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ORIGINAL ARTICLE

Tracing the history and biogeography of the Australian blindsnake radiation

Julie Marin[1]*, Stephen C. Donnellan[2,3], S. Blair Hedges[4], Paul Doughty[5], Mark N. Hutchinson[2], Corinne Cruaud[6] and Nicolas Vidal[1]

[1]Département Systématique et Évolution, Muséum National d'Histoire Naturelle, 75231, Paris Cedex 05, France, [2]South Australian Museum, Adelaide, 5000, Australia, [3]Australian Centre for Evolutionary Biology and Biodiversity, University of Adelaide, Adelaide, 5005, Australia, [4]Department of Biology, Pennsylvania State University, University Park, PA, 16802-5301, USA, [5]Western Australian Museum, Welshpool, Western Australia, 6106, Australia, [6]Genoscope – Centre National de Séquençage, 91057, Évry Cedex, France

*Correspondence: Julie Marin and Nicolas Vidal, Département Systématique et Évolution, UMR 7138, C.P. 26, Muséum National d'Histoire Naturelle, 57 rue Cuvier, F-75231 Paris Cedex 05, France.
E-mail: jumarin@mnhn.fr; nvidal@mnhn.fr

ABSTRACT

Aim We investigated the biogeographical history of Australian blindsnakes (*Ramphotyphlops*) with reference to Australia's palaeoclimatic history over the past 20 Myr, particularly the development of an extensive arid zone over this period. Terrestrial vertebrate lineages dating back to the Miocene or earlier are predicted to display some or all of the following patterns: (1) for taxa including mesic, arid and monsoonal representatives, a mesic distribution should be phylogenetically ancestral; (2) mesic and monsoon tropical lineages should have diverged before the onset of aridification (with arid lineages appearing later); and (3) refuges may have allowed local persistence and diversification of lineages in the monsoon tropical and mesic zones since the mid-Miocene.

Location Continental Australia.

Methods We compiled a molecular data set comprising one mitochondrial and three nuclear genes for 107 individuals belonging to 28 blindsnake species. Phylogenetic relationships were reconstructed using maximum likelihood and Bayesian inference with RAxML and MrBayes, respectively. Divergence times were assessed using multidivtime. Ancestral habitat states (arid and non arid) were reconstructed using the maximum likelihood method implemented in Mesquite.

Results The age of the Australian *Ramphotyphlops* radiation was estimated at 21.9 Ma (95% credibility interval: 30.2–15.1 Ma). Mesic and monsoon tropical lineages are older than the onset of aridification, with mesic distribution appearing as ancestral on phylogenies. After the onset of aridification, lineages persisted and diversified in mesic, tropical and/or rocky refugia. Arid lineages diversified more recently (< 5 Ma).

Main conclusions Australian blindsnakes join several other Australian squamate lineages with tropical-mesic origins that successfully adapted to the expansion of aridity since the mid-Miocene (*c.* 17 Ma) and now show evidence of multiple relatively recent evolutionary radiations across Australia. We further demonstrate that localized refugia permitted persistence and diversification of mesic taxa, with arid lineages diversifying much later (< 5 Ma) when the arid zone was well established.

Keywords
Continental Australia, evolution, historical biogeography, phylogeny, *Ramphotyphlops*, reptile, snake, squamate, timetree.

INTRODUCTION

The global Cenozoic cooling that began in the mid-Miocene (Flower & Kennett, 1994) has been associated with aridification in the Southern Hemisphere (Richardson *et al.*, 2001; Martin, 2006; Ortiz-Jaureguizar & Cladera, 2006; Byrne *et al.*, 2008; Cowling *et al.*, 2009), and its resulting impact on the evolution and biogeography of species. In particular,

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the Australian biota changed dramatically with the development of an extensive arid zone during the last 20 Myr (Martin, 2006; Byrne *et al.*, 2008).

Before the mid-Miocene, the Australian region currently known as the arid zone was warm, humid, characterized by remarkable climate stability, and inhabited by tropical faunas and floras (Alley & Lindsay, 1995). In the mid-Miocene, the fall of sea levels and the extension of Antarctic ice sheets led to the emergence of new conditions, resulting in the aridification of Australia (McGowran *et al.*, 2004; Bowler *et al.*, 2006). By the late Miocene, rain forest was restricted to the north and east, and small patches in the south, as sclerophyllous plant genera and open, dry forests and shrubland expanded in central Australia (Martin, 2006). During the early Pliocene, a brief return to warmer conditions led to the redevelopment of rain forest, but without recovery of the early Miocene biota. Glacial cycles were established in the late Pliocene and intensified at the beginning of the Pleistocene, along with a rapid growth of glaciers in the Northern Hemisphere around 2.5 Ma (Williams *et al.*, 1998; Byrne *et al.*, 2008). The contemporary stony and sandy deserts expanded during glacial intervals (from 4 to 2 Ma), associated with low temperatures that had never prevailed before in this area (Fujioka *et al.*, 2005; Byrne *et al.*, 2008).

Australia currently comprises three major climatic zones (Fig. 1). The mesic zone includes the wet tropics rain forest in the far north-east, extending south along the Great Dividing Range, with an isolated region in the south-west. The arid zone, as defined by Byrne *et al.* (2008), is widespread in central and western Australia. The remaining northern region receives heavy rainfall during summer, and is called the monsoon tropics (Byrne *et al.*, 2011). Throughout these three climatic zones, rocky ranges at the periphery of the Australian continent, such as the Pilbara, Kimberley and Top End, have acted as refugia over the aridification. They permitted the maintenance and the diversification of mesic and tropical squamate lineages such as geckos and skinks (Chapple & Keogh, 2004; Oliver *et al.*, 2010; Doughty *et al.*, 2011a, b; Pepper *et al.*, 2011).

In accordance with the climatic scenario described above, the lineages dating back to the Miocene or earlier should display some or all of the following patterns, already observed in some squamate reptiles: (1) for taxa including mesic, arid and/or monsoonal representatives, a mesic distribution should be phylogenetically ancestral; (2) mesic and monsoon tropical lineages should have diverged before the onset of aridification (with arid lineages appearing later); and (3) monsoon tropical and mesic lineages that have persisted and diversified since the mid-Miocene should be traceable to refugia (Byrne *et al.*, 2008, 2011; Hugall *et al.*, 2008; Fujita *et al.*, 2010; Oliver & Bauer, 2011).

To test these predictions, we investigated a reptile lineage that is widely distributed in the arid, mesic and monsoon tropical zones of Australia: the blindsnakes (Serpentes, Scolecophidia, Typhlopidae). These reptiles are burrowers and small, usually less than 30 cm in length (Vidal *et al.*, 2010). The 42 currently recognized Australian species all belong to *Ramphotyphlops*, a genus of 66 species distributed across South and Southeast Asia, Australasia and Melanesia (as far east as Fiji, and across the Caroline Islands from Palau to Pohnpei), and comprise one of the least-known elements of the Australian herpetofauna (Rabosky *et al.*, 2004). They colonized Australia between 28 (95% credibility interval: 19–39) and 17 (10–26) Ma, by a single dispersal event from Southeast Asia (Vidal *et al.*, 2010). Because they reached Australia before the onset of the aridification process and are widely spread over the three major biomes, they are well suited for testing hypotheses of Neogene biogeographical scenarios within Australia. Accordingly, we constructed a molecular genetic data set for 107 individuals belonging to 28 *Ramphotyphlops* species composed of one mitochondrial and three nuclear markers and performed phylogenetic and molecular clock analyses.

MATERIALS AND METHODS

Taxonomic sampling

Ingroup sampling included 107 individuals belonging to 28 *Ramphotyphlops* species (Appendix S1 in Supporting Information lists the taxa, localities, and accession numbers of the specimens used in the study). For outgroups, we used *Epictia columbi* (Leptotyphlopidae), *Gerrhopilus mirus* (Gerrhopilidae), *Xenotyphlops grandidieri* (Xenotyphlopidae), *Typhlops reticulatus*, *T. arator*, *T. jamaicensis*, *T. vermicularis*, *Ramphotyphlops acuticaudus*, *R. braminus*, an undescribed species of *Ramphotyphlops* from Moyo Island, Lesser Sundas (hereafter, '*R.* sp.'), and *Acutotyphlops subocularis* (all Typhlopidae), *Anilius scytale* and *Tropidophis* sp. (Alethinophidia: Amerophidia), *Boa constrictor* and *Laticauda colubrina* (Alethinophidia: Afrophidia), and the lizard *Shinisaurus crocodilurus* (Anguimorpha: Shinisauridae).

Molecular markers

One mitochondrial protein-coding gene and three nuclear protein-coding genes were used. The mitochondrial marker, cytochrome *b* (*cytb*), was chosen for its potential to resolve the most recent nodes. The three nuclear genes are the prolactine receptor (*PRLR*), the brain-derived neurotrophic factor (*BDNF*) and the bone morphogenetic protein 2 (*BMP2*). These genes have already been used for inferring higher-level squamate phylogenies (Noonan & Chippindale, 2006; Townsend *et al.*, 2008; Wiens *et al.*, 2008; Vidal *et al.*, 2009, 2010). For this work, 91% of the sequences were newly determined: 436 sequences were deposited in GenBank under accession numbers JQ910201–JQ910636.

DNA extraction, amplification, and sequencing

DNA extraction was performed with the DNeasy Tissue Kit from Qiagen (Courtaboeuf, France). Amplification and sequencing was performed using primers listed in Table 1. For

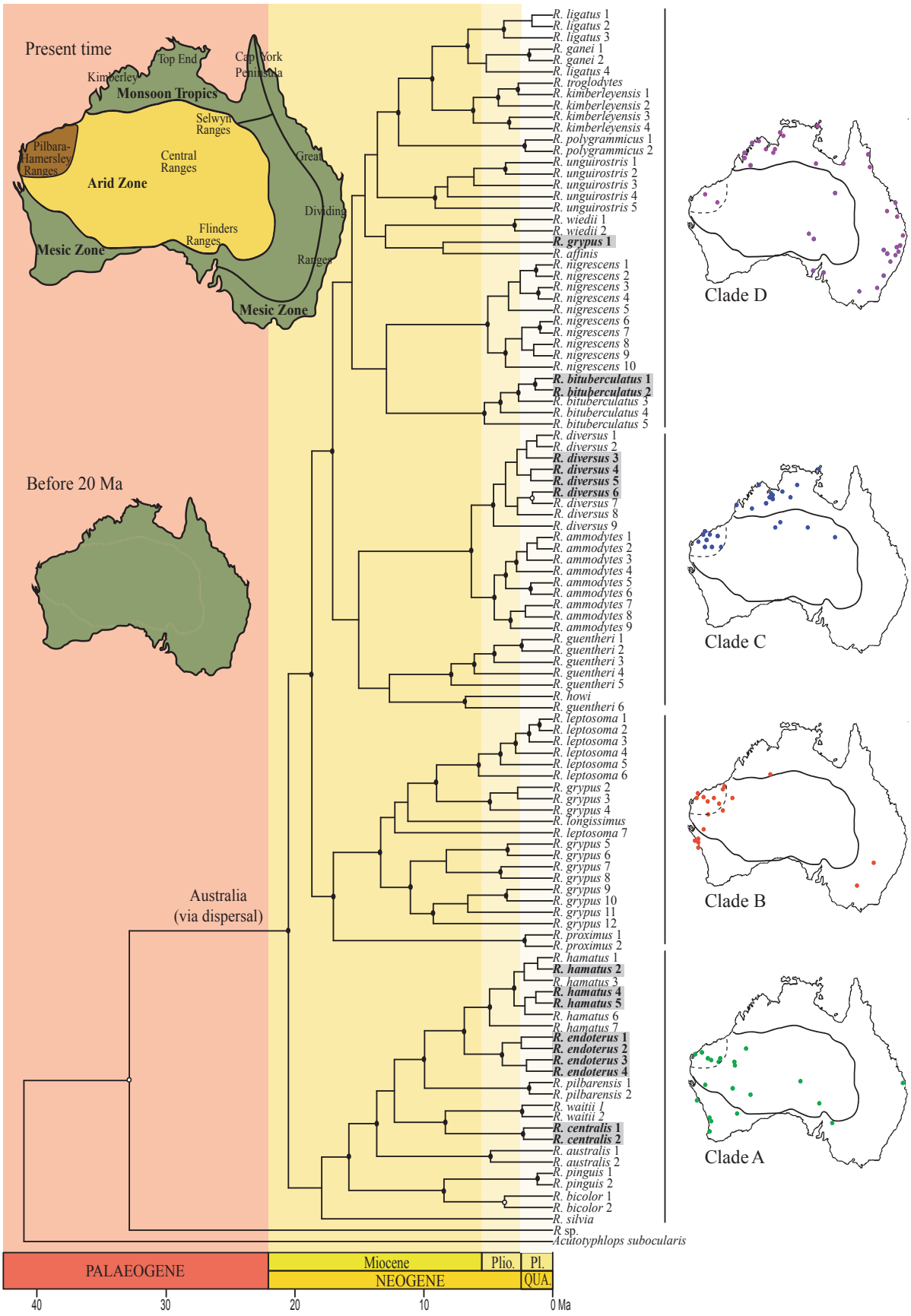

Table 1 List of primers used in this study.

Gene	Primer		Authors
cytb	CS1L	GAAAAACCGCYRTTGTWWTTCAACTA	Adalsteinsson *et al.* (2009)
	LTyph2R	AGYTTGTTTGGGATKGCTCGTAGRAT	Adalsteinsson *et al.* (2009)
	L14910	GACCTGTGATMTGAAAACCAYCGTTGT	Burbrink *et al.* (2000)
	H16064	CTTTGGTTTACAAGAACAATGCTTTA	Burbrink *et al.* (2000)
PRLR	PRLR_f1	GACARYGARGACCAGCAACTRATGCC	Townsend *et al.* (2008)
	PRLR_f2	AAGAGTCRCCCAYATAAAAA	this study
	PRLR_r3	GACYTTGTGRACTTCYACRTAATCCAT	Townsend *et al.* (2008)
	PRLR_r4	AAGAACYTCTCTGGAGGT	this study
	PRLR_r5	ATCCATTGGYTTTGYAGACA	this study
BDNF	BDNF_F	GACCATCCTTTTCCTKACTATGGTTATTTCATACTT	Noonan & Chippindale (2006)
	BDNF_R	CTATCTTCCCCTTTTAATGGTCAGTGTACAAAC	Noonan & Chippindale (2006)
BMP2	f6	CAKCACCGWATTAATATTTATGAAA	Wiens *et al.* (2008)
	r3	ACYTTTTCGTTYTCRTCAAGGTA	Wiens *et al.* (2008)
	BMP2_f7	TTCTCAARTCAGAAAGAGAG	this study
	BMP2_f8	GAAAGAGAGGCCTCCAAG	this study
	BMP2_r4	TCAAGGTACARCATWGAGATGG	this study
	BMP2_r5	CAGTTCTGTCGGCACACA	this study

the four markers, DNA amplification was performed by polymerase chain reaction (PCR) in a final 21-μL volume containing 1 μL dimethyl sulfoxide, 0.8 μL of dNTPs at 6.6 mM, 0.12 μL of Taq DNA polymerase (MP Biomedicals, Illkrich, France or Qiagen), using 2.5 μL of the 10× buffer provided by the manufacturer, and 0.32 μL of each of the two primers at 10 pM; 1 mL of DNA extract was added. The PCRs were performed under the following conditions: an initial denaturation at 94 °C for 3 min followed by 40 cycles (3 min at 94 °C, 40 s at 50 °C, 1 min at 72 °C) and a final elongation at 72 °C for 10 min, using a PCR System 2700 thermocycler (Applied Biosystems, Courtaboeuf, France). Amplification products were visualized on ethidium bromide stained agarose gels. Sequencing was performed by the National Centre for Sequencing (Genoscope) at Évry using the same primers.

The two strands obtained for each sequence were combined using SEQUENCHER 4.9 (GeneCodes, Ann Arbor, MI). Sequence alignment was performed with CLUSTALW2 (Larkin *et al.*, 2007) implemented in BIOEDIT (Hall, 1999) and then manually refined with MEGA 5 (Tamura *et al.*, 2011) using amino acid translations.

Saturation analysis

Saturation of the three codon positions of the two most variable genes, *cytb* and *LRPR*, was evaluated by plotting neighbour-joining uncorrected distances (branch length sum) against corrected distances estimated under the model GTR+I+G, using PAUP* 4.0b10 (Swofford, 2003). Third codon positions in *cytb* were removed in divergence-time analyses because they were judged to be saturated (Lukoschek *et al.*, 2012).

Phylogenetic analyses

Phylogenetic analyses were conducted for each gene separately, for the nuclear data set only, and for the total combined data set. We built phylogenies using maximum likelihood (ML) and Bayesian inference. ML analyses were performed with RAxML 7.2.8 (Stamatakis, 2006; Stamatakis *et al.*, 2008), and Bayesian analyses were performed with MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003). We treated each of the codon positions of the *cytb*, *BDNF*, *LRPR* and *BMP2* genes as a separate partition, such that the combined data set included 12 partitions. Bayesian analyses were performed by running 10,000,000 generations in four chains, saving the current tree every 100 generations, with a GTR+I+G model applied to each partition. The last 90,000 trees were used to construct a 50% majority rule consensus tree. For the ML analysis, we used the same 12 partitions and performed 1000 bootstrap replicates. Trees were visualized with FIGTREE 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Divergence time estimation

Bayesian molecular clock analyses were conducted with the combined data set, excluding the third codon positions in *cytb* (11 partitions), using MULTIDIVTIME T3 (Thorne & Kishino, 2002; Yang & Yoder, 2003). The Bayesian topology

Figure 1 Timetree of Australian *Ramphotyphlops* based on the analysis of DNA sequences from one mitochondrial and three nuclear protein-coding genes. The four major clades (A–D) are indicated by vertical bars next to taxon names. Representatives inhabiting the central arid zone are written in bold and framed in grey. Nodes with black circles are supported by posterior probability > 0.95 and maximum likelihood (ML) bootstrap probability > 75%. Nodes with white circles are supported by posterior probability > 0.90 and ML bootstrap probability > 70%. The insets on the left show continental Australia before (20 Ma) and after (present day) the aridification. On the right, the four distribution maps correspond to the four main clades; points represent the locations of the samples genotyped for this study. Labels on the time scale are Plio. (Pliocene), Pl. (Pleistocene), Qua. (Quaternary).

obtained from the combined data set (12 partitions) was used as the input tree, with the monophyly of the *guentheri–howi* clade constrained, as this clade is supported by the nuclear data, and is coherent with taxonomic and geographical data. PAML 4 (Yang, 2007) was used to estimate model parameters for the MULTIDIVTIME analyses. Bayesian 95% credibility intervals (CI), which are posterior probability intervals analogous to confidence intervals, were calculated for time estimates.

The prior for the *rttm* parameter was set at 130 Ma, intermediate between the oldest fossil snake (100 Ma) and the oldest fossil anguimorph (166 Ma) (Vidal *et al.*, 2009). The three *rttm* settings (100, 130 and 166 Ma) used by Vidal *et al.* (2010) yielded less than 1% difference in time estimates, so the intermediate *rttm* was used here. The prior *bigtime* was set at 200 Ma (Triassic–Jurassic boundary). Other priors followed the recommendations accompanying the software.

The split between the alethinophidian snake taxa Afrophidia and Amerophidia was constrained at 105.8 Ma (Vidal *et al.*, 2009), consistent with geological data for the opening of the Atlantic Ocean (Gradstein *et al.*, 2004). The oldest caenophidians are from the Cenomanian (100–94 Ma) (Rage & Werner, 1999) and therefore the divergence of Elapidae (Caenophidia) and Boidae ('Henophidia') was set at a minimum of 94 Ma. As the use of the latter calibration has been criticized by Sanders & Lee (2008), we also ran analyses with that calibration removed. In the absence of other available fossil calibrations, we calibrated a different node in the tree, the leptotyphlopid/typhlopid divergence, using a time estimate (153 Ma) obtained by excluding the 94-Ma calibration point (Vidal *et al.*, 2009). We used the extremes of the 95% credibility interval (19–39 Ma) as calibrations for the node uniting the Australian *Ramphotyphlops* and *R.* sp. (Vidal *et al.*, 2010). The last calibration used was a maximum corresponding to geological dates when West Indian islands rose above sea level. The node uniting all West Indian taxa was constrained at a maximum of 37.2 Ma (Iturralde-Vinent & MacPhee, 1999).

All analyses were run for 1,100,000 generations, with a sample frequency of 100 after a burn-in of 100,000 generations.

Ancestral area reconstruction

Ancestral character state reconstructions of habitats were conducted using the maximum-likelihood method implemented in MESQUITE 2.75 (Maddison & Maddison, 2011). Recognized species were assigned different habitat codes according to their distributions (Wilson & Swan, 2006): arid (0), arid and non-arid (1), non-arid (2). Ancestral states were reconstructed for all Bayesian trees retained from the analysis of the combined data set and their mean likelihood was then plotted on the maximum clade credibility tree.

RESULTS

We sequenced 2424 aligned nucleotide sites across the four genes. For *cytb*, 678 sites were obtained with 432 variable sites. For *LRPR*, 379 of 483 obtained sites were variable; for *BDNF*, 188 of 672 sites were variable; and for *BMP2*, 361 of 591 sites were variable.

Phylogenetic analyses

There is congruence [no conflicting nodes supported by ML bootstrap (BS) > 70% and posterior probability (PP) > 0.9] between the nuclear and mitochondrial data for both Bayesian and ML analyses. The nuclear genes resolved many basal nodes for which mtDNA was uninformative. Bayesian and ML trees obtained from the combined data set are also largely congruent (see Appendix S2). Of the 22 species represented by at least two individuals, 17 were found to be monophyletic with high support (Fig. 1). Exceptions were as follows: the robust *R. kimberleyensis* clade (BS = 100%/PP = 1.00) includes *R. troglodytes*; the clade comprising *R. guentheri* and *R. howi* is well supported (BS = 72%/PP = 0.98) and the node uniting *R. howi* and one individual of *R. guentheri* (number 6) is strongly supported (BS = 100%/PP = 1.00); *R. leptosoma* is polyphyletic as well as *R. grypus* (which occurs in three places – paraphyletic with *R. leptosoma* and sister to *R. affinis*), and *R. ligatus* is paraphyletic, indicating the presence of cryptic species.

Australian *Ramphotyphlops* are a clade, as reported previously (Vidal *et al.*, 2010), with four major subclades. The basal subclade (clade A, Fig. 1), to which the restricted central east coast species *R. silvia* belongs with weak support (BS = 52%/PP = 0.47), includes *R. australis*, *R. bicolor*, *R. centralis*, *R. endoterus*, *R. hamatus*, *R. pilbarensis*, *R. pinguis* and *R. waitii* (BS = 82%/PP = 0.99). This clade extends from the Pilbara region southwards in western Australia, and eastwards across southern Australia, with the exception of *R. centralis* and *R. endoterus* from the arid zone and *R. hamatus* distributed in both mesic and arid areas. The second clade (clade B) is strongly supported (BS = 91%/PP = 1.00) and comprises *R. grypus*, *R. leptosoma*, *R. longissimus* and *R. proximus.* It is mainly distributed along the western coast including the Pilbara region, with the exception of *R. proximus*, a mesic south-eastern species. Although the last two clades (C and D) are not well supported (BS = 40% and 58%/PP = 0.93 and 0.94, respectively), the monophyly of the node uniting them is strongly supported (BS = 88%/PP = 1.00). The third clade (clade C) includes *R. ammodytes*, *R. diversus*, *R. guentheri* and *R. howi*. This clade is distributed in the monsoonal tropical region with outliers in the Pilbara (*R. ammodytes*) and northern desert (*R. diversus*) regions. The fourth clade (clade D) comprises *R. affinis*, *R. bituberculatus*, *R. ganei*, *R.* '*grypus*' (from arid Queensland), *R. kimberleyensis*, *R. ligatus*, *R. nigrescens*, *R. polygrammicus*, *R. troglodytes*, *R. unguirostris* and *R. wiedii*. This clade is largely distributed in the monsoonal tropical region to the south-east mesic region, with outliers in the Pilbara (*R. ganei*), northern desert (*R.* '*grypus*') and southern desert (*R. bituberculatus*).

It is noteworthy that several species (*R. australis*, *R. ammodytes*, *R. bituberculatus*, *R. diversus*, *R. endoterus*, *R. grypus*,

R. guentheri, *R. hamatus*, *R. kimberleyensis*, *R. ligatus*, *R. leptosoma*, *R. nigrescens* and *R. unguirostris*) harbour deep branching patterns and at least four 'species' (*R. guentheri*, *R. grypus*, *R. kimberleyensis* and *R. ligatus*) are polyphyletic, suggesting hidden species diversity.

Divergence time estimation

Similar divergence times were estimated with and without the 94-Ma calibration point, so we kept it in our primary analysis. The colonization of Australia occurred between 35.1 (38.9–26.8) and 21.9 (30.2–15.1) Ma, which is consistent with geological connections, considering that the collision of the Australian plate with the Asian plate started 25 Ma (Hall, 2002), leading to the narrowing of the ocean gap between these two landmasses and the development of volcanic island arcs in the gap (Metcalfe, 1998). Indeed, faunal exchanges via dispersals between the Australian and Asian plates began around 30 Ma (Filardi & Moyle, 2005; Moyle *et al.*, 2006; Pasquet *et al.*, 2007; Lecompte *et al.*, 2008).

Our age for the intra-Australian *Ramphotyphlops* diversification, 21.9 (30.2–15.1) Ma, is slightly older than that estimated in a previous study – 17 (25.8–10.5) Ma (Vidal *et al.*, 2010). The early diversification of Australian *Ramphotyphlops* appears to have occurred relatively rapidly (short branch lengths and poor support for some nodes) around the early and mid-Miocene, between 22 and 16 Ma for the four major clades. The arid lineages appeared more recently. The well resolved nodes linking the arid species *R. centralis* and *R. endoterus* date back to 2.4 (6.28–0.14) and 4.2 (8.46–1.1) Ma. Species distributed across mesic and arid regions (i.e. *R. bituberculatus*, *R. diversus* and *R. hamatus*) diversified 1.45 (4.1–0.06), 4.9 (8.65–2.24) and 3.2 (6.39–1.05) Ma, respectively.

Ancestral area reconstruction

Non-arid habitat (mesic and monsoon tropical areas) was reconstructed as the ancestral habitat for Australian *Ramphotyphlops*, with the node uniting Australian *Ramphotyphlops* assigned a non-arid character state (Fig. 2).

DISCUSSION

In Australia, aridification in response to global Cenozoic cooling (Flower & Kennett, 1994) is thought to have profoundly influenced the evolution of terrestrial organisms. The onset of arid conditions in central and western Australia 20 Ma caused the restriction of mesic biomes to coastal areas (Martin, 2006; Byrne *et al.*, 2008, 2011). Because the Australian and Asian plates collided 25 Ma (Byrne *et al.*, 2011), some mesic organisms reached Australia from Asia around that time. Blindsnakes colonized Australia 35.1–21.9 Ma by oceanic dispersal from mesic regions (Southeast Asia) (Vidal *et al.*, 2010). In support of this idea, our analyses reconstructed mesic and monsoon tropical areas as the ancestral habitats for Australian *Ramphotyphlops* (Fig. 2).

Deep divergences within the four major clades, and even within many of the recognized species, reflect long-term isolation since the mid-Miocene (*c.* 17 Ma) in mesic and monsoonal tropical zones, and also the Pilbara. The contraction and fragmentation of mesic forest early in the aridification process (Martin, 2006) seems to have accompanied the origin of *Ramphotyphlops* diversification. Indeed, during fragmentation, the population size decreased, accelerating the process of genetic diversity loss by genetic drift (Hedrick, 2011). Moreover, during the localized expansion phases, a loss of genetic diversity through the founder effect may occur in the case of the colonization of new niches (for example, Maruyama & Fuerst, 1984), resulting in taxa with deep coalescent histories (Hewitt, 1999).

As the arid zone expanded in central Australia, mesic lineages were isolated along the east coast and south-west – the monsoonal tropical zone – which served as refugia. Although located in the arid zone, the Pilbara region was also a refugium. These areas were able to retain relative climatic stability during periods of unpredictable climate (Hewitt, 1999), especially peripheral rocky areas protected from climatic extremes because of their proximity to the coast. The Kimberley, Top End and Selwyn ranges in the monsoonal tropics, the Hamersley Range in the Pilbara, and the Central Ranges have been suggested as significant refugia (Byrne *et al.*, 2008; Fujita *et al.*, 2010; Oliver *et al.*, 2010; Pepper *et al.*, 2011). The complex topography of the Great Dividing Range along the east coast and the Flinders Ranges to the south are also potential refugia (Byrne *et al.*, 2011).

Coastal and rocky regions also acted as discrete refugia during the glacial cycles (Pliocene–Pleistocene). Much of the extant Australian blindsnake diversity originated in the last 4 Myr, between a brief return to warmer conditions in the early Pliocene and the intense glacial cycles of late Pliocene and the Pleistocene (Fig. 1). Lineages were probably widespread and then later fragmented among multiple discrete refugia, similar to a proposal by Fujita *et al.* (2010), who found comparable results for a gecko species complex with a near continent-wide distribution.

Arid-zone colonization occurred several times, mostly from mesic and monsoon tropical ancestors between 9 and 3 Ma (Figs 1 & 2). Despite differences in their phylogenetic and geographical positions, arid lineages diversified very recently, between 4.9 and 1.45 Ma. The formation of contemporary stony and sandy deserts, which occurred during glacial intervals of intense climatic cycles since 4 Ma (Fujioka *et al.*, 2005), is consistent with our estimate of the timing of arid lineage diversification.

CONCLUSIONS

Our results on the diversification of Australian *Ramphotyphlops* are consistent with evolutionary and biogeographical hypotheses underlying Australian aridification. Blindsnakes originated from mesic Asian ancestors 35–21 Ma, and their diversification was subsequently driven by forest fragmenta-

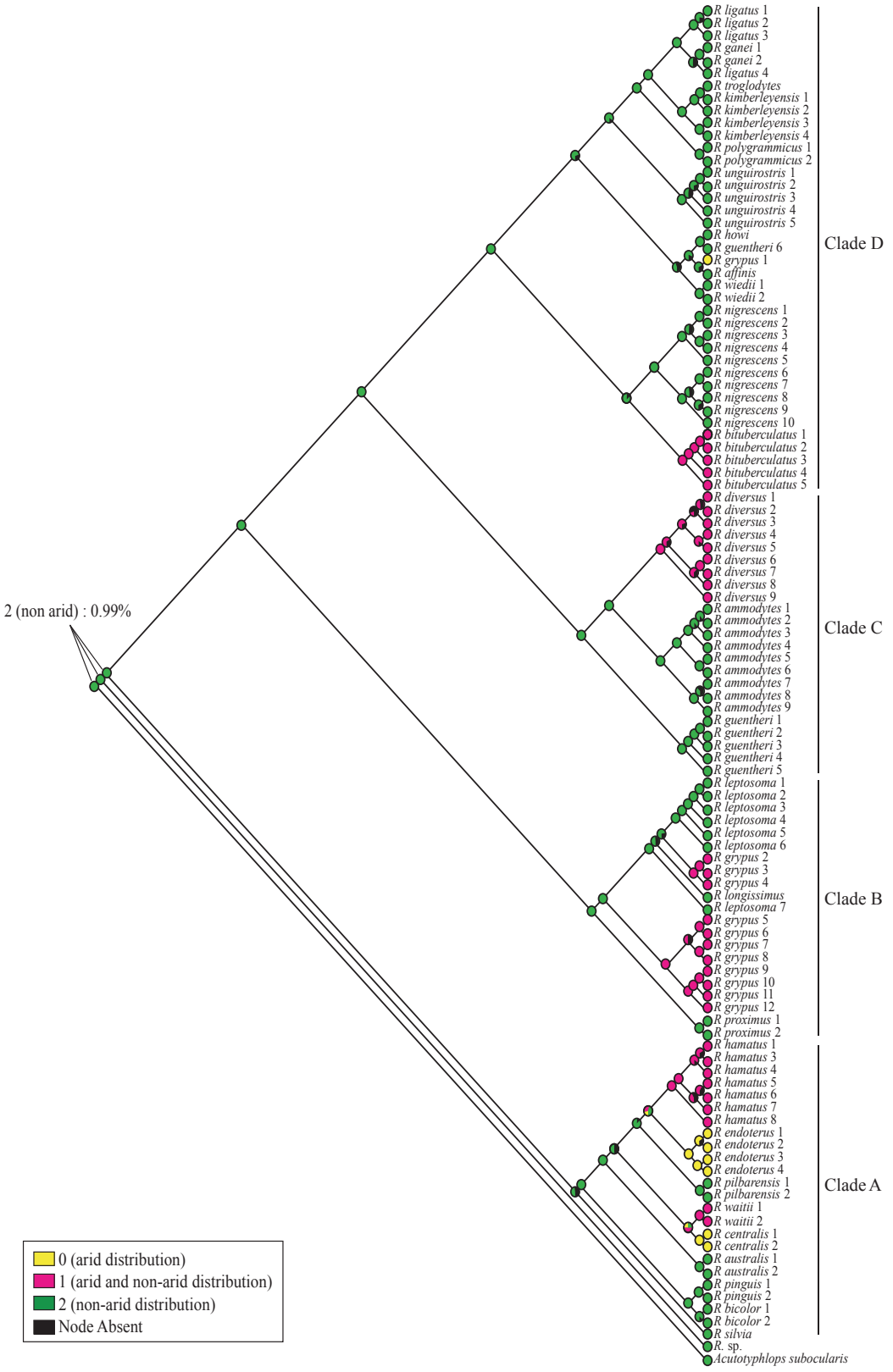

Figure 2 Ancestral area reconstruction of Australian *Ramphotyphlops*. Bayesian cladogram of the combined data set with maximum likelihood estimates of ancestral habitat states. Pie charts correspond to average likelihoods for each state. Percentage values are given for some nodes of interest.

tion resulting from the aridification of Australia beginning 20 Ma. After the onset of aridification, lineages persisted and diversified in mesic, tropical and/or rocky refugia. Arid lineages emerged later, well after the arid zone was established. This pattern is also evident in other squamate lineages: skinks (Rabosky *et al.*, 2007), agamid lizards (Hugall *et al.*, 2008) and geckos (Oliver & Sanders, 2009; Fujita *et al.*, 2010; Oliver & Bauer, 2011; Pepper *et al.*, 2011). Thus, typhlopid snakes appear to be one of several Australian squamate lineages with northern tropical origins that persisted in isolated refugia during the expansion of open and arid environments during the Miocene, with multiple invasions (< 10 Ma) of the arid zone followed by recent diversification (< 5 Ma).

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REFERENCES

Adalsteinsson, S.A., Branch, W.R., Trape, S. & Vitt, L.J. (2009) Molecular phylogeny, classification, and biogeography of snakes of the Family Leptotyphlopidae (Reptilia, Squamata). *Zootaxa*, **2244**, 1–50.

Alley, N.F. & Lindsay, J.M. (1995) Tertiary. *The geology of South Australia*, Vol. 2, *Phanerozoic* (ed. by J.F. Drexel and W.V. Preiss), pp. 151–215. Bulletin 54, Geological Survey of South Australia, Adelaide.

Bowler, J.M., Kotsonis, A. & Lawrence, C.R. (2006) Environmental evolution of the Mallee region, western Murray Basin. *Proceedings of the Royal Society of Victoria*, **118**, 161–210.

Burbrink, F.T., Lawson, R. & Slowinski, J.B. (2000) Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution*, **54**, 2107–2118.

Byrne, M., Yeates, D.K., Joseph, L., Kearney, M., Bowler, J., Williams, M.A.J., Cooper, S., Donnellan, S.C., Keogh, J.S., Leys, R., Melville, J., Murphy, D.J., Porch, N. & Wyrwoll, K.-H. (2008) Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology*, **17**, 4398–4417.

Byrne, M., Steane, D.A., Joseph, L., Yeates, D.K., Jordan, G.J., Crayn, D., Aplin, K., Cantrill, D.J., Cook, L.G., Crisp, M.D., Keogh, J.S., Melville, J., Moritz, C., Porch, N., Sniderman, J.M.K., Sunnucks, P. & Weston, P.H. (2011) Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. *Journal of Biogeography*, **38**, 1635–1656.

Chapple, D.G. & Keogh, J.S. (2004) Parallel adaptive radiations in arid and temperate Australia: molecular phylogeography and systematics of the *Egernia whitii* (Lacertilia: Scincidae) species group. *Biological Journal of the Linnean Society*, **83**, 157–173.

Cowling, R.M., Procheş, Ş. & Partridge, T.C. (2009) Explaining the uniqueness of the Cape flora: incorporating geomorphic evolution as a factor for explaining its diversification. *Molecular Phylogenetics and Evolution*, **51**, 64–74.

Doughty, P., Kealley, L. & Donnellan, S.C. (2011a) Revision of the Pygmy Spiny-tailed Skinks (*Egernia depressa* species-group) from Western Australia, with descriptions of three new species. *Records of the Western Australian Museum*, **26**, 115–137.

Doughty, P., Rolfe, J.K., Burbidge, A.H., Pearson, D.J. & Kendrick, P.G. (2011b) Herpetologial assemblages of the Pilbara biogeographic region, Western Australia: ecological associations, biogeographic patterns and conservation. *Records of the Western Australian Museum, Supplement*, **78**, 315–341.

Filardi, C.E. & Moyle, R.G. (2005) Single origin of a pan-Pacific bird group and upstream colonization of Australasia. *Nature*, **438**, 216–219.

Flower, B.P. & Kennett, J.P. (1994) The middle Miocene climatic transition: East Antarctic ice sheet development, deep ocean circulation and global carbon cycling. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **108**, 537–555.

Fujioka, T., Chappell, J., Honda, M., Yatsevich, I., Fifield, K. & Fabel, D. (2005) Global cooling initiated stony deserts in central Australia 2–4 Ma, dated by cosmogenic ^{21}Ne–^{10}Be. *Geology*, **33**, 993–996.

Fujita, M.K., McGuire, J.A., Donnellan, S.C. & Moritz, C. (2010) Diversification and persistence at the arid–monsoonal interface: Australia-wide biogeography of the Bynoe's gecko (*Heteronotia binoei*; Gekkonidae). *Evolution*, **64**, 2293–2314.

Gradstein, F.M., Ogg, J.G. & Smith, A.G. (2004) *A geologic time scale 2004*. Cambridge University Press, Cambridge.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Hall, R. (2002) Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. *Journal of Asian Earth Sciences*, **20**, 353–431.

Hedrick, P.W. (2011) *Genetics of populations*, 4th edn. Jones & Bartlett Learning, Boston, MA.

Hewitt, G.M. (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, **68**, 87–112.

Hugall, A.F., Foster, R., Hutchinson, M. & Lee, M.S.Y. (2008) Phylogeny of Australasian agamid lizards based on

nuclear and mitochondrial genes: implications for morphological evolution and biogeography. *Biological Journal of the Linnean Society*, **93**, 343–358.

Iturralde-Vinent, M. & MacPhee, R.D.E. (1999) Paleogeography of the Caribbean region: implications for Cenozoic biogeography. *Bulletin of the American Museum of Natural History*, **238**, 1–95.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. & Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, **23**, 2947–2948.

Lecompte, E., Aplin, K., Denys, C., Catzeflis, F., Chades, M. & Chevret, P. (2008) Phylogeny and biogeography of African Murinae based on mitochondrial and nuclear gene sequences, with a new tribal classification of the subfamily. *BMC Evolutionary Biology*, **8**, 199.

Lukoschek, V., Keogh, J.S. & Avise, J.C. (2012) Evaluating fossil calibrations for dating phylogenies in light of rates of molecular evolution: a comparison of three approaches. *Systematic Biology*, **61**, 22–43.

Maddison, W.P. & Maddison, D.R. (2011) *Mesquite: a modular system for evolutionary analysis, version 2.75*. Available at: http://mesquiteproject.org (accessed 13 March 2012).

Martin, H.A. (2006) Cenozoic climatic change and the development of the arid vegetation in Australia. *Journal of Arid Environments*, **66**, 533–563.

Maruyama, T. & Fuerst, P.A. (1984) Population bottlenecks and nonequilibrium models in population genetics. I. Allele numbers when populations evolve from zero variability. *Genetics*, **108**, 745–763.

McGowran, B., Holdgate, G.R., Li, Q. & Gallagher, S.J. (2004) Cenozoic stratigraphic succession in southeastern Australia. *Australian Journal of Earth Sciences*, **51**, 459–496.

Metcalfe, I. (1998) Palaeozoic and Mesozoic geological evolution of the SE Asian region: multidisciplinary constraints and implications for biogeography. *Biogeography and geological evolution of SE Asia* (ed. by R. Hall and J.D. Holloway), pp. 25–41. Backhuys Publishers, Leiden.

Moyle, R.G., Chesser, R.T., Prum, R.O., Schikler, P. & Cracraft, J. (2006) Phylogeny and evolutionary history of Old World suboscine birds (Aves: Eurylaimides). *American Museum Novitates*, **3544**, 1–22.

Noonan, B.P. & Chippindale, P.T. (2006) Dispersal and vicariance: the complex evolutionary history of boid snakes. *Molecular Phylogenetics and Evolution*, **40**, 347–358.

Oliver, P.M. & Bauer, A.M. (2011) Systematics and evolution of the Australian knob-tail geckos (*Nephrurus*, Carphodactylidae, Gekkota): plesiomorphic grades and biome shifts through the Miocene. *Molecular Phylogenetics and Evolution*, **59**, 664–674.

Oliver, P.M. & Sanders, K.L. (2009) Molecular evidence for Gondwanan origins of multiple lineages within a diverse Australasian gecko radiation. *Journal of Biogeography*, **36**, 2044–2055.

Oliver, P.M., Adams, M. & Doughty, P. (2010) Molecular evidence for ten species and Oligo-Miocene vicariance within a nominal Australian gecko species (*Crenadactylus ocellatus*, Diplodactylidae). *BMC Evolutionary Biology*, **10**, 386.

Ortiz-Jaureguizar, E. & Cladera, G.A. (2006) Paleoenvironmental evolution of southern South America during the Cenozoic. *Journal of Arid Environments*, **66**, 498–532.

Pasquet, E., Pons, J.-M., Fuchs, J., Cruaud, C. & Bretagnolle, V. (2007) Evolutionary history and biogeography of the drongos (Dicruridae), a tropical Old World clade of corvoid passerines. *Molecular Phylogenetics and Evolution*, **45**, 158–167.

Pepper, M., Ho, S.Y.W., Fujita, M.K. & Keogh, J.S. (2011) The genetic legacy of aridification: climate cycling fostered lizard diversification in Australian montane refugia and left low-lying deserts genetically depauperate. *Molecular Phylogenetics and Evolution*, **61**, 750–759.

Rabosky, D.L., Aplin, K.P., Donnellan, S.C. & Hedges, S.B. (2004) Molecular phylogeny of blindsnakes (*Ramphotyphlops*) from western Australia and resurrection of *Ramphotyphlops bicolor* (Peters, 1857). *Australian Journal of Zoology*, **52**, 531–548.

Rabosky, D.L., Donnellan, S.C., Talaba, A.L. & Lovette, I.J. (2007) Exceptional among-lineage variation in diversification rates during the radiation of Australia's most diverse vertebrate clade. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 2915–2923.

Rage, J.-C. & Werner, C. (1999) Mid-Cretaceous (Cenomanian) snakes from Wadi Abu Hashim, Sudan: the earliest snake assemblage. *Palaeontologia Africana*, **35**, 85–110.

Richardson, J.E., Weitz, F.M., Fay, M.F., Cronk, Q.C.B., Linder, H.P., Reeves, G. & Chase, M.W. (2001) Rapid and recent origin of species richness in the Cape flora of South Africa. *Nature*, **412**, 181–183.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Sanders, K.L. & Lee, M.S.Y. (2008) Molecular evidence for a rapid late-Miocene radiation of Australasian venomous snakes (Elapidae, Colubroidea). *Molecular Phylogenetics and Evolution*, **46**, 1165–1173.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, **57**, 758–771.

Swofford, D.L. (2003) *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Version 4.0b10. Sinauer Associates, Sunderland, MA.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Thorne, J.L. & Kishino, H. (2002) Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology*, **51**, 689–702.

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

Vidal, N., Rage, J.-C., Couloux, A. & Hedges, S.B. (2009) Snakes (Serpentes). *The timetree of life* (ed. by S.B. Hedges and S. Kumar), pp. 390–397. Oxford University Press, New York, NY.

Vidal, N., Marin, J., Morini, M., Donnellan, S., Branch, W.R., Thomas, R., Vences, M., Wynn, A., Cruaud, C. & Hedges, S.B. (2010) Blindsnake evolutionary tree reveals long history on Gondwana. *Biology Letters*, **6**, 558–561.

Wiens, J.J., Kuczynski, C.A., Smith, S.A., Mulcahy, D.G., Sites, J.W., Jr, Townsend, T.M. & Reeder, T.W. (2008) Branch lengths, support, and congruence: testing the phylogenomic approach with 20 nuclear loci in snakes. *Systematic Biology*, **57**, 420–431.

Williams, M., Dunkerley, D., De Deckker, P., Kershaw, P. & Chappell, J. (1998) *Quaternary environments*. Hodder Arnold, London.

Wilson, S. & Swan, G. (2006) *A complete guide to reptiles of Australia*, 2nd edn. New Holland Publishers, Sydney.

Yang, Z. (2007) PAML 4: phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution*, **24**, 1586–1591.

Yang, Z. & Yoder, A.D. (2003) Comparison of likelihood and Bayesian methods for estimating divergence times using multiple gene loci and calibration points, with application to a radiation of cute-looking mouse lemur species. *Systematic Biology*, **52**, 705–716.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Taxa, localities and accession numbers of the specimens used in this study.

Appendix S2 Additional results (phylogenetic trees and estimated divergence times).

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BIOSKETCH

The authors have broad interests in molecular evolution and herpetology. Their research groups study biogeographical patterns and aim also to discover and classify species.

Author contributions: J.M., S.C.D., S.B.H. and N.V. conceived the idea; S.C.D., M.N.H., and P.D. provided samples; J.M. produced the data, and J.M. and N.V analysed the data. J.M. and N.V. led the writing with every co-author contributing.

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