

Python phylogenetics: inference from morphology and mitochondrial DNA

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We used nucleotide sequences from four mitochondrial genes and structural features of the mitochondrial control region, combined with a revised, previously published, morphological data set to infer phylogenetic relationships among the pythons. We aimed to determine which of two competing hypotheses of relationships of the genera *Aspidites* and *Python* best explains the evolutionary and biogeographical history of the family. All analyses of the combined data recover a set of relationships in which (1) the genus *Python* is paraphyletic with the two east Asian species, *P. reticulatus* and *P. timoriensis*, as the sister lineage to the seven Australo-Papuan python genera. We support recognition of a distinct genus for the *P. reticulatus* + *P. timoriensis* clade; (2) the remaining species of the genus *Python* form a clade which is the sister lineage to the remainder of the family; (3) the genus *Aspidites* is embedded among the Australo-Papuan genera. The seemingly primitive characteristics of *Aspidites* may be better interpreted as reversals or specializations that have accompanied a switch to burrowing in this genus. Resolution of the relationships among the Australo-Papuan lineages is weak, possibly because of rapid diversification early in the history of the radiation. We assessed the tempo of the Indo-Australian python radiation using a maximum likelihood framework based on the birth–death process. We find strong support for elevated speciation rates during the period when Australia collided with the proto-Indonesian archipelago. The data support an origin for pythons outside Australia, followed by a radiation into Australia during the mid-Tertiary. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, 93, 603–619.

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INTRODUCTION

Pythons range from western and southern Africa, through the tropical rainforests of south-east Asia, eastwards as far as New Guinea and into the cooler climates of southern Australia. They differ from the generally similar boas in reproductive mode (viviparous boas, oviparous pythons) and anatomically by the presence in pythons of a novel bone, the supra-orbital, on the dorsal margin of the orbit. Pythons can be terrestrial, arboreal, fossorial or semi-aquatic and vary in size from 0.5 m, for example, *Antaresia perthensis* (Smith, 1985) to approximately 10 m in

length, for example, *Python reticulatus* (Minton & Minton, 1973). Most python genera and species are restricted to the Australo-Papuan region. Of the eight genera recognized by Kluge (1993), only the genus *Python* is not found in Australia or New Guinea. Of the other seven genera, three (*Leiopython*, *Liasis* and *Morelia*), are found in Australia and New Guinea and two each are restricted to Australia (*Antaresia* and *Aspidites*) or New Guinea and associated islands (*Apodora* and *Bothrochilus*).

All recent phylogenetic studies, both morphological (e.g. Underwood & Stimson, 1990; Kluge, 1991) and molecular (Slowinski & Lawson, 2002; Wilcox *et al.*, 2002; Lawson, Slowinski & Burbrink, 2004; Vidal & Hedges, 2004; Lee & Hugall, 2006; Noonan & Chippindale, 2006), recognize the pythons as a

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well-supported clade, but their precise relationships to other snake clades remain uncertain. Conventionally regarded as the sister group of boines, molecular studies suggest that they appear to be even more closely related to some other archaic macrostomatan snakes (*Loxocemus*, Wilcox *et al.*, 2002; Noonan & Chippindale, 2006), while boines are closer to erycines.

The number of python species recognized has increased significantly over recent decades, and some species boundaries among the pythons have been controversial (Stull, 1932; McDowell, 1975; Underwood, 1976; Cogger, Cameron & Cogger, 1983; Smith, 1985; Storr, Smith & Johnstone, 1986; Underwood & Stimson, 1990; Kluge, 1993). In particular, *Liasis fuscus* and *Morelia bredli* have not been recognized as species by some authors (Smith, 1985; Fyfe, 1990; Kluge, 1993). We follow Barker & Barker (1994), Harvey *et al.* (2000) and Keogh, Barker & Shine (2001) in recognizing 33 extant species with the inclusion of *Liasis fuscus* and *Morelia bredli* and an unnamed sibling taxon of *M. viridis* from northern New Guinea (Rawlings & Donnellan, 2003).

Interest in the generic arrangement for pythons increased following the largely intuitive summary of evolution within pythons of McDowell (1975). Two explicitly phylogenetically based studies sought to provide a rigorous systematic and biogeographical framework. Underwood & Stimson (1990) and Kluge (1993) used morphological and behavioural data sets with significant overlap, but reached opposing conclusions about the relationships among species (Fig. 1).

Underwood & Stimson (1990) found a primary dichotomy between the Afro-Asian genus *Python* and all other pythons, which are confined to the Australo-Papuan region (Fig. 1A). In their phylogeny, the anomalous *Aspidites*, which lacks thermoreceptive pits and has fossorial rather than scansorial habits, was embedded within the Australian radiation. In direct contrast, Kluge (1993) placed *Python* well within an otherwise Australo-Papuan clade and identified *Aspidites* as the sister to all other pythons (Fig. 1B). Kluge criticized the placement of *Aspidites* of both McDowell (1975) and Underwood & Stimson (1990) based on their a priori assumption that the absence in *Aspidites* of some characteristics (e.g. thermoreceptive labial pits) is as a result of secondary loss.

In the phylogenetic analysis of Lawson *et al.* (2004), based on mitochondrial Cytochrome *b* nucleotide sequences of just 13 taxa, few nodes were strongly supported but the tree topology is consistent with the Underwood & Stimson (1990) hypothesis in recovering a monophyletic Australo-Papuan clade to the exclusion of the four species of *Python* sequenced (Fig. 1C). However, in contrast to the study of Under-

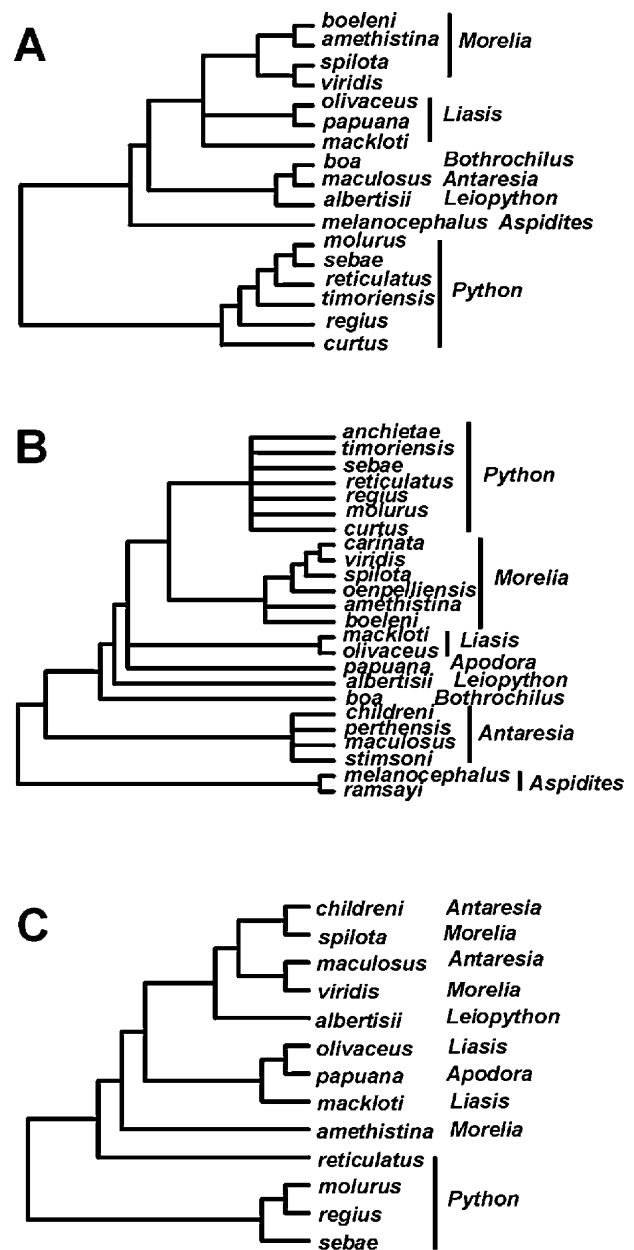


Figure 1. Phylogenetic relationships among pythons determined from: (A) Underwood & Stimson (1990) morphological analysis showing the division between the Moreliini and the Pythonini; (B) Kluge, 1993) morphological analysis; (C) Lawson *et al.* (2004) mitochondrial nucleotide sequence analysis.

wood & Stimson (1990), *Python* was not monophyletic but instead comprised two clades, with *P. reticulatus* as the sister to the Australo-Papuan clade.

Biogeographical implications that flow from the above findings are either: (1) that pythons arose in Africa or Asia and moved southwards through the Asian land-bridge into the Australo-Papuan region

(implied by Underwood & Stimson 1990 and Lawson *et al.*, 2004) or (2) that pythons arose in Gondwana and moved northwards into Asia (implied by the analysis of Kluge 1993). Python-like booid snakes are known from the Eocene and Oligocene of Germany and France (Szyndlar & Böhme, 1993) and fossils attributed to *Morelia* and a second form attributable to *Liasis*, or possibly *Python*, have been found in Miocene deposits in Australia (Scanlon, 2001). While the fossil record does not unequivocally support either hypothesis, Scanlon (2001) points out that the non-appearance of six lineages supposedly more basal than *Morelia* in the Miocene of Australia is more consistent with phylogenetic hypotheses, implying a relatively recent extra-Australian, Tertiary origin for Australian pythons.

Given the conflicting interpretations of the comprehensive morphological studies of Underwood & Stimson (1990) and Kluge (1993), progress on the recovery of the evolutionary history of pythons is likely to come from the development of new character sets. Analyses of nucleotide sequences and relative evolutionary rate tests provide tools with which alternative evolutionary scenarios can be tested. The recent development of a likelihood framework for combined phylogenetic analysis of molecular and morphological data presents an opportunity to reassess python relationships based on new nucleotide sequence and existing morphological data (Tuffley & Steele, 1997; Lewis, 2001). We present a phylogenetic analysis of nucleotide sequence data from four mitochondrial genes with differing rates of molecular evolution, the faster-evolving control region (*CR*) and the medium- to fast-evolving coding gene, Cytochrome *b* (*cytb*) and the slower evolving *12S* and *16S rRNA* genes and a revised version of the morphological/behavioural data of Kluge (1993). We evaluate the characters that were used to distinguish *Aspidites* from other pythons and assess the implications of our findings for the biogeography of the pythons and rates of divergence for this lineage.

MATERIAL AND METHODS

SPECIMENS EXAMINED

Individuals which were sequenced in this study are [^m indicates extractions enriched for mitochondrial DNA, institution abbreviations follow Leviton *et al.* (1985), and ABTC is the Australian Biological Tissue Collection, South Australian Museum]: *Antaresia childreni* SAMA R21411^m; *A. maculosa* ABTC 68227; *A. perthensis* ABTC 68276; *A. stimsoni* SAMA R38794; *Apodora papuana* ABTC 68240; *Aspidites melanocephalus* ABTC 68246; *A. ramsayi* SAMA R19831^m; *Bothrochilus boa* AMS R129533; *Leiopython*

albertisii AMS R124481^m; *Liasis mackloti* SAMA R21422^m; *Liasis fuscus* ABTC 68263, 73012^m; *Liasis olivaceus* ABTC 6503; *Morelia amethystina* AMS R115347^m; *M. boeleni* BPBM 11611; *M. bredli* ABTC 68339; *M. carinata* ABTC 51987; *M. oenpelliensis* ABTC 68277; *M. spilota* SAMA R26878^m; *M. viridis* AMS R115348^m (southern New Guinea), BPBM 11617 (northern New Guinea); *Python brongersmai* ABTC 24797; *P. molurus* ABTC 67159; *P. regius* ABTC 55433, *P. reticulatus* SAMA R28533; *P. sebae* SAMA R26137; *P. timoriensis* ABTC 68326 and the outgroups *Xenopeltis unicolor* CAS 212014 and *Candoia aspera* AMS R115337^m. Samples of *Morelia clastolepis*, *M. kinghorni*, *M. nauta*, *M. tracyae*, *Python anchietae*, *P. curtus* and *P. breitensteini* were not available for inclusion in the phylogenetic analyses in the present study, but limited *cytb* data were available from GenBank and colleagues for the lineage through time (LTT) analyses.

DNA EXTRACTION, PCR AND SEQUENCING

Genomic DNA was extracted with a salting-out method (Miller, Dykes & Polesky, 1988). The polymerase chain reaction (PCR) was used to amplify partial transfer *RNA^{Thr}/CR*, *cytb*, *12S rRNA* and *16S rRNA* gene products. Details of all PCR primers can be found online in the Supplementary Material Table S1. To preferentially PCR amplify the *CR* instead of the control region-like gene that is present in some snakes (Kumazawa *et al.*, 1996), nested PCR was used as described by Kumazawa *et al.* (1996). Two overlapping partial *cytb* products of approximately 300 and 900 bp were amplified using primers L14841 and H15149 (Kocher *et al.*, 1989) for the short product and either L14973 or Snake 12 (L) with H15916 (Kumazawa *et al.*, 1996) for the longer product. Both strands of PCR products were sequenced with the PCR primers and for the *CR* products also with the nested primers, Snake 1 (L), Snake 6 (L) and Snake 7 (H). The potential for each of the mitochondrial primer pairs to amplify mitochondrial genes rather than nuclear paralogues was tested as per Donnellan, Hutchinson & Saint (1999). Mitochondrial DNA (mtDNA) isolated with a CsCl gradient method (Dowling *et al.*, 1996) and total cellular DNA of *Liasis mackloti* SAMA R21422, *Morelia spilota* SAMA R26878 and *M. viridis* AMS R115348 were used to test the whether the *CR* primers amplified mitochondrial genes. Enriched mtDNA and total cellular DNA of *L. fuscus* ABTC 73012, *Morelia spilota* SAMA R26878, *M. viridis* AMS R115348 and *C. aspera* AMS R115337 were used to test the *cytb* primers.

CR sequences were initially aligned in Clustal W (Thompson, Higgins & Gibson, 1994) under varying

gap penalties and insertions and deletions (indels) were incorporated to optimize the alignments. Regions of sequence alignment that varied under differing gap penalties were considered to be of ambiguous alignment and were excluded from the final analyses. Incorporated in this region of ambiguous sequence alignment are three structural features of the *CR*, which were coded as binary characters for inclusion in the analysis.

Complete *cytb* gene sequences were retrieved from GenBank for *Aspidites melanocephalus*, *Antaresia childreni*, *Apodora papuana*, *Liasis mackloti*, *L. olivaceus*, *Leiopython albertisii*, *Morelia amethystina*, *M. spilota*, *M. viridis*, *Python molurus*, *P. regius*, *P. reticulatus*, *P. sebae*, *Loxocemus bicolor*, *Xenopeltis unicolor* (accession numbers: U69741, 751, 760, 835, 837, 839, 842, 843, 847, 851, 853, 857, 860, 863, AY099993, AY121369). The *M. spilota* sequence (U69851) had a stop codon present because of an autapomorphy in a first codon position; this nucleotide position was coded as missing for the present study. The *cytb* sequences for the remaining taxa were amplified and sequenced with primers listed in Supplementary Material Table S1. Because it has been shown that in snakes the control region-like (*CRL*) sequence that is present between the *ND1* and *ND2* genes is typically indistinguishable from the *CR* sequence for the region of the *CR* sequenced here (Kumazawa *et al.*, 1996, Kumazawa *et al.*, 1998), and the *CR* sequence for *Python regius* was not available, we used the published *CRL* sequence (GenBank accession number D84258) in the *CR* data set. The *12S rRNA* and *16S rRNA* sequences for *Loxocemus* and *Xenopeltis* were taken from GenBank (accession numbers AF544755, AF512737, AF544752, AF544825). Nucleotide sequences for the data that we generated are available on GenBank (accession numbers EF545015–107) and the complete aligned data set is available from the corresponding author.

MORPHOLOGICAL CHARACTERS

The morphological/behavioural data comprised the 121 character set of Kluge (1993). Kluge (1993) found 16 morphological characters, of which 12 were cranial, that supported the sister relationship of *Aspidites* with all other pythons (Supplementary Material Table S2). Python crania in the South Australian Museum collection were examined for 11 of the 12 cranial characters (the 12th character was damaged on the only *Aspidites* skull available) in order to assess the reliability, that is, non-ambiguity, with which the character states could be determined. Crania of the following taxa were examined: *Antaresia childreni* (SAMA R26973), *A. stimsoni* (SAMA R26998, R49333), *Aspidites ramsayi* (SAMA R08110),

Boa constrictor (SAMA R29579), *Candoia aspera* (SAMA R45853), *Liasis olivaceus* (SAMA R03906), *Morelia amethystina* (SAMA R00359), *M. boa* (SAMA R45854), *M. spilota* (SAMA R26955, R33495), *M. viridis* (SAMA R04803), *Python molurus* (SAMA R36021), *P. reticulatus* (SAMA R27307), *P. sebae* (SAMA R26137) and *Xenopeltis unicolor* (SAMA R36861). Each character was evaluated with respect to four criteria. (1) Was the character correctly scored? Can the character states be verified in other specimens? (2) Are the character states anatomically identical? (3) Are the character states discrete rather continuous? (4) Are the characters independent? That is, do the states of characters forming parts of a common structure vary independently? Characters that conformed to these criteria were considered to be strong candidate characters to use in considering the relationships of *Aspidites* to other pythons. Phylogenetic support for those strong characters that define the *Aspidites* lineage could then be tested using the Bremer decay index.

The full data set of Kluge (1993) was also reanalysed with a modified out-group comprising a set of five out-group taxa each as an individually coded operational taxonomic unit (OTU) rather than using Kluge's common ancestor approach. The five out-groups (after Kluge) are: (1) boids; (2) erycines and 'advanced snakes' which includes tropidophiines, bolyeriines, *Acrochordus* and 'higher snakes' [colubroids as per Marx & Rabb (1970)]; (3) *Loxocemus*; (4) *Xenopeltis*; and (5) anilioids, which includes *Anilius*, *Cylindrophis* and the uropeltines. Reliability of the resulting tree topology was evaluated by bootstrapping, from 2000 pseudoreplicates and Bremer support.

PHYLOGENETIC ANALYSIS

We preferred to assess data incongruence in a combined data analysis framework because hidden support in individual data sets may only become apparent on a combined analysis framework (see Gatesy, O'Grady & Baker, 1999; Lee & Hugall, 2003). Partitioned branch support (PBS) values were calculated in TreeRot version 2 (Sorenson, 1999) in order to summarize the amount of support or conflict at a particular node on the combined data maximum parsimony (MP) tree(s) contributed by individual data partitions. PBS is used as an indicator of node-specific support contributed by data partitions. Searches were performed in PAUP* as described in Baker & deSalle (1997). Minimum length constrained topologies were derived from heuristic searches with 100 random addition replicates and Tree-bisection-reconnection (TBR) branch swapping. For MP analysis, gaps were treated as a fifth character state. The robustness of

the MP trees was evaluated by non-parametric bootstrap analysis from 1000 pseudoreplicates with 20 random addition heuristic searches of each pseudoreplicate.

Modeltest version 3.0 (Posada & Crandall, 1998) was used to determine the appropriate nucleotide substitution models for the Bayesian analyses of the molecular data with the Akaike Information Criterion. The appropriate model was used for each partition and the model parameters were unlinked and estimated separately for each partition. Bayesian analyses were implemented with MrBayes version 3.1 (Ronquist & Huelsenbeck, 2003). Using default priors, that is, Dirichlet priors for base frequencies (1,1,1,1) and for GTR parameters (1,1,1,1) scaled to the G-T rate, a Uniform (0.05,50.00) prior for the Γ shape, and an Exponential (10.0) prior for branch lengths. All topologies were a priori equally probable. Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) sampling was performed with four incrementally heated chains that were simultaneously run for 1 000 000 generations, using default priors as starting values for model parameters. Bayesian posterior probabilities (PP) were obtained from 50% majority rule consensus of trees sampled every 100 generations after trees recovered prior to stationarity being reached were discarded as the 'burn-in' stage. Multiple runs were performed to assess that all parameters were not considerably different at stationarity.

The ability to test alternative hypotheses on mixed model data that incorporate molecular and morphological data is limited at the moment to maximum parsimony-based tests such as the Templeton test (Templeton, 1983), because currently available ML implementations, for example, PAUP*, do not support mixed model analyses. We are reluctant to base tests of the ability of our data to distinguish between alternative phylogenetic hypotheses solely on parsimony-based tests, as MP is less able to cope with the impact of saturation of nucleotide substitutions than model-based analyses. In particular, as the principal alternative hypotheses that we would like to have tested involve basal branches, the impact of saturation is likely to be higher.

ANALYSIS OF DIVERSIFICATION RATES

We obtained partial *cytb* sequences (720 bp) for the remaining Indo-Australian species from GenBank: *M. clastolepis* (GenBank AF241401), *M. nauta* (AF241382), *M. kinghorni* (AF241386), *M. tracyae* (AF241384) and J.S. Keogh: *P. curtus*, and *P. breitensteini* (Keogh *et al.*, 2001). We aligned these sequences manually with *cytb* for the other python species and coded all other data (i.e. the remainder of *cytb* and the three other mitochondrial genes) for these species as

missing. We repeated the Bayesian MCMCMC analysis as described above on this 33-taxon data matrix.

Scanlon (2001) provides a strongly supported calibration point for divergence within the Australian python radiation. *Morelia riversleighensis* is a well-characterized fossil taxon, inferred as morphologically intermediate in branching position between *M. oenpelliensis* and *M. spilota*. Its remains are known from the end of the early Middle Miocene (c. 18 Myr BP) back to the latest Oligocene (25–26 Myr BP). We used the existence of *M. riversleighensis* to set the split between its two living relatives, *M. oenpelliensis* and *M. spilota*, at 25 Myr BP.

To estimate divergence times, we applied penalized likelihood rate smoothing (PL; Sanderson, 2002) to the consensus phylogram from the Bayesian analysis using the software package r8s (Sanderson, 2004). Prior to PL analysis, we pruned all taxa that were not nested within the Indo-Australian python radiation. We used the cross-validation procedure described in Sanderson (2002) to select an optimal smoothing parameter, and we compared these results to the cross-validation score obtained when divergence times were estimated using the Langley-Fitch algorithm in r8s.

Molecular phylogenies with branch lengths calibrated to absolute or relative timescales can be used to test for temporal variation in lineage diversification rates (Pybus & Harvey, 2000; Rabosky, 2006b; Nee, 2007). We used a maximum likelihood framework based on the birth–death process (Rabosky, 2006b) to test whether diversification rates in pythons have varied over time. This approach fits a candidate set of rate-variable and rate-constant models of diversification to phylogenetic data using maximum likelihood. The test statistic, $\Delta\text{AIC}_{\text{RC}}$, is calculated as the difference in Akaike Information Criterion (AIC) scores between the best-fit rate-constant and rate-variable models of diversification. Lower AIC scores imply better fitting models, and a positive $\Delta\text{AIC}_{\text{RC}}$ statistic thus suggests that a rate-variable model of diversification provides the best fit to the data. The $\Delta\text{AIC}_{\text{RC}}$ statistic is compared with a distribution generated under the null hypothesis of rate constancy; this distribution is tabulated from phylogenies simulated under a rate-constant model of diversification (Rabosky, 2006b).

The likelihood of the PL-calibrated python phylogeny was computed under four diversification models described in Rabosky (2006b): (1) the one-parameter pure-birth model (speciation rate $\lambda > 0$, with extinction rate $\mu = 0$); (2) a constant-rate birth–death model (two parameters, $\lambda > 0$; $\mu = 0$); (3) a pure birth rate-variable model where speciation rate λ_1 shifts to rate λ_2 at some time t_s (three parameters: λ_1 , λ_2 , t_s); and (4) a rate-variable model with two speciation rates and

two extinction rates, but constrained such that the extinction fraction μ/λ remains constant (four parameters: t_s ; $\lambda_1, \lambda_2 > 0$; $\mu_1, \mu_2 = 0$; but $\mu_1/\lambda_1 = \mu_2/\lambda_2$). Models (3) and (4) imply discrete shifts in diversification rates, and we added two density-dependent models to better approximate the possibility of a gradual change in diversification rates. In this case, the speciation rate is a logistic or exponential function of the number of lineages in existence at any point in time (Nee, Mooers & Harvey, 1992; Rabosky, 2006a). These provide a tractable alternative to models where the speciation rate varies continuously over time, which pose a much more challenging problem in non-linear optimization.

We also computed the γ -statistic (Pybus & Harvey, 2000) for the PL-calibrated python phylogeny. The γ -statistic measures the extent to which speciation times in a reconstructed phylogeny follow an exponential distribution; negative γ -values imply an excess of early branching events and a corresponding temporal decline in net diversification rates. Positive γ -values indicate an excess of recent speciation events and can be caused by increased diversification rates or constant diversification rates with non-zero extinction. Analyses of diversification rates and phylogenetic simulation were conducted using source code modified from the LASER package for the R programming environment (Rabosky, 2006a).

RESULTS AND DISCUSSION

TEST FOR PARALOGOUS SEQUENCES

All the mitochondrial primer pairs amplified PCR products at a dilution of $\geq 10^{-4}$, while the nuclear primers amplified products to a dilution of 10^{-2} . Partial *CR* and *cytb* sequences amplified from enriched mitochondrial DNA and from total cellular DNA for *C. aspera* AMS R115337, *Liasis mackloti* SAMA R21422, *Liasis fuscus* ABTC 73012, *Morelia spilota* SAMA R26878 and *M. viridis* AMS R115348 were indistinguishable, providing no evidence that the primers amplified paralogous sequences.

CR STRUCTURAL FEATURES

Three structural features were present in the 5' region of the *CR* in Australo-Papuan pythons and some species of *Python* (Rawlings, 2001). The first feature is an indel at the 5' end of the region adjacent to the *tRNA^{Pro}* gene that is approximately 20 bp of the amino acid acceptor stem and the T ψ C arm of the *tRNA^{Ile}* gene. The second feature is a 15-bp hairpin found adjacent to the isoleucine pseudogene. These two features are present in all of the Australo-Papuan pythons and *P. reticulatus* and *P. timorensis*, although there are only 14 bp of the isoleucine tRNA sequence

present in the two species of *Aspidites* and only 10 bp present in *P. timoriensis*. The third feature, a 15-bp partial repeat of the hairpin in the region 5' to the hairpin, is present in *Antaresia childreni* and *A. stimsoni*. None of these features is present in *P. brongersmai*, *P. molurus*, *P. regius*, *P. sebae* or the out-groups *Candoia* and *Xenopeltis* and consequently the *CR* is considerably shorter for these taxa. For the phylogenetic analysis, we coded these three features as binary characters.

COMBINED DATA ANALYSIS

The aligned partial *CR*, *cytb*, *12S rRNA*, *16S rRNA* sequences, and *CR* structural features and Kluge's 121 morphological characters were jointly analysed using PAUP*4.0b2a. For each of the data partitions, the number of characters included (after exclusion of ambiguously aligned sites in the molecular partitions) and the number of parsimony informative characters, respectively, were: *CR* – 768/175, *cytb* – 1114/401, *12S rRNA* – 372/84, *16S rRNA* – 498/63, morphology – 117/113, *CR* structural features – 3/3.

Heuristic searches under using the MP criterion of optimality found two trees of 4247 steps each (Fig. 2A). The two trees differed solely in the relationships between *Apodora papuana* and *Liasis olivaceus*, with *Apodora* and *L. olivaceus* as sister taxa in one tree or in the second tree with *Apodora* as a sister lineage to a *L. olivaceus* plus the *L. fuscus/L. mackloti* clade. The model of nucleotide substitution found for the combined nucleotide sequence data set, using Modeltest3, was GTR + I + Γ . The Bayesian inference tree is shown in Figure 2B, with posterior probabilities indicated.

In both MP and Bayesian analyses, similar tree topologies were recovered, with most internal branch lengths shorter than terminal branches (Fig. 2). In terms of relationships supported by both sets of analyses, the genus *Python* was paraphyletic, with the Afro-Asian species as the sister to a clade (hereafter called the Indo-Australian clade) that includes the two *Python* species from east of Wallace's Line, *P. reticulatus* and *P. timoriensis*, and the seven Australo-Papuan genera. Both the Afro-Asian *Python* and the Indo-Australian clades are well supported in both the MP and Bayesian analyses. Within the Afro-Asian *Python* clade, relationships among the four taxa are well supported in both analyses. Within the Indo-Australian clade, the two *Python* species form a well-supported clade, with the Australo-Papuan genera as a second also well-supported clade. Relationships among the Australo-Papuan genera are sensitive to the method of analysis and predictably are not well supported in either analysis where they show conflict. While both the MP (Fig. 2A) and Bayesian

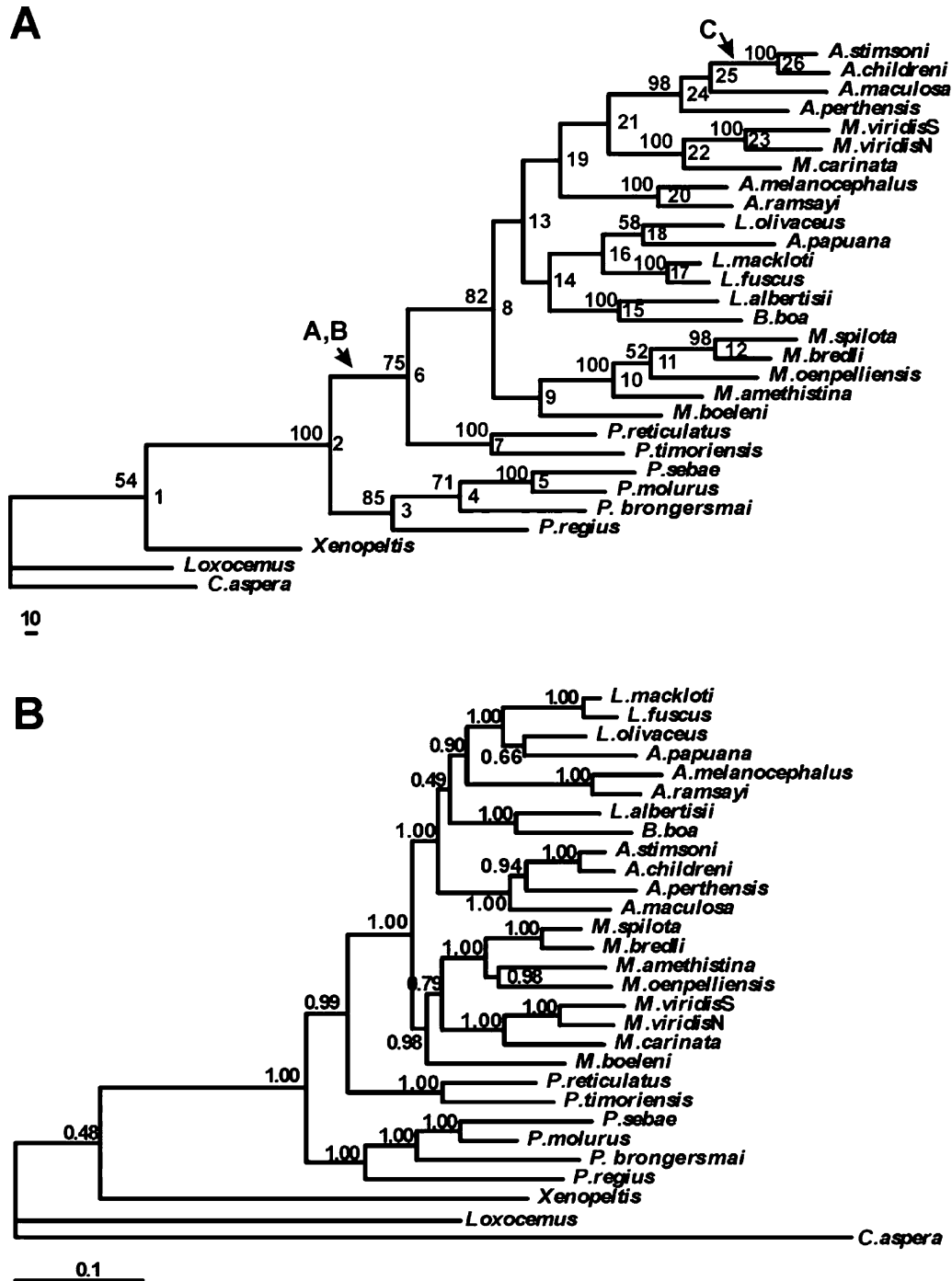


Figure 2. Combined data trees. (A) One of two MP phylograms found from a heuristic search showing proportion of bootstrap pseudoreplicates (above branches) and nodes numbered for reference to Supplementary Material Table S3 (at left of the relevant node). The two MP trees differ solely in the relationships of *Apodora papuana* and *L. olivaceus*. Inferred evolutionary origins of three CR secondary structural elements: A, *tRNA^{le}* pseudogene; B, 5' ~30 bp hairpin; and C, 15 bp ½-hairpin indel. (B) Bayesian tree with posterior probabilities at nodes.

(Fig. 2B) trees show different topologies within the Australo-Papuan part of the tree, there is agreement on the major subclades. Both analytical methods support *Antaresia* and *Aspidites* as they are currently

recognized. Other clades consistently recognized are [(*Liasis fuscus*, *L. mackloti*) *L. olivaceus*, *Apodora papuana*]; (*Bothrochilus boa*, *Leiopython albertisii*); 'typical' *Morelia* [(*M. bredli*, *M. spilota*) *M. amethis-*

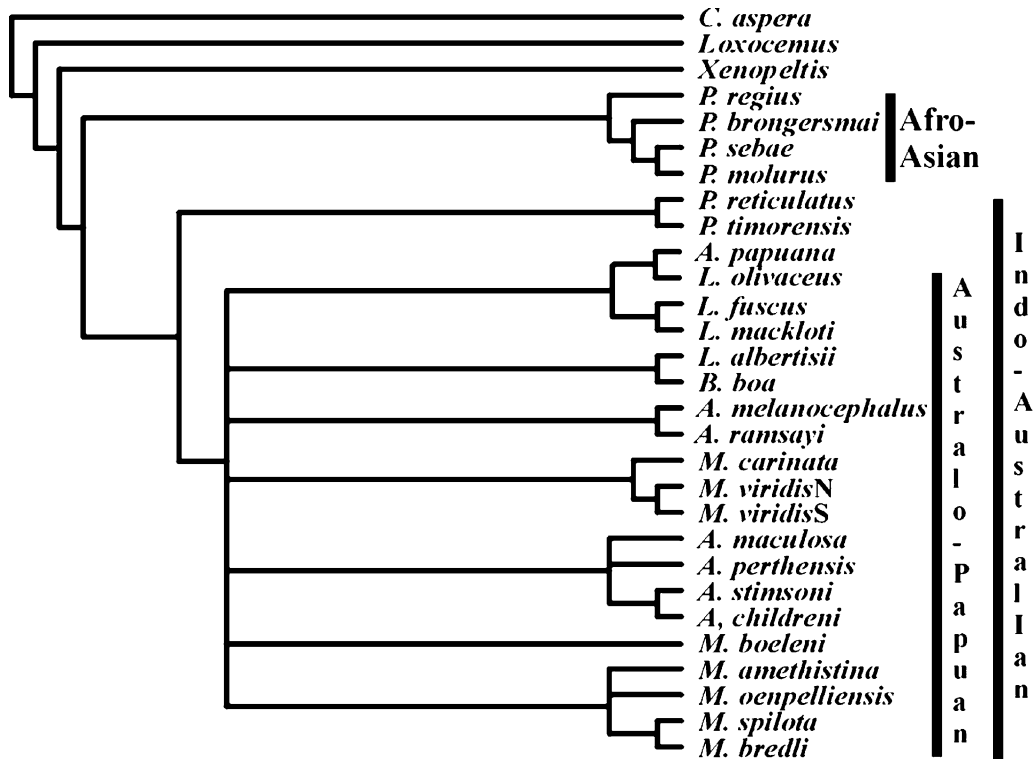


Figure 3. Strict consensus tree showing relationships that were present in both the MP and Bayesian analyses.

tina, *M. boeleni*, *M. oenpelliensis*]; and the rough-scaled and green pythons [*M. carinata*, (*M. viridis* N, *M. viridis* S)]. The last two clades, collectively *Morelia* (*sensu* McDowell), were found as a single clade in the Bayesian analysis but not the MP analysis.

To determine the support contributed by each data partition to the phylogenetic analysis, PBS indices were determined for each of the two equally most parsimonious trees (Supplementary Material Table S3). PBS indices show that the morphological data support four nodes concordantly with the molecular data, and while 16 nodes show conflict between the morphology and at least three of the molecular partitions, while for two nodes the morphological data neither support nor conflict (Supplementary Material Table S3, Fig. 2A). Where the morphological data show conflict with the molecular data partitions, the *16S rRNA* partition is in many cases (12 nodes) also in conflict with the other molecular partitions. When the morphology partition was discordant with at least three of the molecular partitions, it had negative values of a similar magnitude to the largest positive values among the molecular partitions at 14 nodes.

PBS values for the Afro-Asian *Python* clade (node 3) are low or uninformative for all but the *cytb* partition, which is negative and low. PBS values for

the Indo-Australian clade (node 6) are high or uninformative for four of the five molecular partitions, but negative and high for the morphological partition and negative but low for the *16S rRNA* partition (Supplementary Material Table S3). PBS values for the *P. reticulatus* and *P. timorensis* clade (node 7) are all positive. The *CR* and *cytb* partitions had high positive values for the Australo-Papuan clade (node 8) in contrast with a highly conflicting morphological partition.

PHYLOGENETIC ANALYSIS OF MORPHOLOGICAL CHARACTERS

Our evaluation of the reliability or utility of the morphological characters found by Kluge (1993) to support the sister relationship of *Aspidites* to all other pythons is presented in Supplementary Material Appendix S1. In summary, eight of the 11 characters were considered of doubtful value for phylogenetic inference and excluded from subsequent analyses. The four characters that were retained and used in our reanalyses were: 31 (the separation of the supraorbital from the parietal), 45 (the relative length of the quadrate), 50 (the number of palatine teeth per ramus in an adult) and 55 (width of the maxillary process).

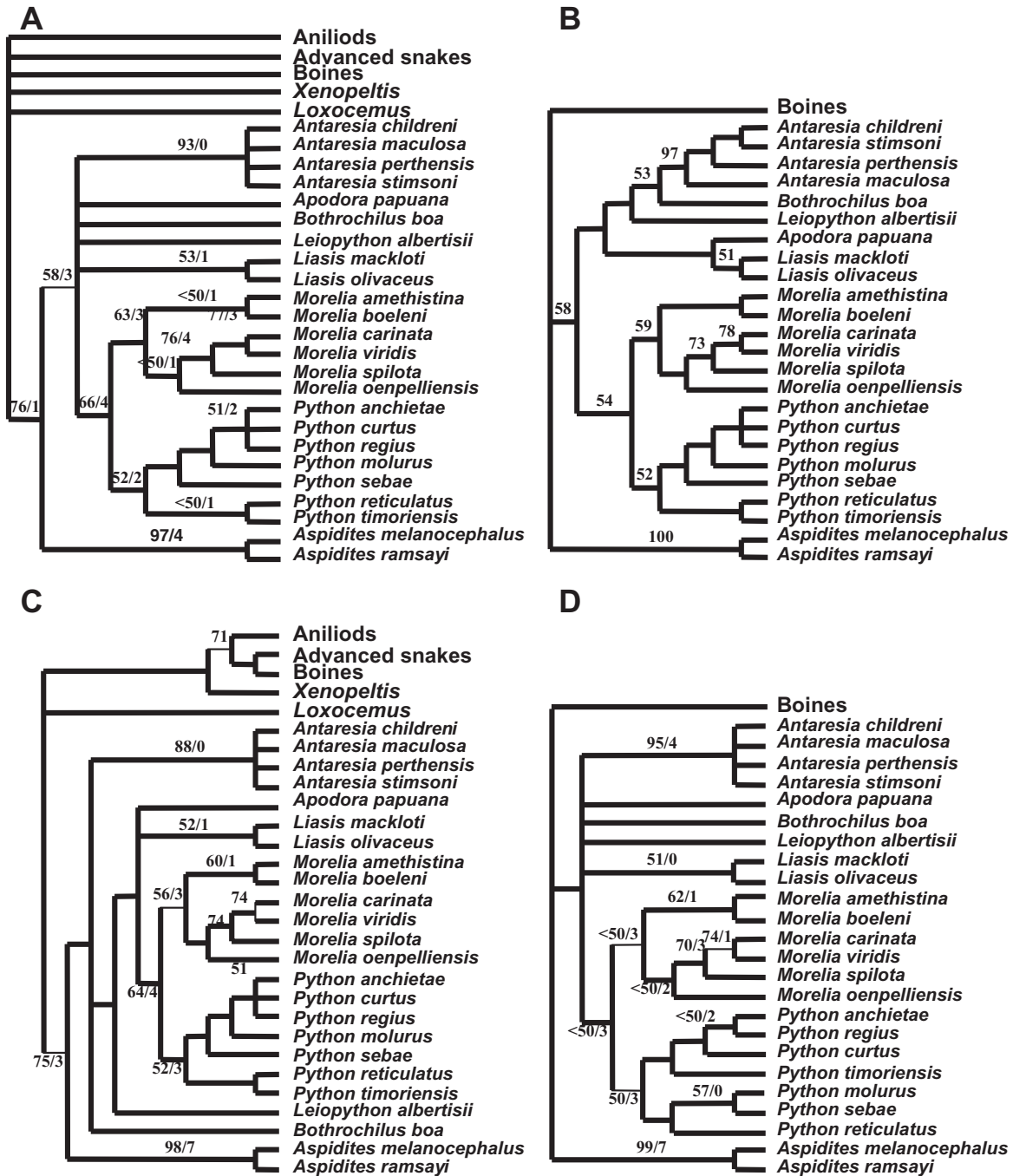


Figure 4. Strict consensus trees of MP analysis of Kluge, 1993) morphological data. (A) and (C) include an expanded set of out-groups, (B) and (D) incorporate a single common ancestor as per Kluge’s analysis. (C) and (D) exclude eight potentially ambiguous morphological characters that Kluge considered significant to the definition of *Aspidites* (see text). Numbers at nodes represent bootstrap pseudoreplicate proportions (left) and Bremer decay indices (right).

Using MP, the data of Kluge (1993) were reanalysed with an expanded set of five out-groups. A heuristic search of 121 characters (119 parsimony informative, two parsimony uninformative) with simple stepwise addition and tree bisection–reconnection found 60

equally most-parsimonious trees of length 464 steps. A strict consensus tree is shown in Figure 4A. Two equally most-parsimonious trees of length 410 steps were found using a generalized ‘boine’ out-group (consensus tree shown in Fig. 4B). Each search was also

repeated with the eight characters mentioned above excluded from the analysis. For the analysis with five out-groups, there were 20 equally most-parsimonious trees of length 422 steps (Fig. 4C), compared with seven equally most-parsimonious trees of length 375 steps found with a single out-group (Fig. 4D).

In each of the analyses that used all the characters, *Aspidites* was placed as the sister taxon to all other pythons with weak bootstrap support [58%, decay index (d) of 1] (Fig. 4). All of the polytypic genera were each monophyletic, but there was only strong bootstrap support for the *Antaresia* and *Aspidites* clades, 93% (d = 5) and 97% (d = 4), respectively, with expanded out-groups and 97 and 100%, respectively, with a generalized out-group. *Python* and *Morelia* formed sister clades with bootstrap proportions of 66 and 54% with expanded out-groups and generalized out-group, respectively. Decay indices were all five or less with the strongest support for the *Antaresia* clade.

Our reanalyses of Kluge's morphological data, with and without expanded out-groups, are consistent with the placement of *Aspidites* as the sister group to all other pythons. However, bootstrap support for this arrangement is only strong (76%) for the original Kluge (1993) data set. With expanded out-groups, the bootstrap support for *Aspidites* as a sister group drops to 58% and hypothesis testing shows that the data do not unequivocally support Kluge's hypothesis. The sister relationship of *Morelia* to *Python* is supported in each analysis with only low (50–64% pseudoreplicates) bootstrap support.

The monophyly of *Aspidites* is one of the best-supported nodes (16 synapomorphies; Supplementary Material Table S3) in the morphological analysis (both Kluge's original work and our reanalysis), yet the basal position for this clade found in the 'best' morphological trees is not supported by the molecular data, and is less strongly supported by Kluge's own data set than was at first apparent. We suggest that the apparent strong support for a basal *Aspidites* would be considerably weakened if these 16 characters were not independent, and earlier authors have noted this possibility. The placement of *Aspidites* of McDowell (1975) and Underwood & Stimson (1990) reflects their a priori assumption that the presence or absence in *Aspidites* of some characteristics (e.g. thermoreceptive labial pits and the horizontal part of the nasal bone lying above the nostrils) is because of secondary loss or secondary acquisition of these traits, respectively. While this may have been a procedural error on the part of Underwood and Stimson (justifiably criticized by Kluge, 1993), it is nevertheless true that *Aspidites* is unique among pythons in its burrowing habits, and could be expected to show a suite of characters co-evolved for this mode of life. Of

Kluge's 16 synapomorphies which unite all pythons exclusive of *Aspidites*, at least seven (the functional significance of most of the remainder is unknown) can be argued as being typical of the anatomical changes which occur in the skulls and scalation of limb-reduced squamates which burrow (Greer, 1979; Rieppel, 1984; Greer, 1985). These characters are linked to the formation of a burrowing rostrum (characters 1 and 5), shortening and strengthening of the facial bones (characters 50 and 55), reduction of the cross-sectional area of the head (character 73), fusion of head shields (character 85) and in the specific case of pythons, absence (secondary loss) of the forward-opening thermoreceptive pits (character 106).

SYSTEMATIC IMPLICATIONS

The lack of divergence found in morphological analyses is also reflected at a molecular level. Genes such as *12S rRNA* and *16S rRNA*, often used to determine deeper evolutionary histories (e.g. Hedges & Poling, 1999) do not have sufficient phylogenetic signal to be useful in python systematics, as seen in their low, frequently slightly negative decay values.

The molecular data suggest that the primary split among pythons is between the genus *Python* and the other genera, with *Aspidites* included within the Australian radiation (Fig. 3). This is in concordance with the findings of Underwood & Stimson (1990) and Schwaner & Dessauer (1981), who placed the genus *Python* as the sister lineage to all of the Australo-Papuan genera, and is fundamentally different from the set of relationships proposed by Kluge (1993), which placed the genus *Python* as a nested clade among the Australo-Papuan genera. The second finding is that the genus *Python* is paraphyletic, with *P. reticulatus* and *P. timoriensis* forming the sister clade to the Australo-Papuan genera. McDowell (1975) made the observation that *P. reticulatus* appeared to be more closely related to the Australo-Papuan genus *Liasis* (now *Morelia amethystina*) than to the other African and Asiatic python species and the recent molecular phylogeny of Lawson *et al.* (2004) provides further support. This relationship is also supported by the presence of two mitochondrial genomic changes, a *tRNA^{Leu}* pseudogene and a partial 5' hairpin in the *CR* that *Python reticulatus* and *P. timoriensis* have in common with the Australo-Papuan pythons.

Rare genomic changes are excellent candidates for 'high-quality' phylogenetic markers because of their rarity and assumed low rate of convergence, and the precise secondary loss of the character is likely to be extremely rare for most large-scale mutations (Rokas & Holland, 2000). Gene order changes and rearrangements in the mitochondrial genome are rare in most

animal groups, making these markers useful for higher level phylogenetics (Boore, Lavrov & Brown, 1998, Boore, Daehler & Brown, 1999), but can be relatively frequent and a potentially more appropriate marker for lower-level phylogenetics (Kurabayashi & Ueshima, 2000). Pythons are the only snake lineage examined to date that has an extended 5' section of the *CR* (Kumazawa *et al.*, 1996, 1998). The isoleucine tRNA pseudogene and 15-bp hairpin are not found in the xenopeltine and boid out-groups *Xenopeltis* and *Candoia*, nor are they present in colubrid or viperid snakes (Kumazawa *et al.*, 1996, 1998). This presence/absence phylogenetic pattern of these structures is also found in the duplicated copy of the *CR* that is present elsewhere in the mitochondrial genome of snakes (Kumazawa *et al.*, 1996, 1998). Therefore, the presence of rare genomic changes in the 5' section of the *CR* of *Python reticulatus* and *P. timoriensis* and the Australo-Papuan pythons constitutes strong evidence of phylogenetic affinities of these taxa. The formal taxonomic description of a new genus for the *P. reticulatus* + *P. timoriensis* clade is presented at the end of the Discussion.

Kluge placed *Aspidites* as the sister lineage to all pythons, McDowell (1975) placed *Aspidites* as the sister lineage to a *Bothrochilus/Leiopython, Antaresia* and *Liasis* clade and Underwood & Stimson (1990) placed *Aspidites* as the sister lineage to all pythons apart from the genus *Python*. Our data are not conclusive regarding the position of *Aspidites*, but our analyses favour an origin for *Aspidites* within the Australian radiation, rather than being its sister lineage. Our analyses show short branch lengths at deeper divergences, with much longer terminal branches between species within genera, but the poor resolution at the base of the Australian radiation suggests rapid diversification of these python lineages. Further probing of this area of divergence with independent and more slowly evolving genetic sequences (e.g. from nuclear introns) might be informative in unravelling the base of the Australian radiation.

The relationships among *Python* species agree in showing the short, stout *P. regius* at the base of the python clade, suggesting giant forms evolved twice, once in *P. reticulatus*, and once in the lineage leading to the Asian and African giants, *P. sebae* and *P. molurus*, respectively.

The remaining pythons show some apparent cases of conflict with Kluge's taxonomic arrangement. In the Bayesian analyses, monophyly of *Morelia* is well supported, but the genus is diphyletic in the MP analyses with the *M. carinata* + *M. viridis* clade as sister to *Antaresia*. However, support in the MP analysis is virtually non-existent for any deeper relationships, including all those concerning species

of *Morelia*. So, in conclusion, our data are minimally consistent with monophyly of *Morelia*. We find three lineages within *Morelia*: *M. boeleni*, the *M. carinata* + *M. viridis* clade, and an *M. amethistina* + *M. bredli* + *M. oenpelliensis* + *M. spilota* clade, which concurs with Kluge only in the sister relationship between *M. carinata* and *M. viridis* and the somewhat remote position of *M. boeleni*.

Kluge (1993) tentatively placed *papuana* in the monotypic *Apodora*, designated as *sedis mutabilis* because of the lack of resolution of the relationships between *Apodora*, *Liasis mackloti* and *L. olivaceus*. Our analyses also found a relationship between *Apodora* and *Liasis*, but, in contrast to Kluge's analysis, were consistent in showing [(*Apodora*, *L. olivaceus*) (*L. fuscus*, *L. mackloti*)] but with strong support only for the pairing of *L. fuscus* and *L. mackloti*.

The current use of monotypic genera for *Bothrochilus boa* and *Leiopython albertisii* is based on Kluge's analyses which placed them as successive sister species of his *Liasis* + *Morelia* + *Python* clade. In contrast, our study also concurs with that of McDowell (1975) in placing them as sister taxa. McDowell (1975) considered that there were so many similarities between *B. boa* and *L. albertisii* that separate generic status was unwarranted. There is considerable pattern variation in *B. boa*, with distinctive orange and black striped markings being the most striking and the most common in collections (Kluge, 1993; O'Shea, 1996). However, there is also a uniformly dark form similar to *L. albertisii* (McDowell, 1975; Kluge, 1993; O'Shea, 1996) and a range of variations in between (Kluge, 1993). Our data strongly support the use of a single generic name (*Bothrochilus*) for this species pair.

DIVERSIFICATION RATES

We restricted our analysis of diversification rates to the monophyletic Indo-Australian python radiation (all Australo-Papuan genera plus *P. reticulatus* and *P. timoriensis*). Cross-validation analysis of PL trees with different smoothing parameters and of the Langley-Fitch calibrated tree indicated optimal performance of PL with a smoothing parameter of 32 (Fig. 5A). To visually assess the tempo of diversification in the Indo-Australian pythons, we constructed a log-lineage-through-time (LTT) plot (Nee *et al.*, 1992). This represents the number of lineages in existence as a function of the time from the root node in the ultrametric PL tree. The python LTT plot clearly shows an excess of early diverging lineages (Fig. 5B), implying that diversification rates have declined over time.

Likelihood analysis of the PL tree strongly rejects the null hypothesis that diversification rates in the

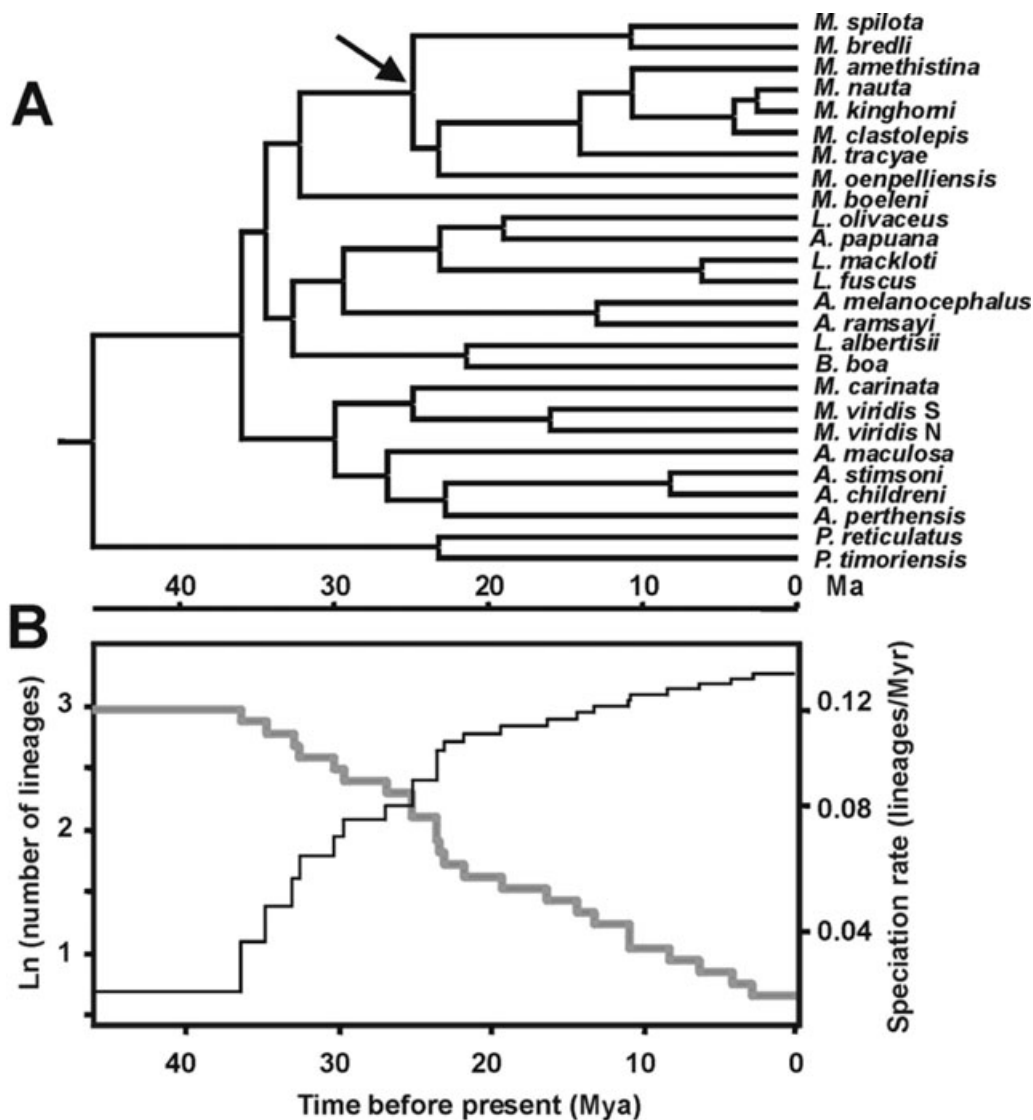


Figure 5. (A) Penalized likelihood (PL) chronogram derived from the Bayesian consensus phylogram for all members of the Indo-Australian python radiation. Arrow indicates fossil calibration point. (B) Black line: log-lineage through time plot for nodal divergence times inferred from the PL analysis. Grey line indicates the maximum likelihood estimate of the speciation rate through time under the best-fit rate-variable model of diversification (DDL; density-dependent logistic).

Indo-Australian pythons have been constant through time ($\Delta\text{AIC}_{\text{RC}} = 11.04$; $P = 0.005$; Table 1). The best-fit rate-variable model was the two parameter density-dependent logistic model (DDL; $\text{AIC} = 76.05$), and the best-fit rate-constant model was the one-parameter pure-birth model ($\text{AIC} = 87.10$). Despite the appearance of a pronounced rate-shift at approximately 23 Mya in the LTT plot (Fig. 5B), there is no evidence that a discrete rate-shift model fits the data better than the DDL model, which specifies a gradual decline in speciation rates (Table 1). It is clear that the DDL model provides a better fit to the data than

the density-dependent exponential model (DDX; $\text{AIC} = 81.49$; Table 1). These models differ considerably in the expected rate of lineage accumulation through time: the DDL model proposes a linear decay in diversification rates, such that the addition of each new lineage results in a constant decrement to the speciation rate. Under the DDX model, in contrast, most of the decline in the speciation rate occurs with early diverging lineages, and this model should provide a better fit when explosive diversification occurred very early in the radiation (e.g. Lovette and Bermingham, 1999). Taken together, estimates of the

Table 1. Model-based analysis of the tempo of Indo-Australian python diversification

Model type*	NP†	LnL (AIC)‡	ΔAIC§	Model¶
Pure birth (RC)	1	-42.55 (87.1)	11.04	$\lambda = 0.041$
Birth-death (RC)	2	-42.55 (89.1)	13.04	$\lambda = 0.041, \mu = 0$
DDX	2	-36.03 (76.05)	0	$\lambda(t) = 0.281(n_t^{-0.730})$
DDL	2	-38.75 (81.49)	5.44	$\lambda(t) = 0.130\left(1 - \frac{n_t}{27.024}\right)$
Discrete shift (pure birth)	3	-35.73 (77.46)	1.40	$\lambda_1 = 0.104, \lambda_2 = 0.022, t_s = 21.41$ Mya
Discrete shift (birth-death)	4	-34.85 (77.69)	1.64	$\lambda_1 = 0.324, \lambda_2 = 0.029, \mu = 0.89^*\lambda, t_s = 22.82$ Mya

The difference in AIC scores between the best-fit rate-constant and rate-variable models (ΔAIC_{RC}) is 11.04 ($P < 0.005$). We determined the probability of this value under the null hypothesis by tabulating ΔAIC_{RC} statistics for 2000 phylogenies of the same size as the Indo-Australian python clade ($N = 26$ taxa) simulated under the pure-birth model and finding the percentile of this distribution corresponding to the observed ΔAIC_{RC} statistic.

*(RC) denotes rate-constant model; DDL and DDX correspond to density-dependent logistic and exponential models, respectively.

†Number of parameters in each model.

‡Log-likelihood and AIC scores of the python data under each model.

§Difference in AIC scores between each model and the overall best-fit model.

¶Parameters of each model estimated using maximum likelihood: λ = speciation rate; μ = extinction rate; $\lambda(t)$ is the speciation rate at time t as a function of the number of lineages in existence at that point in time (n_t); t_s = inferred time of rate shift for discrete shift models in millions of years before present (Mya). Rates for λ and μ parameters are in units of lineages/million years.

speciation rate for the DDL (Fig. 5B) and discrete shift models (Table 1) suggest that diversification rates in the pythons have decreased at least fourfold over the course of their estimated 45-Myr history in the Indo-Australian archipelago.

It is probable that we cannot infer the true magnitude of the shift in speciation rates over time because of the confounding effect of background extinction. AIC scores for discrete shift models with and without extinction are approximately equal. However, the model with extinction specifies a 10-fold reduction in the speciation rate over time vs. a modest fourfold reduction under the model without extinction. This is as a result of the ‘pull of the present’, whereby background extinction results in an apparent excess of recently diverged lineages (Nee *et al.*, 1994a, Nee, May & Harvey, 1994b). If the Indo-Australian python radiation was characterized by high levels of background extinction, we would have reduced power to detect temporal declines in diversification rates under models that specify $\mu = 0$, such as the DDX, DDL and pure-birth discrete shift models. The magnitude of the decline in speciation rates through time inferred under models without extinction thus represent minimum estimates of the true decline in the speciation rate.

The calculated γ -statistic for the Indo-Australian pythons ($\gamma = -3.152$) is inconsistent with a rate-constant diversification process ($P < 0.001$) and corroborates our finding that diversification rates have declined over time. It is well known that incomplete

taxon sampling can result in a perceived temporal decline in diversification rates (Pybus & Harvey, 2000). Although we included all nominate members of the Indo-Australian python radiation in our analysis, it is possible that undescribed or undetected morphologically cryptic species could have resulted in a spurious decline in diversification rates over time. To explore the effects of missing species on our analysis, we determined the number of missing lineages that would render the observed γ -statistic (-3.152) insignificant. We simulated sets of 1000 phylogenies under a pure-birth model of cladogenesis to a final clade size of $N_T = 30, 35, 40 \dots 90, 95$ and 100 lineages, then randomly pruned each simulated tree to the same number of lineages in the Indo-Australian python tree ($N = 26$). We calculated the γ -statistic for each simulated tree and determined the 0.05%ile of the distribution of γ for each N_T ; this value corresponds to the lower bound of the 95% confidence interval around the null hypothesis that γ is not significantly less than zero. Results indicate that the true size of the Indo-Australian python clade would have to exceed 95 species to observe $\gamma = -3.152$ if this value is not significantly less than zero ($\alpha = 0.05$).

Our results suggest comparatively rapid divergence of python lineages in the Indo-Australian archipelago during the Eocene to Miocene periods. To the extent that our calibration of the *M. spilota/M. oenpelliensis* split at 25 My is accurate, this implies that over half of all extant Indo-Australian python lineages existed

by the early Miocene. This suggests that the lack of python fossils predating the mid-Miocene (Rage, 1987; Scanlon, 1996) may reflect a real sampling gap in the palaeontological record for this group.

BIOGEOGRAPHICAL IMPLICATIONS

On the basis of the most ancestral taxon in each study, Underwood & Stimson (1990) and Kluge (1993) formulated biogeographical hypotheses for the pythons. Underwood & Stimson (1990) suggested that the pythons originated in south-east Asia, the genus *Python* differentiating there and dispersing west into Africa, while a second, south-easterly dispersal of the ancestral python stock founded the Australo-Papuan radiation. In contrast, Kluge (1993) concluded an Australia–New Guinea origin of pythons with subsequent radiation into south-east Asia and Africa.

The phylogenetic results of the present study place the Afro-Asian pythons as the sister group to all other pythons, and show deep divergences among these species, compared with the shallow divergences and poor resolution among the Australian radiation. This suggests that pythons arose in Africa or Asia and dispersed eastwards through Malaysia and Indonesia, arriving relatively recently in Australia and New Guinea. The paraphyletic split within *Python* occurs amongst the Asian pythons, with *P. reticulatus* and *P. timoriensis* being divergent from the Asian *P. brongersmai* and *P. molurus* and the African *P. regius* and *P. sebae*.

The frequent discovery that biogeography reflects phylogeny more accurately than morphological inferences (e.g. Schulte, Melville & Larson, 2003; Noonan & Chippindale, 2006) is again reiterated here, even although the reasons for the disparity are as a result of either parallel adaptive radiations (e.g. Losos *et al.*, 1998) or shared plesiomorphic features, as is the case here. The origin of the Australian python radiation from probable Oligocene colonizers from Asia fits an emerging pattern that describes the origins of several other significant Australian region squamate radiations. The study of Keogh (1998) of Australian elapids, and studies by Fuller, Baverstock & King (1998) and Ast (2001) on varanids, and work on agamids (Schulte *et al.*, 2003; Hugall & Lee, 2004) also point to a similar time frame for the radiation of these lineages in Australia. Geological evidence presented by Metcalfe (1998) shows that, during the Oligocene, about 30 Myr BP, Australia's northward drift into the proto-Indonesian archipelago narrowed the open ocean gap between the Australian and Asian continental masses, and also generated volcanic island arcs in the gap, making over-water dispersal by terrestrial taxa increasingly survivable. Such survival would have been enhanced also if the taxa concerned were relatively competent swimmers, scan-

orial, physiologically robust, metabolically low-gearred and opportunistic predators. In all respects, pythons fit this profile, as do varanids, *Physignathus*-like agamids and *Laticauda*-like elapids.

TAXONOMY

McDowell (1975) pointed out that the genus *Python* (in his case also including the genus *Morelia*) was divisible into two morphological groups, the *reticulatus* group (*reticulatus*, *timoriensis* and *Morelia*) and the *molurus* group (the remaining Afro-Asian species of *Python*). The two were diagnosed on the basis of the arrangement of the thermoreceptive pits, morphology of the ectopterygoid and hemipenes, and colour pattern of the upper labial area. McDowell's dichotomy lacked an explicit phylogenetic structure, but our combined data and *CR* synapomorphy now provide strong evidence that these two morphotypic groups constitute independent lineages. Accordingly, we support limiting the generic name *Python* (type species *Coluber molurus* Linnaeus 1758) to the species of Africa and Asia, but excluding *reticulatus* and *timoriensis*. Hoser (2004) has recently proposed the genus *Broghammerus* for *Python reticulatus*. Our analyses provide a phylogenetic basis for recognizing this genus, and further indicate that *reticulatus* and *timoriensis* are sister species, as McDowell (1975) suggested. We redefine *Broghammerus* and expand it to include *timoriensis*.

BROGHAMMERUS HOSER, 2004

Constrictor Wagler 1830, Nat. Syst. Amph., p. 168. Type species '*Constrictor schneideri* Wagl.' (erroneous citation of *Python schneideri* Merrem 1820, = *Python reticulatus* (Schneider 1801)), designated by Fitzinger 1843, Syst. Rept., p. 24. Primary homonym of *Constrictor* Laurenti 1768. (synonymy from McDiarmid, Campbell & Touré, 1999).

Broghammerus Hoser, 2004, p. 21. Type species *Boa reticulata* Schneider (1801), *Hist Amph.* 2, p. 264.

Definition: The clade comprising *Broghammerus reticulatus* (Schneider 1801), and all species that share a more recent common ancestor with *Broghammerus reticulatus* than with *Python molurus*.

Diagnosis (from McDowell, 1975 and Kluge, 1993): A genus of pythonine snakes, of large to gigantic size (adult total length reportedly to 10 m). Differentiated from *Python* (s.s.) by having the supralabial thermoreceptive pits less well defined than the infralabial pits (converse arrangement in *Python*); by infralabial pits set in a longitudinal groove defined ventrally by a longitudinal fold; colour pattern of the suborbital

supralabial region similar to the rest of the supralabials, compared with *Python*, in which there is a dark suborbital patch; elongate medial anterior process of the ectopterygoid, which extends much further anteriorly than the lateral anterior process, compared with subequal processes in *Python* (excluding *P. curtus*); and by hemipenial morphology (McDowell *et al.*, 1975); not known for *timoriensis*). Otherwise most similar to *Morelia* and *Liasis*, from which it can be differentiated (along with species of *Python*) by having the suborbital portion of the maxilla without any lateral flare or projection; the mandibular foramen of the compound bone lying below the posterior end of the dentary tooth row, rather than fully posterior to it; a large medially divided frontal; high midbody scale count (54 or more).

Included species: reticulatus (Schneider 1801), *timoriensis* (Peters 1876).

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Table S1. Primers used for amplification and sequencing.

Table S2. Sixteen characters found by Kluge (1993) that defined the position of *Aspidites*.

Table S3. Partitioned decay indices for combined data parsimony analysis.

Appendix S1. Evaluation of Kluge's (1993) cranial morphological characters.

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