Covaris Ultrasonicator, indexed library preparation using a Beckman-Coulter Biomek FXp liquid-handling robot, size selection via SPRIselect beads, and pooled enrichment (12–16 samples) using an Agilent Custom SureSelect kit with custom designed baits (Ruane et al. 2015). Following enrichment, we sequenced pooled samples on one lane of an Illumina HiSeq 2500 PE150 at the College of Medicine Translational Laboratory at Florida State University.

We merged the overlapping reads following Rokyta et al. (2012) and assembled the loci using the approach of Hamilton et al. (2016), but with *Anolis carolinensis* (Alföldi et al. 2011) and *Calamaria pavementata* (Ruane et al. 2015) genomes as references. We formed consensus sequences from assembled clusters containing > 250 mapped reads. We subsequently determined orthology using a neighbor-joining clustering approach (see Hamilton et al. 2016 for details), and orthologues were aligned using MAFFT v7.023 (Kato and Standley 2013). Alignments were auto-trimmed/masked following Hamilton et al. (2016), but with MINGOODSITES=14, MINPROPSAME=0.4, and MISSINGALLOWED=24. Finally, we visually inspected the auto-trimmed/masked alignments in Geneious R9 (Biomatters Ltd., Kearse et al. 2012) to verify that any misaligned regions were removed. The final dataset comprised 376 loci with an average length of 1635 bp.

Additionally, we reconstructed the mitochondrial genomes from the raw reads for each sample using a wrapper R script based on MITObim version 1.9 (Hahn et al. 2013). A link to these scripts can be found in at [www.github.com/IanGBrennan/mitoGenome\\_Assembly](http://www.github.com/IanGBrennan/mitoGenome_Assembly). As a reference, we used the mitochondrial genome of *Python regius* (Dong and Kumazawa 2005). We aligned the reconstructed sequences using MAFFT version 7.3 (Kato and Standley 2013) and then carefully inspected and edited the alignment by eye. For further phylogenetic inference we only analysed the coding regions of the mitochondrial genomes.

### *Phylogenetic hypotheses*

*Concatenation and gene trees.* In order to evaluate the robustness of our results, we performed several different approaches of phylogenetic inference. First, we used the concatenated alignment of 376 loci to perform Maximum Likelihood (ML) phylogenetic inference using RAxML version 8.2 (Stamatakis 2014). We used the more complex GTRGAMMA model and partitioned the alignment with the partitioning scheme inferred above. We used the option “-f a” to perform a rapid bootstrap analysis with 1,000 replicates and search for the highest scoring ML tree simultaneously and set *Xenopeltis unicolor* as the outgroup. This approach was used for the nuclear and mitochondrial alignments separately. Then, we performed a Bayesian phylogenetic inference using MrBayes version 3.2 (Ronquist et al. 2012). We used the same alignment and partitions as above but used the best substitution model estimated by PartitionFinder 2 for each partition. We ran 2 independent analyses with 3 heated and one cold chains each, for 20 million generations, sampling every 1,000 generation and a burnin of 5 million generations. We assessed the convergence of all the parameters of the runs by inspecting the ESS (Effective Sample Size).

For subsequent analyses on species trees, we generated individual gene trees on each of the loci with ML inference using RAxML with the same partitioning and specifications as the concatenated analysis, but with only 100 bootstrap replicates and no outgroup, since the analysis described below (Astral) requires unrooted trees.

Finally, we also estimated the individual gene trees for each locus running random sets of 10 loci (an arbitrary number that allows practical computational times) on StarBeast2 (Ogilvie et al. 2017) to eventually obtain gene trees for all the loci. We unlinked the loci substitution models and set all sites to a GTR+G model. We linked the clocks for each locus under a strict molecular clock with a prior of substitution rate at 0.001 sites per million years, which has been estimated for nuclear exons in snakes (Hugall et al. 2007). We used strict instead of relaxed clocks because strict clocks have been found sufficient for phylogenomic studies when estimating substitution rates is not of direct interest (Ogilvie et al. 2017). We ran the MCMC chains for each analysis for 2 billion generations, sampling every 50,000, and checked that the chains had proper mixing and convergence with Tracer v.1.6.0 (Rambaut et al. 2014) and RWTY v.1.0.1 (Warren et al. 2017), confirming an ESS of over 200 for every parameter including tree topology.

## **Appendix II: Fossil calibrations**

There were four fossil calibrations we could use to achieve convergence. The dates calculated between all the fossils were within each other's 95% HPD, except for the dates estimated

using only *Procerophis sahnii* which were around half the age of the rest (Table S3).

Therefore, for our final dating analysis we excluded this calibration from the analysis.

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1. *Titanoboa cerrejonensis* was used to calibrate the split between Erycinae and Boinae (Head et al. 2009), for which we used an exponential distribution with an offset of 58 Ma and a mean of 3, to model a minimum hard age of 58 and a maximum soft age of 64 Ma (Jaramillo et al. 2007; Woodburne et al. 2014; Head 2015).
2. *Procerophis sahnii* to calibrate the divergence between the Colubriiformes and Acrochordidae+Xenodermatidae, with a uniform distribution between 50.5 and 72.1 Ma (Head et al. 2016).
3. Undescribed python fossils from the middle Eocene from Germany (Szyndlar 1994; Szyndlar and Rage 2003) can be used as stem pythonid fossils to calibrate the node splitting Loxocemidae and Pythonidae to a minimum age of 48.5 Ma. “*Ogmophis*” *compactus* can also be used to calibrate the divergence between Loxocemidae and Pythonidae using an exponential distribution with an offset of 35.2 Ma and a mean 3, to describe a hard minimum age of 35.2 and an indeterminate maximum (Smith 2013; Head 2015). Since the fossil of *Ogmophis compactus* cannot be unequivocally assigned to Loxocemidae, we decide to use the middle Eocene pythonid fossils from Europe to calibrate this split.
4. The fossil of *Morelia riversleighensis*. This taxon is difficult to place as a calibration in a phylogeny since it has been assigned to a definition of *Morelia* that is now considered polyphyletic (Kluge 1993; Head 2015). Specimens now assigned to this taxon were first described as *Montityphonoides riversleighensis* and *Morelia antiqua* (Smith and Plane 1985) but later assigned to the extant *Morelia spilota* and *Liasis olivaceus* respectively (Kluge 1993). Nevertheless, both taxa were suggested

later to belong to the same extinct species, *Morelia riversleighensis* (Scanlon 2001). The definition of *Morelia* used by these and other authors is now considered polyphyletic (Pyron et al. 2013; Reynolds et al. 2014; Head 2015), and the split it could calibrate, between *Liasis* and *Morelia*, is the initial split within the Australo-Papuan pythons since each genus is in a different clade. Head (2015) suggests using this fossil to put a minimum age on Australo-Papuan pythons of 12.5 Ma, based on the holotype specimens from Henk's Hollow Site in System C of the Riversleigh fossil sites., However, Scanlon (2001) describes fossils from this taxon from Systems A and B, which are as old as the late Oligocene, based on faunal fossil correlates (Archer et al. 1989), which places pythons in Australia at least 23 Ma.

Other fossils have been assigned to extant genera. For example (Scanlon and Mackness 2001) described *Liasis dubundingala* from the early Pliocene, at a time when the concept of *Liasis* included *Bothrochilus* and *Leiopython*. As our phylogeny shows that the former concept of *Liasis* is paraphyletic with *Aspidites*, it is unclear where *Liasis dubundingala* could be justifiably placed. Therefore, the only fossil we can use within pythons is *Morelia riversleighensis*.

Two important fossils, one from Saint-Maximine (Duffaud and Rage 1997) and another from the Paris Basin (Augé et al. 1997), both in France, have been attributed based on the Mammal Paleogene Zone (MP) to the Lutetian, 40.4-48.6 Ma (Gibbard et al. 2010) and the Ypresian, 48.6-55.8 Ma, respectively. These fossils correspond to a palatine with a maxillary foramen, which is characteristic of pythonids. However, the posterior part of these palatines is placed very posteriorly to the choanal and maxillary processes, a trait unknown in modern pythons, therefore they could belong to stem pythonid taxa and we still cannot confidently attribute

them to pythonids (Szyndlar and Rage 2003). The oldest fossils confidently attributed to pythonids based on cranial and post-cranial bones are dated to the middle Eocene from the Messel and Geiseltal sites in Germany (Szyndlar 1994; Szyndlar and Rage 2003). The Geiseltal site corresponds to 42.7-48.5 Ma (Vandenberghe et al. 2012), while Messel corresponds to 47.5-48 Ma (Mertz and Renne 2005). These are attributed to pythons due to their high vertebral count (over 400), a palatine not projected posteriorly, and a maxillary foramen lying completely within the palatine (Szyndlar and Böhme 1993; Szyndlar 1994).

### Appendix III: Taxonomic implications

Kluge (1993) produced a detailed phylogeny of Australo-Papuan pythons based primarily on morphology and also some behavioral characters. He used this phylogeny as the basis for revising the group and he split them in to a number of genera, but he also acknowledged that more work was needed within group (p. 50): “I suggest attributing names to more inclusive groups of pythonines await complete resolution of relationships and confirmation of that overall pattern with additional evidence”. Nevertheless, most of his generic arrangement is in common usage. We have taken a conservative approach and use his framework to maintain taxonomic stability and have revised only where necessary based on our phylogeny. Detailed reviews on the taxonomic history of the Pythonidae have been done recently (Schleip and O'Shea 2010; Reynolds et al. 2014; Barker et al. 2015), and the different taxonomic arrangements proposed in the major works on python systematics are summarized in Table S7.

An important finding of our study is the placement of the Papuan olive python, *Apodora papuana*, in our phylogeny. This species was originally described as *Liasis papuanus* Peters & Doria 1878, and although an affinity with the water python (*Liasis mackloti*) was pointed out (Peters and Doria 1878), this was when *Liasis* had a broader taxonomic definition. Over a century later, Kluge (1993) described the monotypic *Apodora* for the species *Apodora papuana*. Later, molecular studies found *Apodora papuana* to be sister to *Liasis olivaceus* (Rawlings et al. 2008; Pyron et al. 2013; Reynolds et al. 2014), rendering *Liasis* paraphyletic. For this reason, Reynolds et al. (2014) subsumed *Apodora* into *Liasis*. In our study, with both nuclear and mitochondrial data, we find strong support of *Apodora papuana* being sister to *Liasis*, and due to the morphological and biological differences between these two lineages

(Barker et al. 2015), we continue to recognise *Apodora*. Similarly, *Leiopython* has been synonymized with *Bothrochilus* (Rawlings et al. 2008; Pyron et al. 2013; Reynolds et al. 2014). Our results do support a very close relationship and a divergence of around 5 Ma between the two lineages, but due to the strong morphological differences between the two we retain both of these genera (Kluge 1993; Barker et al. 2015).

Based on the polyphyly of *Morelia sensu* Kluge (1993), Reynolds et al. (2014) resurrected *Simalia* to accommodate the species that would not belong to *Morelia* anymore (see Table S7). Our analyses support this arrangement, except for the case of *oenpelliensis* (see below).

Using mitochondrial DNA, the water pythons (*Liasis mackloti/fuscus*) had been found to include eastern (Queensland and New Guinea) and western (Northern Territory and Indonesian islands) clades (Rawlings et al. 2004), and it was suggested that the name *L. fuscus* be used for the eastern clade and the name *L. mackloti* for the western clade. Given that the population of *L. mackloti* from Northern Territory, Australia, is recovered as sister to *Liasis fuscus* from Queensland, Australia, we propose that all populations from Australia and New Guinea be referred to as *Liasis fuscus*, and *Liasis mackloti* is used for Indonesian islands.

#### *Unresolved issues*

Our study resolves the relationships between the python species and genera. However, relatively little attention has been given to intra-generic relationships and species delimitation. Several groups would benefit from thorough phylogenomic studies to determine how many truly independent lineages there are, in particular the species complexes found



around *Python molurus*, *P. sebae*, *Antaresia* and *Leiopython*. Additionally, some groups that have been studied using small mitochondrial fragments or morphological and behavioral data, like the *S. amethystina* (Harvey et al. 2000), *P. curtus* (Keogh et al. 2001), *M. viridis* (Rawlings and Donnellan 2003), *L. mackloti* (Rawlings et al. 2004; Carmichael et al. 2007), *M. reticulatus* (Auliya et al. 2002; Murray-Dickson et al. 2017), *Leiopython* (Schleip 2008) and *M. spilota* (Ciavaglia et al. 2014) complexes would certainly benefit from phylogenomic studies.

Our nuclear data agree with placing the ball python (*P. regius*) as the sister to all other Afro-Asian pythons (Reynolds et al. 2014). However, we do not have the other African species in our nuclear dataset, and our mitochondrial analyses infer all the African taxa as monophyletic, and sister to the Asian taxa.

#### *The Australian scrub python*

Stull (1933) described the subspecies *Liasis amethystinus kinghorni* with type locality Lake Barrine, Queensland, Australia (Stull 1933), and provided a brief diagnosis from the nominal subspecies from New Guinea. In their systematic review and molecular study, Harvey et al. (2000) divided the *amethystina* complex into several species, and elevated *Morelia kinghorni* to full species and placed *Liasis clarki* Barbour, 1914 from the Torres Strait (between Australia's Cape York and New Guinea) as a junior synonym of *Morelia amethystina*. Cogger (2014) argued that Harvey et al. (2000) did not provide a diagnosis for *M. kinghorni* (which was already provided by Stull in the original description) and that the synonymy of *Liasis clarki* into *M. amethystina* was arbitrary. He further argued that although likely a valid

species, *M. kinghorni* should be considered a junior synonym of *M. amethistina* until the status of *Liasis clarki* was resolved.

The group still requires major work in establishing the species delimitation, particularly since the name *Simalia amethistina* is still applied to a polyphyletic group of populations. Although we did not include samples from southern New Guinea, our data demonstrate that the Australian populations are not West New Britain's closest relatives, but instead are the closest relatives to *Simalia nauta* from Yamdena Island in Maluku, Indonesia. Therefore, for the moment do not support the proposal by Cogger (2014) to synonymize *Simalia kinghorni* into *S. amethistina* (Cogger 2014) until further studies on this group are done. Further studies also need to include samples from the Torres Strait because if these populations are found to be conspecific, then the name *Simalia clarki* (Barbour, 1914) would have priority for the Australian scrub python.

#### *Morelia, Simalia, and the Oenpelli python*

The Oenpelli python, might be the rarest python on Earth. Only found in the remote Arnhem Land escarpments of northern Australia, and with a unique combination of morphological traits, it was described scientifically only in the late 1970s (Gow 1977), and has been very hard to place in the phylogeny of pythons. In its original description, this snake was assigned to the *reticulatus* group, back then in *Python* (Gow 1977). Nevertheless, Gow's definition of the *reticulatus* group is today known to be polyphyletic, including *Malayopython reticulatus*, *Simalia amethistina*, *Simalia boeleni* and *Morelia spilota*. Later, in the first phylogenetic study to include the Oenpelli python, Kluge placed it within *Morelia*, along with *boeleni*,

*amethystina*, *carinata*, *spilota* and *viridis* (Kluge 1993). This was later confirmed by Rawlings et al. (2008) using a combined dataset of Kluge's morphological data and mitochondrial genes. A later study using mitochondrial sequences inferred the clades we call here *Morelia*, *Simalia* and *Nawaran* gen. nov. as monophyletic, but they did not include species from other groups (Ciavaglia et al. 2014). However, by using a more complete sampling and relying purely on molecular genetic data, that traditional definition of *Morelia* was found polyphyletic, with *amethystina*, *boeleni* and *oenpelliensis* in one clade and *spilota*, *viridis* and *carinata* in another (Pyron et al. 2013). Finally, Reynolds et al. (2014), finding the same results, placed the first three (*amethystina*, *boeleni* and *oenpelliensis*) in *Simalia*. In our results, we do find support for these two independent clades. However, *oenpelliensis* actually is more closely related to *Morelia* than *Simalia*. The divergence between *Morelia*, *Antaresia* and the Oenpelli python lineages seems to have been very rapid and the relationships are hard to resolve. Because of this, the fact that it is a unique and independent lineage, and that the Oenpelli python is morphologically divergent to an obvious degree, we describe the new genus *Nawaran* gen. nov. for this species.

Wells & Wellington (1985) proposed the generic name *Nyctophilopython* for *oenpelliensis* but we do not recognize their name. They produced no evidence in their brief description for a unique lineage and it is well understood that their self-published work does not adhere to good practices in taxonomy (Kaiser et al. 2013). Further, the name has never come into common usage. The Australian Society of Herpetologists has recommended that new names should be published when peer-reviewed scientific evidence warrants such recognition (ASH, 2016: <http://www.australiansocietyofherpetologists.org/position-statements/>).



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#### Appendix IV: Pythonidae Fitzinger, 1826 species list

Python species list grouped by clade. Species with \* missing from the study and species with ° are only represented with mitochondrial data.

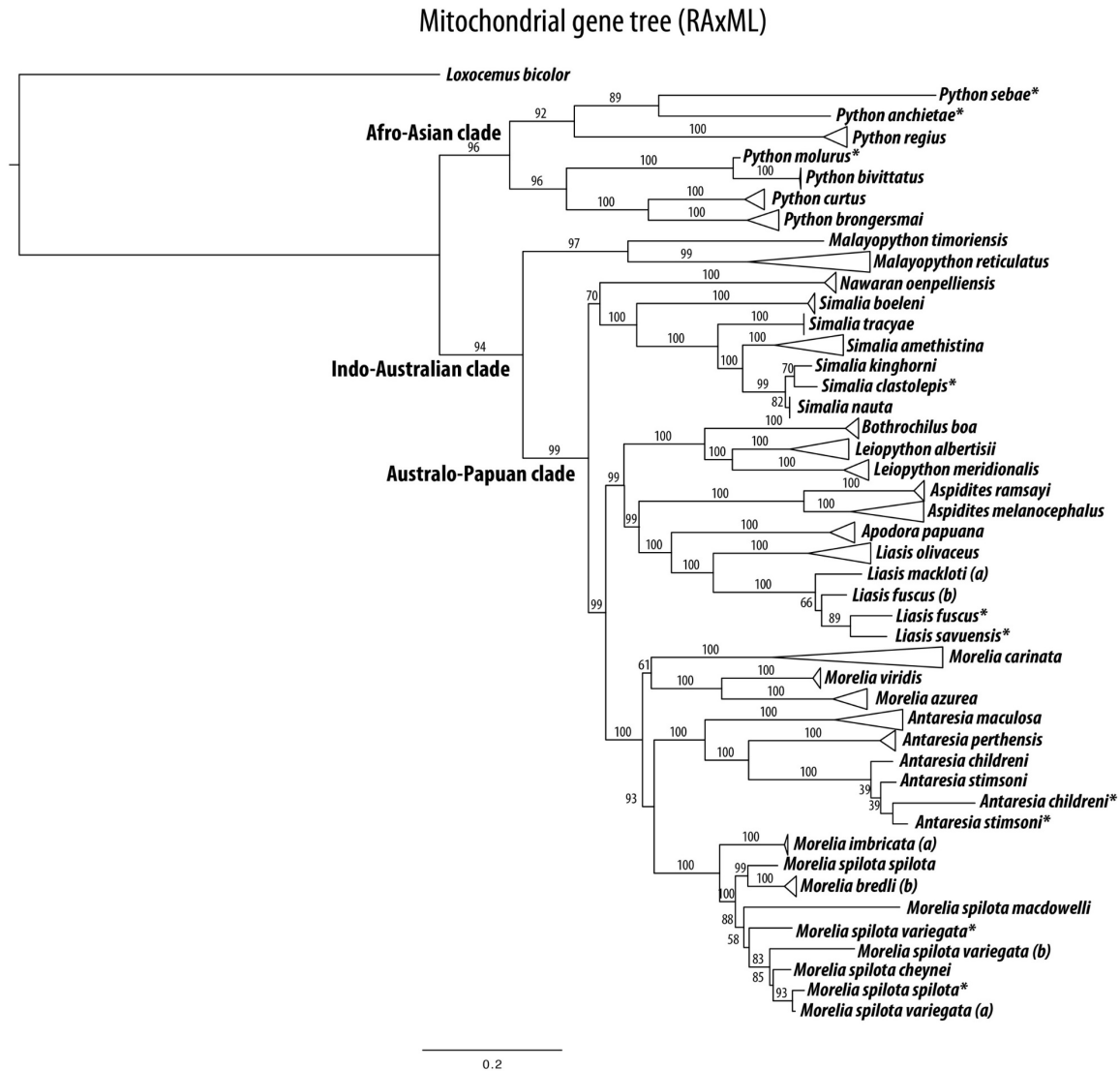
Clade I. Afro-Asian clade		
	<i>Python</i> Daudin, 1803 - 1. African clade	<i>Python anchietae</i> Bocage, 1887° <i>Python natalensis</i> Smith, 1840* <i>Python regius</i> (Shaw, 1802) <i>Python sebae</i> (Gmelin, 1789)°
	<i>Python</i> Daudin, 1803 - 2. Asian clade	<i>Python bivittatus</i> Kuhl, 1820 <i>Python breitensteini</i> Steindachner, 1881 <i>Python brongersmai</i> Stull, 1938 <i>Python curtus</i> Schlegel, 1872 <i>Python kyaictiyo</i> Zug, Gotte & Jacobs, 2011* <i>Python molurus</i> (Linnaeus, 1758)°
Clade II. Indo-Australian clade		
1. Wallacean clade	<i>Malayopython</i> Reynolds, Niemiller & Revell, 2014	<i>Malayopython reticulatus</i> (Schneider, 1801)
2. Australo-Papuan clade	a. <i>Morelia</i> clade	<i>Malayopython timoriensis</i> (Peters, 1876)
	<i>Antaresia</i> Wells & Wellington, 1984	<i>Antaresia childreni</i> (Gray, 1842) <i>Antaresia maculosa</i> (Peters, 1873) <i>Antaresia perthensis</i> (Stull, 1932) <i>Antaresia stimsoni</i> (Smith, 1985)
	<i>Morelia</i> Gray, 1842	<i>Morelia azurea</i> (Meyer, 1874) <i>Morelia bredli</i> (Gow, 1981) <i>Morelia carinata</i> (Smith, 1981) <i>Morelia imbricata</i> Smith, 1981 <i>Morelia spilota</i> (Lacépède, 1804) <i>Morelia viridis</i> (Schlegel, 1872)

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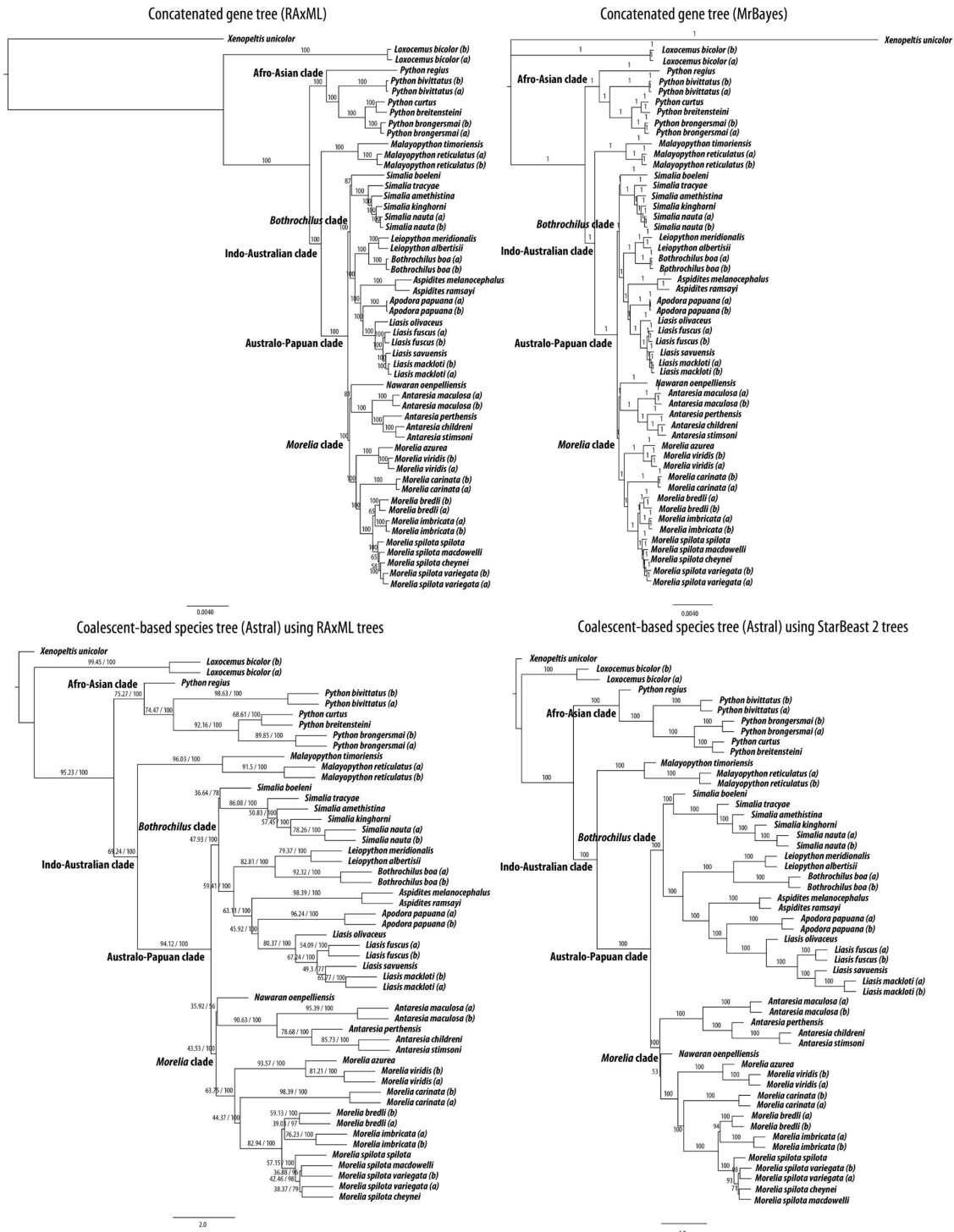
	<i>Nawaran</i> Esquerré, Donnellan, Brennan, Lemmon, Lemmon, Zaher, Grazziotin & Keogh, 2018	<i>Nawaran oenpelliensis</i>
b. <i>Bothrochilus</i> clade	<i>Apodora</i> Kluge, 1993	<i>Apodora papuana</i> (Peters & Doria, 1878)
	<i>Aspidites</i> Krefft, 1864	<i>Aspidites melanocephalus</i> (Krefft, 1864)
	<i>Bothrichilus</i> Fitzinger, 1843	<i>Aspidites ramsayi</i> (Macleay, 1882)
	<i>Leiopython</i> Hubrecht, 1879	<i>Bothrochilus boa</i> (Schlegel, 1837)
		<i>Leiopython albertisii</i> (Peters & Doria, 1878)
		<i>Leiopython biakensis</i> Schleip, 2008*
		<i>Leiopython fredparkeri</i> Schleip, 2008*
		<i>Leiopython huonensis</i> Schleip, 2008*
		<i>Leiopython meridionalis</i> Schleip, 2014
		<i>Leiopython montanus</i> Schleip, 2014*
	<i>Liasis</i> Gray, 1842	<i>Liasis dunni</i> Stull, 1832*
		<i>Liasis fuscus</i> Peters, 1873
		<i>Liasis mackloti</i> Duméril & Bibron, 1844
		<i>Liasis olivaceus</i> Gray, 1842
		<i>Liasis savuensis</i> Brongersma, 1956
	<i>Simalia</i> Gray, 1849	<i>Simalia amethystina</i> (Schneider, 1801)
		<i>Simalia boeleni</i> (Brongersma, 1956)
		<i>Simalia clastolepis</i> (Harvey, Barker, Ammerman & Chippindale, 2000)°
		<i>Simalia kinghorni</i> (Stull, 1933)
		<i>Simalia nauta</i> (Harvey, Barker, Ammerman & Chippindale, 2000)
		<i>Simalia tracyae</i> (Harvey, Barker, Ammerman & Chippindale, 2000)

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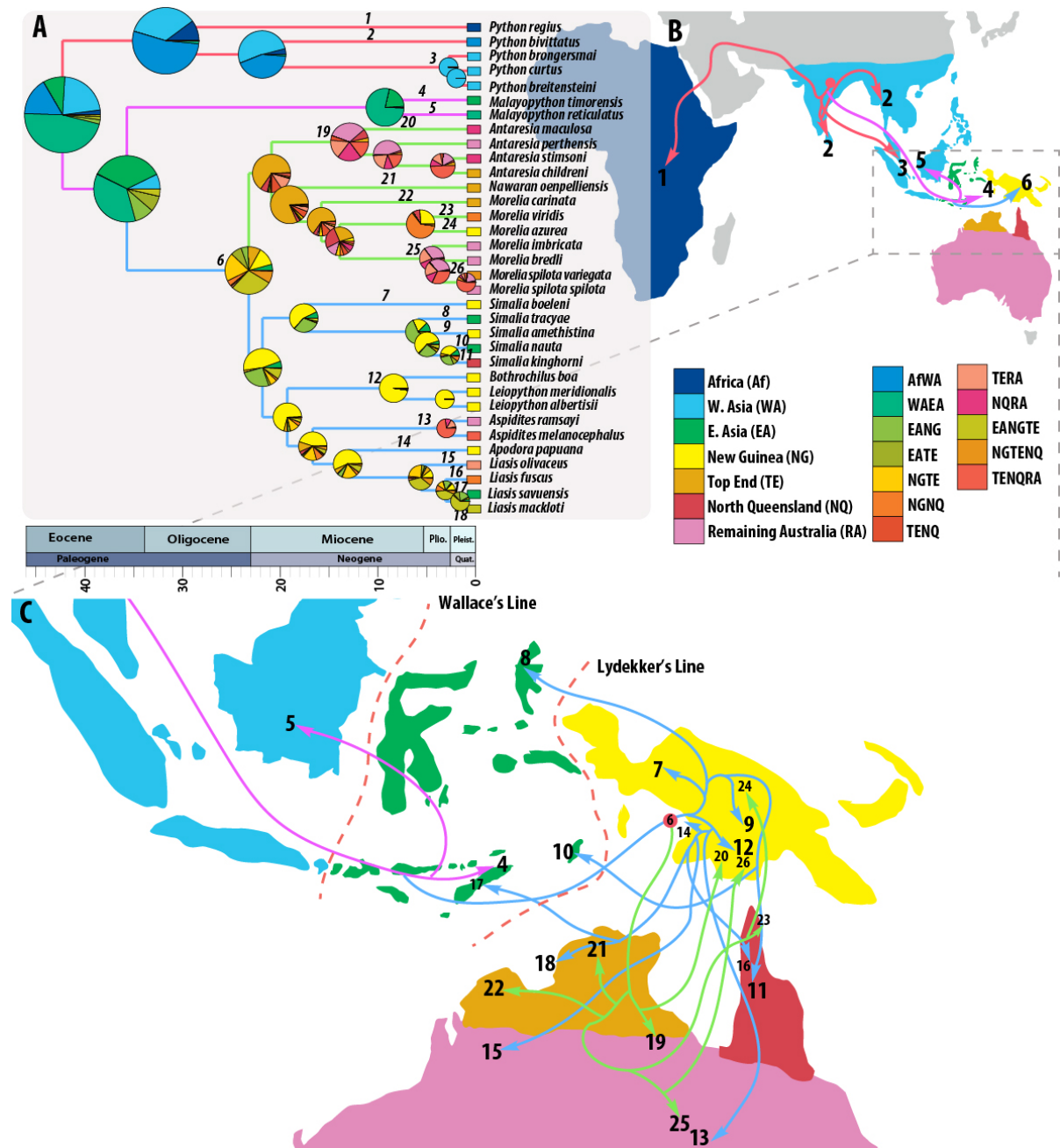
**Figure S1.** Maximum Likelihood tree using the mitochondrial genomes (our study) and the mitochondrial loci from Reynolds et al. (2014). Clades with multiple samples of the same taxon have been collapsed for ease of visualization. Tips with an asterisk indicate data from Reynolds et al. (2014). Branch labels indicate bootstrap support based on 1,000 replicates.



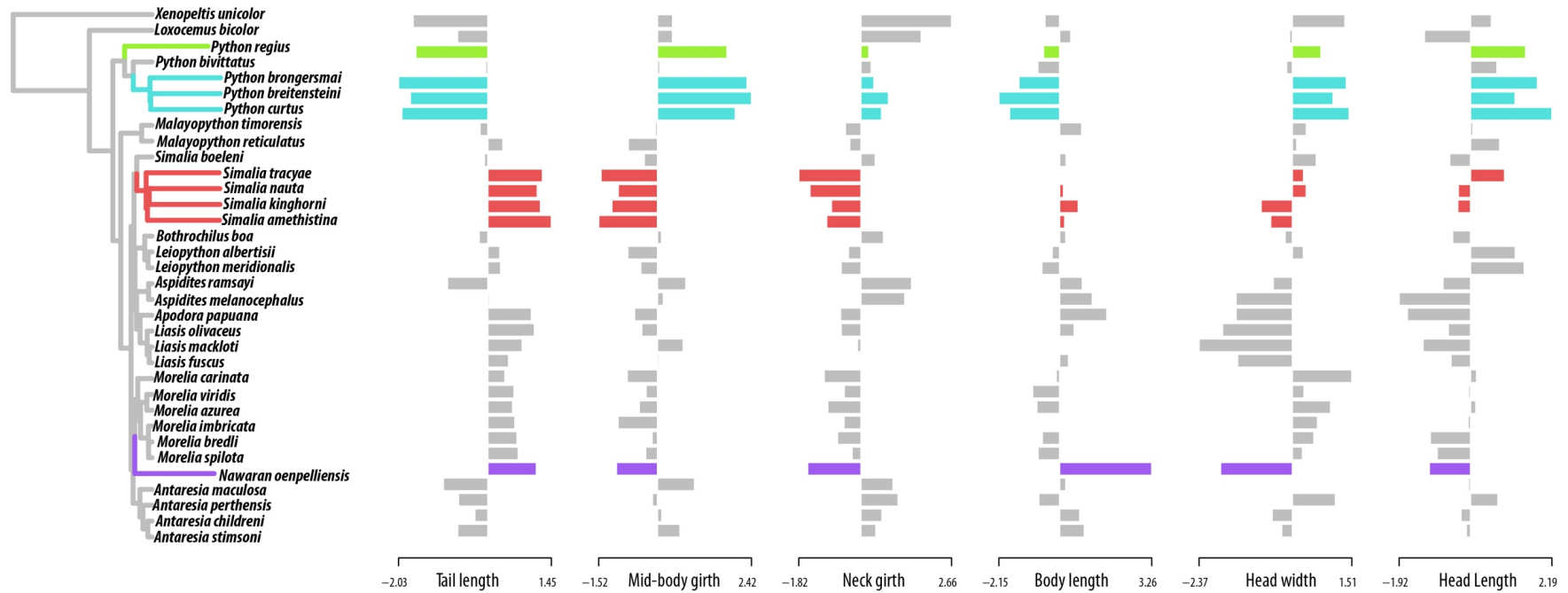
**Figure S2.** Concatenated and partitioned gene trees inferred by RAXML and MrBayes (top left and right respectively) and Coalescent-based species trees inferred by Astral using the individual gene trees from RAXML and StarBeast 2 (bottom left and right respectively). Branch support annotated as bootstrap support on RAXML tree and posterior probability in MrBayes tree. Branch support on Astral tree with RAXML is annotated as local posterior support / multi-locus bootstrap support and Astral tree with StarBeast 2 is annotated as multi-locus bootstrap.



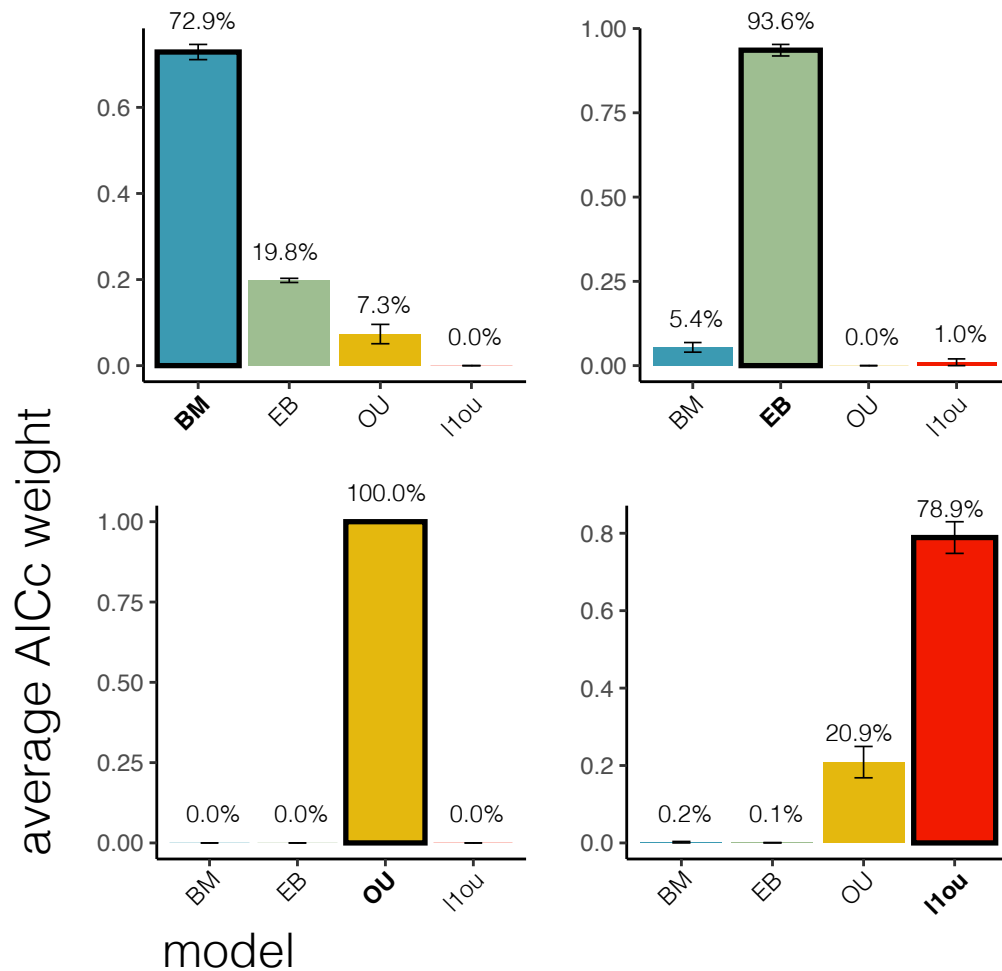
**Figure S3.** Biogeographic history inference if *P. bivittatus* was considered Afro-Asian, accounting for the hypothesis that the giant pythons of Africa (*P. sebae* and *P. natalensis*) and Asia (*P. molurus* and *P. bivittatus*) are a clade.



**Figure S4.** Phenotypic adaptive shifts (in color) in body shape found by *llo*, along with the values of body shape log-shape ratios for each body measurement.



**Figure S5.** Small tree size does not appear to adversely affect the statistical power of the python phylogeny and phenotypic dataset. We accurately recover all four models of evolution with modest false positive rates. 100 datasets were simulated under (top-to-bottom, left-to-right): Brownian Motion (BM), Early Burst (EB), single-optimum Ornstein Uhlenbeck (OU), and multi-optima OU (*l1ou*) models using empirical parameter estimates as a guide. Generating models are **bolded** and their corresponding bar graphs are outlined in black, and confidence intervals (95%) are noted on each bar.





**Table S1.** Specimens used in the molecular genetic analyses.

Genus	Species	Voucher	Locality	Country
<b>Pythonidae</b>				
<i>Antaresia</i>	<i>childreni</i>	ABTC 29270 / MAGNT R19042	Guluwuru Island, NT	Australia
<i>Antaresia</i>	<i>maculosa</i> (a)	FI 001	Captive specimen	Australia
<i>Antaresia</i>	<i>maculosa</i> (b)	ABTC 58410 / SAM R46757	Cairns Area, Qld	Australia
<i>Antaresia</i>	<i>perthensis</i>	ABTC 55518 / WAM R83717	3k S Parabardoo Mine, WA	Australia
<i>Antaresia</i>	<i>stimsoni</i>	ABTC 55501 / SAM R26879	Goyder Lagoon, SA	Australia
<i>Apodora</i>	<i>papuana</i> (a)	CTMZ 06784 / CJF 3610	Captive specimen	-
<i>Apodora</i>	<i>papuana</i> (b)	ABTC 68240	Sorong, West Papua	Indonesia
<i>Aspidites</i>	<i>melanocephalus</i>	ABTC 29560 / MAGNT R21365	Musselbrook Reservoir, Qld	Australia
<i>Aspidites</i>	<i>ramsayi</i> (b)	ABTC 70157 / SAM R54050	Tirrawarra Swamp, SA	Australia
<i>Bothrochilus</i>	<i>boa</i> (a)	CTMZ 04605 / COBRA 84877	Captive specimen	-
<i>Bothrochilus</i>	<i>boa</i> (b)	ABTC 50005 / AMS R129533	Amelei, WNPB	Papua New Guinea
<i>Leiopython</i>	<i>albertisii</i>	ABTC 125776	Manokwari, West Papua	Indonesia
<i>Leiopython</i>	<i>meridionalis</i>	ABTC 123501	near Gurney, SW Alotau	Papua New Guinea
<i>Liasis</i>	<i>fuscus</i> (a)	ABTC 73012 / SAM R54429	Burke Development Road 13k ENE Karumba turnoff, Qld	Australia
<i>Liasis</i>	<i>fuscus</i> (b)	ABTC 55511 / SAM R27497	Darwin area, NT	Australia
<i>Liasis</i>	<i>mackloti</i> (a)	FI 010	Captive specimen	-
<i>Liasis</i>	<i>mackloti</i> (b)	ABTC 128044 / WAM R107163	West Timor	Indonesia
<i>Liasis</i>	<i>olivaceus</i>	ABTC 128019	Woodstock Station, WA	Australia
<i>Liasis</i>	<i>savuensis</i>	ABTC 68268	Sawu Island	Indonesia
<i>Malayopython</i>	<i>reticulatus</i> (a)	ABTC 123690 / UMMZ 225974	Kayuadi Island, S of Sulawesi	Indonesia
<i>Malayopython</i>	<i>reticulatus</i> (b)	ABTC 128047	Pantar Island	Indonesia
<i>Malayopython</i>	<i>timoriensis</i>	ABTC 68327	Flores Island	Indonesia
<i>Morelia</i>	<i>azurea</i>	ABTC 125856	Manokwari, West Papua	Indonesia
<i>Morelia</i>	<i>bredli</i>	ABTC 68807 / SAM R52604	Captive specimen	Australia
<i>Morelia</i>	<i>bredli</i>	ABTC 55500 / SAM R26878	Goyder Lagoon, SA	Australia
<i>Morelia</i>	<i>carinata</i> (a)	ABTC 51987	Mitchell Plateau, WA	Australia
<i>Morelia</i>	<i>carinata</i> (b)	ABTC 128234	Bachsten Creek, WA	Australia
<i>Morelia</i>	<i>imbricata</i>	ABTC 62453 / WAM R113305	15 km SE of Karragullen, WA	Australia
<i>Morelia</i>	<i>imbricata</i>	ABTC 68827 / SAM R52647	St Francis Island, SA	Australia
<i>Morelia</i>	<i>spilota cheynei</i>	ABTC 81077	Lamb Range, 5.9k NE Lake Barrine, Qld	Australia
<i>Morelia</i>	<i>spilota macdowellii</i>	ABTC 31935	20 km W of Mackay, Qld	Australia
<i>Morelia</i>	<i>spilota spilota</i>	ABTC 13446	Gosford, NSW	Australia
<i>Morelia</i>	<i>spilota variegata</i> (a)	ABTC 123692 / UMMZ 227833	near Merauke, West Papua	Papua New Guinea
<i>Morelia</i>	<i>spilota variegata</i> (b)	ABTC 55482 / SAM R21456	Darwin, NT	Australia

<i>Morelia</i>	<i>viridis</i> (a)	ABTC 43349 / AMS R115355	Noru, CHP	Papua New Guinea
<i>Morelia</i>	<i>viridis</i> (b)	ABTC 125878	Algodang, Aru Archipalego, Maluku	Indonesia
<i>Nawaran</i>	<i>oenpelliensis</i>	ABTC 29590	ex Territory Wildlife Park, NT	Australia
<i>Python</i>	<i>breitensteini</i>	C9866	Kalimantan, Borneo	Indonesia
<i>Python</i>	<i>brongersmai</i> (a)	ABTC 65521	Bangka Island, Sumatra	Indonesia
<i>Python</i>	<i>brongersmai</i> (b)	CTMZ 04930	Captive specimen	-
<i>Python</i>	<i>curtus</i>	C9860	vicinity of West Rantauprapat, Sumatra	Indonesia
<i>Python</i>	<i>bivittatus</i> (a)	127	Captive specimen	-
<i>Python</i>	<i>bivittatus</i> (b)	ABTC 55498	Captive specimen	-
<i>Python</i>	<i>regius</i>	CTMZ 12714 / ROM field 41191	Captive specimen	-
<i>Simalia</i>	<i>amethystina</i>	ABTC 50041 / AMS R129577	Amelei, WNPB	Papua New Guinea
<i>Simalia</i>	<i>boeleni</i>	ABTC 49652 / BPBM 11611	Mt Kaindi, MP	Papua New Guinea
<i>Simalia</i>	<i>kinghorni</i>	ABTC 102403 / MAGNT R35168	Tully Gorge Road, Tully Gorge National Park, Qld	Australia
<i>Simalia</i>	<i>nauta</i> (a)	ABTC 123688 / UMMZ 227831	near Saumlaki, Yamdena Island, Tanimbar Islands, Maluku	Indonesia
<i>Simalia</i>	<i>nauta</i> (b)	ABTC 128067	Yamdena Island, Tanimbar Islands, Maluku	Indonesia
<i>Simalia</i>	<i>tracyae</i>	ABTC 123693 / UMMZ 225575	Halmahera Island, North Maluku	Indonesia

#### *Loxocemidae*

<i>Loxocemus</i>	<i>bicolor</i> (a)	CTMZ 06834 / JAC 22070	Captive specimen	-
<i>Loxocemus</i>	<i>bicolor</i> (b)	MVZHerp 143487	Santo Domingo Tehuantepec, Oaxaca	Mexico

#### *Xenopeltidae*

<i>Xenopeltis</i>	<i>unicolor</i>	CTMZ 04192 / MZUSP 15163	Captive specimen	-
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#### Outgroups and taxa used for dating calibrations

<i>Aparallactus</i>	<i>weneri</i>	SMG 5406	Captive specimen	-
<i>Coluber</i>	<i>constrictor</i>	FK 2444 / UMMZ 202041	Broward, Florida	USA
<i>Dipsas</i>	<i>catesbyi</i>	CTMZ 00545 / MZUSP 14664	Fazenda da Ceplac, Bahia	Brazil
<i>Acrochordus</i>	<i>javanicus</i>	CTMZ 06782 / USNM 347549	Barrio Banga, north shore of Lake Taal, Talisay, Luzon Is.	Philippines
<i>Bothrops</i>	<i>leucurus</i>	CTMZ 16131 / UFPB 4473	Mata do Buraquinho, Paraíba	Brazil
<i>Fordonia</i>	<i>leucobalia</i>	CTMZ 14553 / CAS 229746	Chaung Wa Pyin Village, Thaye Aei, in Min Dut Chaung	Burma
<i>Pareas</i>	<i>nuchalis</i>	FK 2626	Captive specimen	-
<i>Boa</i>	<i>constrictor</i>	CTMZ 00798 / MZUSP 15558	UHE-Peixe Angical, Tocantins	Brazil
<i>Candoia</i>	<i>aspera</i>	ABTC 48713	Captive specimen	-
<i>Eryx</i>	<i>colubrinus</i>	CTMZ 06811 / CBGM 00835	Captive specimen	-

**Table S2.** Ecological guild and number of specimens measured for head and body shape for every species included in this study.

Genus	Species	Guild	n for head shape	n for body shape
I. Afro-Asian clade				
1. <i>Python</i>	<i>bivittatus</i>	Terrestrial	8	10
2. <i>Python</i>	<i>breitensteini</i>	Terrestrial	4	5
3. <i>Python</i>	<i>brongersmai</i>	Terrestrial	9	9
4. <i>Python</i>	<i>curtus</i>	Terrestrial	2	2
5. <i>Python</i>	<i>regius</i>	Terrestrial	21	21
II. Indo-Australian clade				
1. Wallacean clade				
6. <i>Malayopython</i>	<i>reticulatus</i>	Semi-Arboreal	21	15
7. <i>Malayopython</i>	<i>timoriensis</i>	Semi-Arboreal	4	4
2. Australo-Papuan clade				
a. <i>Morelia</i> clade				
8. <i>Antaresia</i>	<i>childreni</i>	Terrestrial	47	47
9. <i>Antaresia</i>	<i>maculosa</i>	Terrestrial	34	35
10. <i>Antaresia</i>	<i>perthensis</i>	Terrestrial	39	42
11. <i>Antaresia</i>	<i>stimsoni</i>	Terrestrial	50	52
12. <i>Morelia</i>	<i>azurea</i>	Arboreal	14	13
13. <i>Morelia</i>	<i>bredli</i>	Semi-Arboreal	18	19
14. <i>Morelia</i>	<i>carinata</i>	Arboreal	2	2
15. <i>Morelia</i>	<i>imbricata</i>	Semi-Arboreal	7	6
16. <i>Morelia</i>	<i>spilota</i>	Semi-Arboreal	107	109
17. <i>Morelia</i>	<i>viridis</i>	Arboreal	20	22
18. <i>Nawaran</i>	<i>oenpelliensis</i>	Semi-Arboreal	9	9
b. <i>Bothrochilus</i> clade				
19. <i>Aspidites</i>	<i>melanocephalus</i>	Semi-Fossorial	58	59
20. <i>Aspidites</i>	<i>ramsayi</i>	Semi-Fossorial	35	35
21. <i>Bothrochilus</i>	<i>boa</i>	Terrestrial	18	19
22. <i>Leiopython</i>	<i>albertisii</i>	Terrestrial	15	13
23. <i>Leiopython</i>	<i>meridionalis</i>	Terrestrial	32	24
24. <i>Liasis</i>	<i>fuscus</i>	Semi-Aquatic	21	21
25. <i>Liasis</i>	<i>mackloti</i>	Semi-Aquatic	23	22
26. <i>Liasis</i>	<i>olivaceus</i>	Terrestrial	25	23
27. <i>Apodora</i>	<i>papuana</i>	Terrestrial	18	12
28. <i>Simalia</i>	<i>amethistina</i>	Semi-Arboreal	25	13
29. <i>Simalia</i>	<i>boeleni</i>	Semi-Arboreal	13	14
30. <i>Simalia</i>	<i>kinghorni</i>	Semi-Arboreal	35	30
31. <i>Simalia</i>	<i>nauta</i>	Semi-Arboreal	5	5
32. <i>Simalia</i>	<i>tracyae</i>	Semi-Arboreal	2	2
Loxocemidae				
33. <i>Loxocemus</i>	<i>bicolor</i>	Semi-Fossorial	8	8
Xenopeltidae				
34. <i>Xenopeltis</i>	<i>unicolor</i>	Semi-Fossorial	6	5
Total			755	727

**Table S3.** Main node ages and 95% HPD (High Posterior Densities) of the MCMC tree of the dating analysis using all calibrations on their own and combined.

Dating calibrations	<i>Loxocemus</i> / Pythonidae	<i>Python</i> / rest of Pythonidae	<i>Malayopython</i> / Australo-Papuan Pythonidae	Australian / Papuan radiations
Only <i>Ogmophis</i>	37.18 (35.2-43.8)	21.1 (17.65-25.84)	17.75 (14.58-21.84)	11.24 (9.36-13.73)
Only <i>Procerophis</i>	16.82 (13.44-20.71)	9.58 (7.85-11.52)	8.08 (6.49-9.76)	5.18 (4.19-6.16)
Only Eocene python	49.9 (48.5-54.53)	30.86 (26.68-35.98)	25.96 (21.88-30.52)	16.39 (14.2-19.1)
Only <i>Titanoboa</i>	36.07 (30.09 - 43.12)	20.8 (18.03-24.26)	17.41 (14.82-20.82)	11.01 (9.62-12.77)
Only <i>Morelia</i>	76.31 (63.75-91.38)	45.15 (39.98-52.66)	38.02 (32.84-44.5)	24.01 (23-27.5)
<i>Titanoboa</i> + <i>Morelia</i> +Eocene python	66.25 (55.3-76.29)	42.27 (38.87-45.92)	35.69 (32.36-39.01)	23.22 (23-23.95)

**Table S4.** BioGeoBEARS models tested sorted in decreasing model preference according to the AICc. After model name, columns represent LnLikelihood, number of parameters of the model, d, e, j, and w parameters, corrected AIC and AICc weight which gives a sense of the relative probability of each model.

<i>P. bivittatus</i> as Asian								
Model	LnL	n params	d	e	j	w	AICc	AICc wgt
DEC+J+W	-88.35	4	0.016	0.0041	0.16	1.13	186.1	0.42
DIVALIKE+J	-89.97	3	0.02	0.0039	0.15	1	186.7	0.3
DEC+J	-90.7	3	0.023	0.0088	0.035	1	188.2	0.15
BAYAREA+J	-91.56	3	0.012	0.0099	0.16	1	189.9	0.062
DEC	-93.08	2	0.025	0.0097	0	1	190.5	0.045
DEC+W	-93.08	3	0.025	0.0097	0	0.74	193	0.014
DIVALIKE	-94.98	2	0.031	0.0094	0	1	194.3	0.0068
BAYAREALIKE	-110.9	2	0.058	0.081	0	1	226.2	8.10E-10
<i>P. bivittatus</i> as Afro-Asian								
Model	LnL	n params	d	e	j	w	AICc	AICc wgt
DEC+J	-89.44	3	0.021	0.005	0.062	1	185.7	0.49
DEC+J+W	-88.42	4	0.02	0.0044	0.13	0.76	186.2	0.37
DEC	-92.78	2	0.027	0.0088	0	1	189.9	0.058
BAYAREA+J	-92.22	3	0.014	0.01	0.14	1	191.2	0.03
DIVALIKE+J	-92.58	3	0.031	0.0085	0.03	1	192	0.021
DEC+W	-92.78	3	0.027	0.0089	0	0.62	192.4	0.017
DIVALIKE	-94.54	2	0.033	0.0091	0	1	193.5	0.01
BAYAREALIKE	-111	2	0.063	0.083	0	1	226.4	7.00E-10

**Table S5.** Likelihood ratio tests between nested models tested. Model name details are in Table S4. The columns go from left to right as follows: Alternative hypothesis model, null hypothesis model, LnLikelihood for both models, degrees of freedom for the two models and the test, the Dstatistic for the Likelihood ratio test, the *P* value testing the hypothesis that both models have equal likelihood, the AIC for the models, the AIC weights for the models and the AIC weights for the ratios of the models.

<i>P. bivittatus</i> as Asian															
Alternative	Null	LnLalt	LnLnull	DFalt	DFnull	DF	Dstatistic	<i>P</i> value	AICalt	AICnull	AICwt alt	AICwt null	AICwt ratio alt	AICwt ratio null	
DEC+J	DEC	-90.7	-93.08	3	2	1	4.75	0.029	187.4	190.2	0.8	0.2	3.95	0.25	
DEC+W	DEC	-93.08	-93.08	3	2	1	-1.80E-06	1	192.2	190.2	0.27	0.73	0.37	2.72	
DEC+J+W	DEC+J	-88.35	-90.7	3	2	1	4.71	0.03	182.7	185.4	0.8	0.2	3.88	0.26	
DIVALIKE+J	DIVALIKE	-89.97	-94.98	3	2	1	10.02	0.0015	185.9	194	0.98	0.018	55.25	0.018	
BAYAREALIKE+J	BAYAREALIKE	-91.56	-110.9	3	2	1	38.72	4.90E-10	189.1	225.8	1	1.10E-08	9.39E+07	1.10E-08	
<i>P. bivittatus</i> as Afro-Asian															
Alternative	Null	LnLalt	LnLnull	DFalt	DFnull	DF	Dstatistic	<i>P</i> value	AICalt	AICnull	AICwt alt	AICwt null	AICwt ratio alt	AICwt ratio null	
DEC+J	DEC	-89.44	-92.78	3	2	1	6.68	0.0098	184.9	189.6	0.91	0.088	10.36	0.096	
DEC+W	DEC	-92.78	-92.78	3	2	1	-3.10E-06	1	191.6	189.6	0.27	0.73	0.37	2.72	
DEC+J+W	DEC+J	-88.42	-89.44	3	2	1	2.04	0.15	182.8	182.9	0.51	0.49	1.02	0.98	
DIVALIKE+J	DIVALIKE	-92.58	-94.54	3	2	1	3.92	0.048	191.2	193.1	0.72	0.28	2.61	0.38	
BAYAREALIKE+J	BAYAREALIKE	-92.22	-111	3	2	1	37.58	8.80E-10	190.4	226	1	1.90E-08	5.32E+07	1.90E-08	

**Table S6.** Loadings of each Principal Component (PC) of the PCA on the body shape measurements. The last two rows correspond to the proportion of variance explained by each PC for body and head shape.

	PC1	PC2	PC3	PC4	PC5
Tail length	-0.822	-0.034	-0.262	-0.297	0.017
Mid-body girth	0.462	0.190	-0.733	-0.080	0.200
Neck girth	0.242	0.383	0.475	-0.584	-0.247
Body length	-0.140	0.509	0.237	0.690	0.151
Head width	0.143	-0.587	0.331	-0.024	0.598
Head length	0.116	-0.461	-0.048	0.296	-0.720
Prop. Var. body	0.808	0.100	0.051	0.028	0.013
Prop. Var. head	0.599	0.179	0.119	0.044	0.023

**Table S7.** Species included in different genera by the main systematic works on Pythonidae.

Study	Boulenger (1893)	McDowell (1975)	Underwood & Stimson (1990)	Kluge (1993)	Rawlings et al. (2008)	Reynolds et al. 2014	This study
Criteria	Morphological similarity	Morphological similarity	Phenetic dendrograms built from 38 morphological characters	Maximum Parsimony analysis of 121 behavioral and morphological characters	Maximum Parsimony and Bayesian Inference of four mitochondrial loci and Kluge's morphological characters	Maximum Likelihood and Bayesian Inference or a supermatrix alignment of 1-11 mitochondrial and nuclear loci	Different analyses of concatenation and coalescent species trees of 376 nuclear loci, complete mitochondrial genomes and Reynold's et al. (2014) data
<i>Python</i>	<i>amethestina</i> , <i>anchietae</i> , <i>curtus</i> , <i>molurus</i> , <i>regius</i> , <i>reticulatus</i> , <i>sebae</i> , <i>spilota</i> , <i>timoriensis</i>	<i>reticulatus</i> group: <i>amethistina</i> , <i>boeleni</i> , <i>reticulatus</i> , <i>spilota</i> , <i>timoriensis</i> . <i>molurus</i> group: <i>anchietae</i> , <i>curtus</i> , <i>molurus</i> , <i>regius</i> , <i>sebae</i>	<i>anchietae</i> , <i>curtus</i> , <i>molurus</i> , <i>regius</i> , <i>reticulatus</i> , <i>sebae</i> , <i>timoriensis</i>	<i>anchietae</i> , <i>curtus</i> , <i>molurus</i> , <i>regius</i> , <i>reticulatus</i> , <i>sebae</i> , <i>timoriensis</i>	<i>brongersmai</i> , <i>molurus</i> , <i>regius</i> , <i>sebae</i> ,	<i>anchietae</i> , <i>bivittatus</i> , <i>brongersmai</i> , <i>curtus</i> , <i>molurus</i> , <i>regius</i> , <i>sebae</i>	<i>anchietae</i> , <i>bivittatus</i> , <i>breitensteini</i> , <i>brongersmai</i> , <i>curtus</i> , <i>molurus</i> , <i>regius</i> , <i>sebae</i>
<i>Broghammerus</i>					<i>reticulatus</i> , <i>timoriensis</i>		
<i>Malayopython</i>						<i>reticulatus</i> , <i>timoriensis</i>	<i>reticulatus</i> , <i>timoriensis</i>
<i>Antaresia</i>				<i>childreni</i> , <i>maculosa</i> , <i>perthensis</i> , <i>stimsoni</i>	<i>childreni</i> , <i>maculosa</i> , <i>perthensis</i> , <i>stimsoni</i>	<i>childreni</i> , <i>maculosa</i> , <i>perthensis</i> , <i>stimsoni</i>	<i>childreni</i> , <i>maculosa</i> , <i>perthensis</i> , <i>stimsoni</i>
<i>Chondropython</i>	<i>viridis</i>	<i>viridis</i>					
<i>Morelia</i>			<i>albertisii</i> , <i>amethistina</i> , <i>boa</i> , <i>boeleni</i> , <i>bredli</i> , <i>carinata</i> , <i>children</i> , <i>fuscus</i> , <i>mackloti</i> , <i>maculosa</i> , <i>oenpelliensis</i> , <i>olivaceus</i> , <i>papuana</i> , <i>perthensis</i> , <i>spilota</i> , <i>stimsoni</i> , <i>viridis</i>	<i>amethistina</i> , <i>boeleni</i> , <i>carinata</i> , <i>oenpelliensis</i> , <i>spilota</i> , <i>viridis</i>	<i>amethistina</i> , <i>bredli</i> , <i>boeleni</i> , <i>carinata</i> , <i>oenpelliensis</i> , <i>spilota</i> , <i>viridis</i>	<i>bredli</i> , <i>carinata</i> , <i>spilota</i> , <i>viridis</i>	<i>azurea</i> , <i>carinata</i> , <i>imbricata</i> , <i>spilota</i> , <i>viridis</i>
<i>Nawaran</i>							<i>oenpelliensis</i>
<i>Apodora</i>				<i>papuana</i>	<i>papuana</i>		<i>papuana</i>
<i>Aspidites</i>	<i>melanocephalus</i> , <i>ramsayi</i>	<i>melanocephalus</i> , <i>ramsayi</i>	<i>melanocephalus</i> , <i>ramsayi</i>	<i>melanocephalus</i> , <i>ramsayi</i>	<i>melanocephalus</i> , <i>ramsayi</i>	<i>melanocephalus</i> , <i>ramsayi</i>	<i>melanocephalus</i> , <i>ramsayi</i>
<i>Bothrochilus</i>				<i>boa</i>	<i>boa</i>	<i>albertisii</i> , <i>boa</i> , <i>hoserae</i> ( <i>meridionalis</i> )	<i>boa</i>
<i>Leiopython</i>				<i>albertisii</i>	<i>albertisii</i>		<i>albertisii</i> , <i>meridionalis</i>
<i>Liasis</i>	<i>albertisii</i> , <i>childreni</i> , <i>fuscus</i> , <i>mackloti</i> , <i>olivaceus</i> , <i>papuana</i>	<i>olivaceus</i> group: <i>mackloti</i> , <i>olivaceus</i> , <i>papuana</i> . <i>boa</i> Group: <i>albertisii</i> , <i>boa</i> , <i>childreni</i>		<i>mackloti</i> , <i>olivaceus</i>	<i>fuscus</i> , <i>mackloti</i> , <i>olivaceus</i>	<i>fuscus</i> , <i>mackloti</i> , <i>papuana</i> , <i>olivaceus</i>	<i>fuscus</i> , <i>mackloti</i> , <i>olivaceus</i> , <i>savuensis</i>
<i>Nardoa</i>	<i>boa</i>						
<i>Simalia</i>						<i>amethistina</i> , <i>boeleni</i> , <i>oenpelliensis</i> , <i>clastolepis</i> , <i>kinghorni</i> , <i>nauta</i> , <i>tracyae</i>	<i>amethistina</i> , <i>boeleni</i> , <i>clastolepis</i> , <i>kinghorni</i> , <i>nauta</i> , <i>tracyae</i>
No. of species:	18	19	26	24	25	33	36