CSIRO PUBLISHING
www.publish.csiro.au/journals/ajz *Australian Journal of Zoology*, 2004, **52**, 531–548

Molecular phylogeny of blindsnakes (*Ramphotyphlops*) from western Australia and resurrection of *Ramphotyphlops bicolor* (Peters, 1857)

D. L. Rabosky[A,E], *K. P. Aplin*[B], *S. C. Donnellan*[C] *and S. B. Hedges*[D]

[A]Department of Ecology and Evolutionary Biology, Corson Hall, Cornell University, Ithaca, NY 14853-2701, USA.
[B]Australian National Wildlife Collection, CSIRO Sustainable Ecosystems, Crace, ACT 2601, Australia.
[C]Evolutionary Biology Unit and Centre for Evolutionary Biology and Biodiversity, South Australian Museum, North Terrace, Adelaide, SA 5000, Australia.
[D]Department of Biology, Pennsylvania State University, University Park, PA 16802, USA.
[E]Corresponding author. Email: DLR32@cornell.edu

Abstract

Blindsnakes (Typhlopidae) represent one of the least known elements of the Australian herpetofauna. Mitochondrial DNA sequence data and morphology are used here to show that a widespread species of Australian blindsnake, *Ramphotyphlops australis*, comprises two distinct species. *Ramphotyphlops bicolor* (new combination) is resurrected from synonymy with *R. australis* and redescriptions are provided for both species. Mitochondrial DNA sequence variation within *R. australis* indicates that the central and south-coast populations are more closely related to each other than either is to the morphologically distinctive populations at the northern edge of the species' range. Observed levels of differentiation suggest historical isolation of populations from the Kalbarri/Shark Bay region of the western Australian coastline. However, lack of concordance between mitochondrial haplotype phylogeny and morphology for several individuals might reflect limited gene flow between the northern and south–central populations. We note that many taxa show restricted distributions or range disjunctions along the central Western Australian coastal margin, and we discuss possible models to explain population fragmentation in this region. Pliocene–Pleistocene sea-level fluctuations along the western Australian coast could have isolated sand plain communities in the Kalbarri/Shark Bay region from similar communities further south near Geraldton, possibly underlying the phylogeographic pattern in *R. australis*. Data from additional taxa will be needed to fully evaluate this hypothesis.

Introduction

Blindsnakes of the genus *Ramphotyphlops* represent one of the least-known elements of the Australian herpetofauna. The genus is widely distributed throughout the Australasian region and at least 40 species are known from Australia. Little is known of the natural history of these fossorial snakes, but they appear to specialise almost exclusively on ants and termites (Webb and Shine 1993). The general lack of useful taxonomic characters in the Typhlopidae has been a barrier to the recognition of biodiversity in this group. Remarkably little variation is present in meristic and continuous external traits, and virtually nothing is known about the taxonomic utility of osteological and visceral characters in *Ramphotyphlops*. The description of many new species in the past decade (Aplin and Donnellan 1993; Shea and Horner 1996; Aplin 1998) has served to highlight a general lack of knowledge about species diversity and phylogenetic relationships in this group. The taxonomy of *Ramphotyphlops* has changed little since Waite (1918) provided a comprehensive revision of the genus in Australia.

Ramphotyphlops australis is one of the most widely distributed Australian typhlopids, ranging across the southern third of Australia. Considerable morphological variation occurs

 10.1071/ZO04045 0004-959X/04/050531

across the range of *R. australis*, and previous authors have noted differences between 'eastern' and 'western' forms (Shea 1999). Peters (1857) recognised the eastern form as a distinct species, *Onychocephalus* (= *Ramphotyphlops) bicolor*, but Waite (1918) and subsequent authors have considered *O. bicolor* to be a junior synonym of *R. australis*. Peters (1860) and Boulenger (1893) noted that *R. bicolor* possesses a snout with a prominent cutting edge, whereas *R. australis* is distinguished by the presence of a rounded snout. Ontogenetic and geographic variability in this character appear to have resulted in Waite's (1918) synonymy of *R. bicolor* with *R. australis* (Waite 1897).

Adding to the confusion, *R. australis* populations from the extreme north-western portion of the species' range (Shark Bay/Kalbarri regions) are characterised by several distinctive morphological attributes. Many individuals from this region lack pigmentation for 3–10 vertebral scale rows immediately posterior to the ocular scale, resulting in a 'collared' appearance. Furthermore, these populations show reduced vertebral scale counts and have a narrower band of pigmented dorsal scales than southern and central populations.

In this paper, we use DNA sequences of the mitochondrial (mt) 16S rRNA (rRNA) gene to show that *R. australis*, as presently construed, comprises two highly divergent groups corresponding to eastern and western forms of *R. australis*. For clarity and consistency, we provisionally refer to these forms as *R. australis* and *R. bicolor*, respectively, and references to the currently recognised composite form are placed in quotations ('*R. australis*'). These groups are sympatric in Western Australia and display morphological differences consistent with mitochondrial haplotype differentiation, indicating that *R. bicolor* is a valid species. We resurrect *R. bicolor* from synonymy with *R. australis* and provide redescriptions for both species.

We also provide an analysis of sequence variation across the range of *R. australis* to determine whether molecular and morphological evidence warrant recognition of the northern 'collared' forms of *R. australis* as specifically distinct from southern and central populations. We discuss possible biogeographic mechanisms that may have been responsible for the current distribution of *R. australis*.

Materials and Methods

DNA extraction, amplification, and sequencing

Tissue samples were obtained from collections at the Western Australian Museum and South Australian Museum. Morphological characters (discussed below) were used to assign '*R. australis*' samples to *R. australis* and *R. bicolor* subgroups. In total, 11 putative *R. bicolor* and 38 *R. australis* samples were obtained, encompassing virtually the entire range of '*R. australis*' (Fig. 1), as well as samples from the following taxa: *R. ammodytes*, *R. hamatus*, *R. grypus*, *R. waitii*, *R. longissimus*, *R. bituberculatus*, *R. pilbarensis*, *R. leptosoma* and *R. unguirostris*. Museum catalogue numbers for specimens examined, locality data, and GenBank accession numbers are given below (see Appendix).

Genomic DNA was extracted using the ClonTech NucleoSpin Tissue Kit. Primers used follow the nomenclature of Hedges (1994), with the addition of a new primer specific to *Ramphotyphlops* (16H10R: 5′ AGTGGGCCTAAAAGCAGCCA 3′). For all individuals, a 370-bp fragment of the mitochondrial 16S rRNA gene (referred to as 16S-I) was amplified using the primers 16L10/16H10R.

Fifty-microlitre PCR reactions were amplified for 34–45 cycles at 95°C for 15 s, 55°C for 30 s, and 72°C for 45 s. Amplification products were visualised on a 2% low-melt agarose gel and purified using Ultrafree-DA gel filters (Millipore). Cycle sequencing reactions were performed by the Nucleic Acids Facility at Pennsylvania State University using dye-labelled terminators, followed by electrophoresis on an Applied Biosystems Inc. PRISM 3100 Genetic Analyzer. Sequences were aligned by eye using the Eyeball Sequence Editor (ESEE; Cabot and Beckenbach 1998) and algorithmically using CLUSTAL X (Higgins *et al*. 1996) with default parameters. All sites with missing or ambiguous data were removed before analysis.

Following preliminary sequence analysis of the mitochondrial 16S rRNA gene, we selected one sample from each of the principal lineages of interest and supplemented the partial 16S dataset by sequencing an

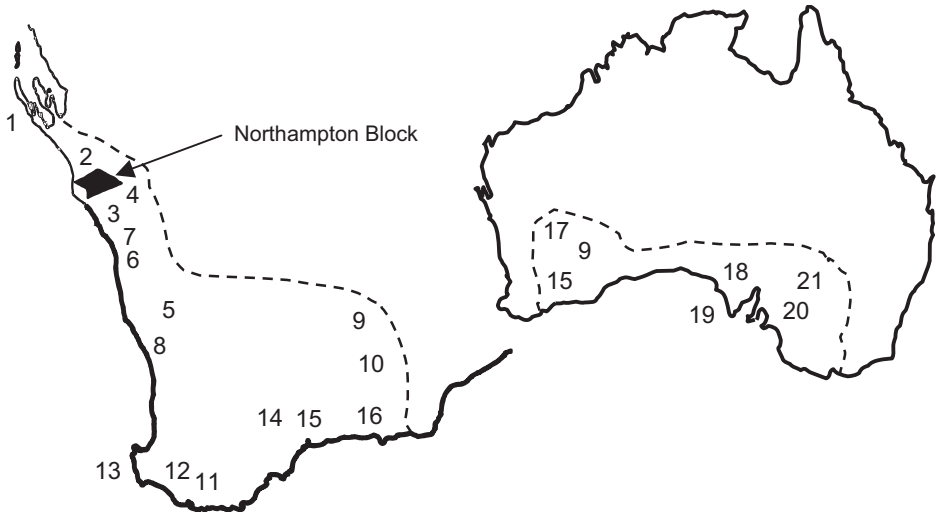

Fig. 1. Localities for *R. australis* and *R. bicolor* samples from which DNA sequence data were obtained: (1) Edel Land; (2) Kalbarri; (3) Geraldton; (4) Yuna; (5) Marchagee; (6) Mt Lesueur; (7) Dongara; (8) Perth; (9) Kalgoorlie; (10) Norseman; (11) Lake Jasper; (12) Black Point; (13) Margaret River; (14) Lake Magenta; (15) Ravensthorpe; (16) Esperance; (17) Mt Jackson, WA; (18) Gawler Ranges, SA; (19) SW Eyre Peninsula, SA; (20) Adelaide region, SA; (21) Dangalli Conservation Park, SA. Dashed lines on maps correspond to ranges for *R. australis* (left) and *R. bicolor* (right). Type locality for *R. bicolor* is Adelaide (20).

overlapping mitochondrial 16S fragment (460 bp) and a 400-bp portion of the mitochondrial 12S rRNA gene. The second 16S fragment (16S-II) was sequenced with the primer pair 16H9/L9 or 16H9/L1 (460 bp) and the 12S fragment was obtained using 12L5/12H4 or 12L5/12H11. The 12S fragment corresponds to positions 570–954 in the mitochondrial genome of the colubrid snake *Dinodon semicarinatus* (+ strand), and the 16S-I and 16S-II fragments correspond to positions 1536–1986 and 1847–2420, respectively.

Therefore, we analysed two datasets. The first one consisted of the 16S-I fragment for 38 *R. australis*, 11 *R. bicolor*, *R. ammodytes*, *R. hamatus*, *R. grypus*, *R. waitii*, *R. longissimus*, *R. bituberculatus*, *R. pilbarensis*, *R. leptosoma* and *R. unguirostris*. A single non-Australian typhlopid, *Typhlops lumbricalis* from Cuba, was included as an outgroup. The second dataset consisted of the combined 12S, 16S-I and 16S-II fragments for 12 lineages of interest: *R. australis*, *R. bicolor*, *R. ammodytes*, *R. hamatus*, *R. grypus*, *R. waitii*, *R. longissimus*, *R. bituberculatus*, *R. pilbarensis*, *R. leptosoma*, *R. unguirostris* and *T. lumbricalis.*

Sequence analysis

To clarify the relationship between *R. australis* and *R. bicolor*, a neighbour-joining (NJ) analysis was performed on the 16S-I dataset for all samples. Because our primary interest in this dataset was to assess the distinctiveness of *R. bicolor* and *R. australis*, and because we address interspecific relationships with a substantially larger combined dataset, we chose to use a relatively simple distance analysis for these data. Simple distance models can perform well when the transition-transversion ratio (R) is not high ($R < 5$), the number of substitutions per site is not large, and the number of nucleotides examined is small, in part owing to the high variance associated with parameter estimation for more complex models of sequence evolution under these conditions (Nei and Kumar 2000; Takahashi and Nei 2000). Owing to the small number of nucleotides (<300 in the full alignment), low transition-transversion ratios ($R = 2.317$ for 16S-I dataset) and low levels of divergence among sequences within *R. australis* and *R. bicolor* subgroups, the Kimura two-parameter model was chosen for analysis (Kimura 1980; Nei and Kumar 2000). The NJ analysis was performed in MEGA 2.1 (Kumar *et al*. 2001), and nodal support was assessed with 2000 bootstrap pseudoreplicates.

Maximum parsimony (MP), maximum likelihood (ML) and Bayesian methods were used to analyse the combined 16S-I, 16S-II and 12S dataset. Modelltest 3.06 (Posada and Crandall 1998) was used to determine

an appropriate model of sequence evolution for ML and Bayesian analyses. Likelihood ratio tests in Modelltest suggested using a general time-reversible model with gamma-distributed rate heterogeneity ($\alpha = 0.506$) and invariant sites (proportion of invariant sites = 0.379).

Maximum parsimony trees and bootstrap support (1000 pseudoreplicates) were evaluated in PAUP* using the branch-and-bound search algorithm. Characters were unordered and assigned equal weights. Maximum likelihood trees were constructed in PAUP* using the heuristic search option with TBR branch-swapping, and nodal support was assessed with nonparametric bootstrapping (1000 pseudoreplicates). Bayesian analyses were performed using the MCMC method in the program MrBayes 3.0 (Huelsenbeck and Ronquist 2001). Four chains were run for 1 000 000 generations each, using default prior probabilities and heating parameters. Likelihood scores stabilised rapidly and stationarity was reached after several thousand generations. Trees were sampled every 100 cycles and the first 1000 trees were discarded as burn-in. Therefore, posterior probabilities were estimated from the remaining 9000 trees. To ensure that the analysis did not become trapped on local optima, the analysis was repeated two times to check for convergence of posterior probabilities.

To estimate genealogical relationships among 38 *R. australis* sequences, a statistical parsimony cladogram was constructed using TCS 1.13 (Clement *et al*. 2000), implementing the method of Templeton *et al*. (1992). This algorithm uses a parsimony criterion to estimate relationships among haplotypes and to infer missing intermediates and probable ancestral haplotypes. This method circumvents several limitations associated with using traditional phylogenetic approaches for population data, including the assumption that ancestral haplotypes are no longer present in the population (Clement *et al*. 2000).

Morphology and taxonomy

One hundred and twenty-nine preserved specimens of '*R. australis'* were examined in this study, comprising both *R. australis* and *R. bicolor* forms; these included 72 from South Australia and 57 from Western Australia. Before molecular analysis, specimens of '*R. australis*' that had tissue available were assigned to putative *R. australis* and *R. bicolor* species groups on the basis of snout morphology or pigmentation. Measurements, meristics and terminology generally follow the conventions of Aplin and Donnellan (1993) and Shea and Horner (1996). Total length was measured with a rule; all other measurements were taken to the nearest hundredth millimetre with digital calipers. Vertebral scale counts began at the prefrontal and terminated at the last dorsal scale overlapping the tail-spine. Midbody diameter was measured along the horizontal axis of the snake; measurements were taken at 25%, 50% and 75% of total length and averaged. The tail-spine in some Australian typhlopids is heavily pigmented, with the spine boundary clearly discernable beneath translucent dorsal/ventral scales, and spine diameter was measured in the vertical plane for all individuals with pigmented spines.

For several localities (Ravensthorpe and Kalgoorlie), mitochondrial sequence and morphological data were available for individuals with the snout morphology of either *R. australis* or *R. bicolor*. Concordance between morphology and phylogeny for sympatric individuals was considered sufficient grounds for species recognition (Avise and Ball 1990).

Results

Relationships of R. bicolor *and* R. australis

After removal of gaps and ambiguous sites from the 16S-I fragment, a total of 275 nucleotides were aligned for 38 *R. australis*, 11 *R. bicolor* and nine additional species of *Ramphotyphlops*. The full alignment included 105 variable sites, and 35 and 9 sites were variable within *R. australis* and *R. bicolor*, respectively. A distance matrix of uncorrected pairwise numbers of differences (Table 1) shows that *R. australis* and *R. bicolor* haplotypes are separated by at least 36 mutational differences, a greater number than that separating many other pairs of *Ramphotyphlops* species. These differences are much greater than the maximum number of substitutions separating haplotypes within *R. australis* (15) or *R. bicolor* (7). The NJ tree (Fig. 2) indicates that *R. australis* and *R. bicolor*, as identified by morphology, each comprise exclusive groups with high bootstrap support. All '*R. australis*' specimens from South Australia are contained within the *R. bicolor* group. In light of the concordant morphological evidence, the sympatry of individuals from *R. australis* and *R. bicolor* groups at Kalgoorlie and Ravensthorpe (Localities 9 and 15 in

Table 1. Pairwise uncorrected sequence differences for species of *Ramphotyphlops* examined in this study, based on 275 bp of mitochondrial 16S rDNA

Because multiple haplotypes were available for *R. australis* and *R. bicolor*, numbers reflect minimum differences. Values in parentheses for *R. australis* and *R. bicolor* represent the maximum number of differences separating any combination of haplotypes within each species

	1	2	3	4	5	6	7	8	9	10
1. *R. australis*	(15)									
2. *R. bicolor*	36	(7)								
3. *R. waitii*	23	37								
4. *R. hamatus*	21	39	28							
5. *R. pilbarensis*	25	39	35	27						
6. *R. leptosoma*	39	46	48	43	44					
7. *R. grypus*	34	47	41	36	40	47				
8. *R. longissimus*	30	44	42	34	37	27	29			
9. *R. bituberculatus*	31	41	40	33	38	36	36	40		
10. *R. ammodytes*	37	41	45	40	43	52	41	44	37	
11. *R. unguirostris*	34	38	43	34	38	44	43	38	36	32

Fig. 1), indicates that '*R. australis*', as currently defined, comprises two distinct species. Remarkably little divergence was observed among *R. bicolor* 16S rRNA sequences, and it is difficult at present to comment on the relationships of populations within this species.

Relationships among species of Ramphotyphlops

A total of 1064 bp of the 12S and 16S genes were aligned for 11 species of *Ramphotyphlops* plus *Typhlops lumbricalis*. Of these, 377 sites were variable and 230 sites were parsimony informative. Results of the ML interspecific analysis (Fig. 3) suggest close relationships between the *R. leptosoma* and *R. grypus* (bootstrap support: 70% MP, 88% ML; Bayesian posterior probability: 1.00), and between *R. hamatus* and *R. pilbarensis* (67/84/0.97). Topologies supporting an *R. leptosoma* + *R. grypus* + *R. longissimus* grouping were recovered (64/78/1.00) and *R. bituberculatus* was the closest relative of this group (76/92/1.00). Close relationships may also occur for *R. waitii*, *R. australis*, *R. pilbarensis* and *R. hamatus* (54/67/0.99). Although the position of *R. bicolor* is inconclusive, our results suggest that this species does not share a particularly close relationship with *R. australis*.

Statistical parsimony analysis of R. australis *haplotypes*

After removal of gaps and ambiguous sites, the alignment of the 16S-I fragment for 38 *R. australis* samples contained 313 sites, including 43 variable and 30 parsimony-informative sites. Two haplotype networks were recovered by TCS from the analysis of these *R. australis* sequences from Western Australia. A minimum of 21 mutational steps separates haplotypes of these clades and they could not be joined under the 95% parsimony criterion due to the high level of genetic divergence between their haplotypes. This indicates that there is a significant chance of multiple hits at sites differentiating the two clades and suggests that the observed number of mutational steps between them (21) may be an underestimate.

These haplotype networks are highly congruent with geography, corresponding to northern and south–central clades (Fig. 4). The northern clade contains populations from the Kalbarri, Shark Bay, and Geraldton coastal regions in the northern portion of the

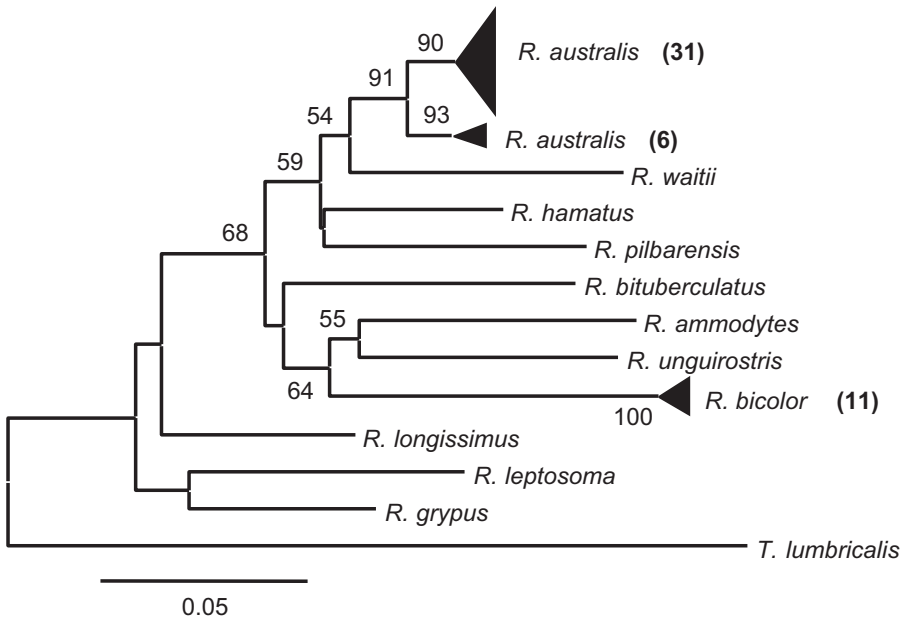

Fig. 2. Neighbour-joining tree for 58 operational taxonomic units (OTUs) of *Ramphotyphlops* based on a 275-bp portion of the mitochondrial 16S rRNA gene. The tree was constructed from a matrix of pairwise Kimura two-parameter distances. Numbers in parentheses after *R. australis* and *R. bicolor* indicate the total number of sequences in each group. The two major groups recovered within *R. australis* are identical to those recovered by the statistical parsimony analysis (see text for details). Relationships among other *R. australis* and *R. bicolor* sequences are not shown, because of low bootstrap support or low sequence divergence (<0.5%).

species' range, and the south–central clade contains individuals from all other populations. These clades overlap near Geraldton in the north, as two individuals from Geraldton (3B, 3C) were placed in the south–central clade and one individual was placed in the northern clade (3A). Furthermore, an individual from Yuna (4A; 75-km north-east and inland from Geraldton) was placed in the south–central clade. Haplotype groupings into northern and south–central clades reflect the two major lineages within *R. australis* recovered by the NJ analysis (Fig. 2), and suggest a high level of differentiation between populations inhabiting these regions.

At least two morphological traits are found predominantly in *R. australis* individuals from the extreme northern portion of the range (Kalbarri, Edel Land and Shark Bay): (1) a 'collared' morphology, with an unpigmented ring of scales around the neck, and (2) reduction in vertebral scale counts relative to *R. australis* from southern and central regions. These characters were mapped on the haplotype network (Fig. 4) to determine whether morphology is congruent with recovered genealogical relationships. Lack of concordance between morphology and mtDNA genealogy was noted for individuals in south–central and northern clades. An individual from Geraldton (3A) was identical in sequence to individuals from the northern clade and did not exhibit a 'collared' morphology; however, this individual did show a substantial reduction in the number of vertebral scales ($n = 290$). Another individual from the northern clade (1B) was unique among all *R. australis* examined in that it possessed the 'collared' type morphology without a concomitant

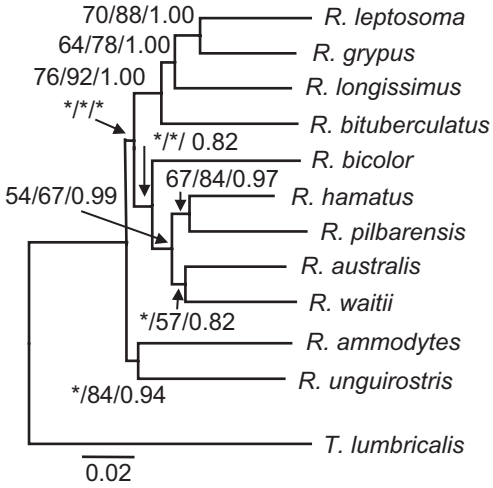

Fig. 3. Maximum-likelihood tree found with heuristic search under a general time-reversible (Γ + I) model showing relationships among Western Australian *Ramphotyphlops*. Tree is based on 1064 bp of combined 12S and 16S mtDNA. Numbers near nodes indicate bootstrap support and posterior probabilities for maximum-parsimony/maximum-likelihood/Bayesian analyses. Asterisks indicate nodes supported in less than 50% of bootstrap replicates. The scale bar is in units of nucleotide substitutions per site.

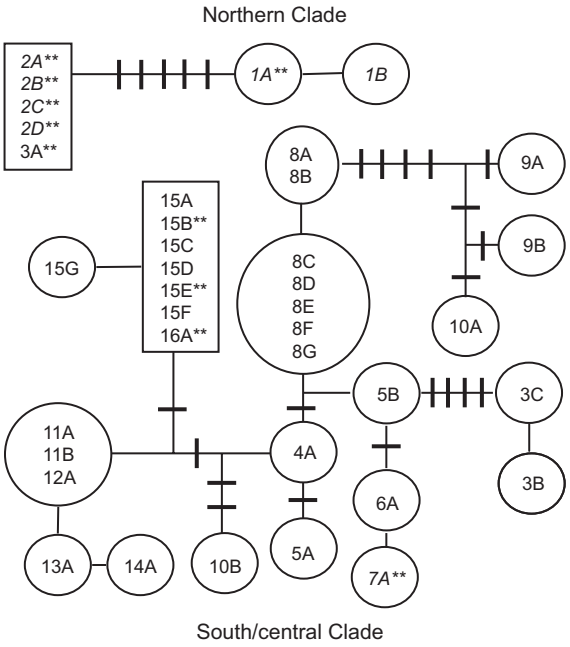

Fig. 4. Unrooted parsimony cladograms for *R. australis* 16S mtDNA sequences. Numbers correspond to sampling localities given in Fig. 1, and individual sequences have a unique number/letter combination (see Appendix). Single base substitutions are denoted by cross-bars and probable ancestral haplotypes are enclosed in boxes. Cladograms for the south–central clade and northern clade could not be connected under the parsimony criterion and are separated by 21 mutational steps. Individuals in italic type show the 'collared' pigmentation pattern, and double asterisks indicate individuals with fewer than 318 vertebral scales.

reduction in the number of vertebral scales (n = 330). Finally, an individual was found in the central coast clade (7A) with a 'collar' and reduced numbers of vertebral scales (n = 312; mean for all *R. australis* examined was 340). This individual was taken from a coastal locality ~75 km south of Geraldton and 175 km south of the locality for the Kalbarri specimens (northern clade). Nonetheless, differences between northern and south–central clades are highly significant for both scale counts ($P < 0.0001$; Wilcoxon two-sample test) and presence/absence of 'collared' pattern ($\chi^2 = 25.8$; df = 1; $P < 0.0001$).

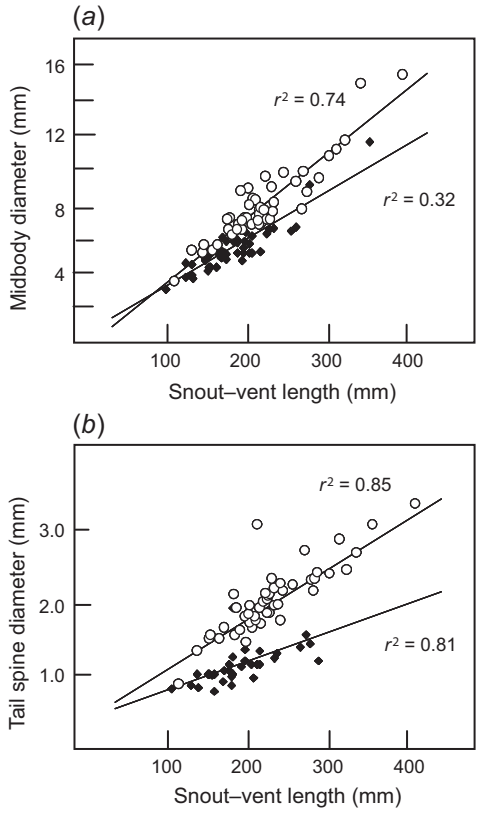

Fig. 5. Regressions of (*a*) tail-spine diameter and (*b*) midbody diameter against snout–vent length for *R. australis* (closed diamonds) and *R. bicolor* (open circles). Slopes of regression lines for both characters are significantly ($P < 0.001$) different.

Morphological differences between R. australis *and* R. bicolor

Slopes of regression lines for midbody diameter and tail-spine diameter against SVL are significantly different between *R. australis* and *R. bicolor* (midbody diameter: $F_{1,90} = 11.57$, $P < 0.001$; tail-spine diameter: $F_{1,78} = 7.74$, $P < 0.001$; Fig. 5).

Taxonomic treatment

Ramphotyphlops australis, Gray (1845) (Fig. 6*a*)

Synonymy: *Anilios australis*, Gray (1845: 135); *Typhlops preissi*, Jan (1864: 15); *Typhlops australis*, Boulenger (1893: 35); *Ramphotyphlops australis*, Storr (1981: 238).

Lectotype: BMNH 1946.1.10.61.

Material examined: See Appendix.

Diagnosis: A moderately large, moderately stout blindsnake with 22 midbody scale rows and 305–401 vertebral scales. Nasal cleft usually joining second supralabial between preocular/postnasal suture and first supralabial; anterior portion of cleft extending less than half the distance between nostril and rostral and not visible from above. *R. australis* is distinguished from *R. bicolor* and *R. hamatus* by a rounded snout lacking a distinct transverse edge. It also lacks the pronounced, heavily pigmented tail-spine of *R. bicolor*; instead, the tail-spine is small and unpigmented, lightly pigmented, or not distinct from dorsal colouration.

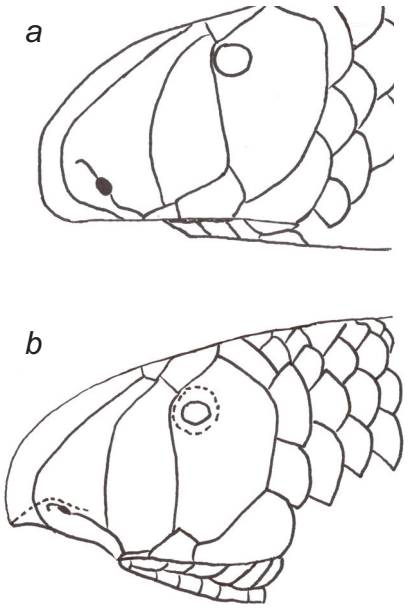

Fig. 6. Head morphology of (*a*) *R. australis* WAM R113304 and (*b*) *R. bicolor* SAMA R56557. Stippled line along anterior margin of snout in *R. bicolor* indicates transverse 'edge'.

Description: Head shape moderately elongate; weakly to moderately trilobed, particularly in large individuals. Mean ratio of rostral width : length equals 0.97 (s.d. = 0.08; $n = 27$). Rostral extends 4/5 of total distance between tip of snout and eye midpoint, with a mean ratio of 0.81 (s.d. = 0.05; $n = 27$). Moderate bilateral indentations at preocular/nasal suture and at rostronasal suture. Rostral appears rounded in frontal, side and dorsal aspect. Rostral might show rounded bulge at anterior portion of ventral surface.

Nostrils inferior. Nasal cleft typically extends posterolaterally to second supralabial, but rarely joins preocular/second supralabial junction, junction of first and second supralabials, or the preocular (frequencies for these variants are given in Storr 1981). Cleft curves upward and terminates no more than halfway between the nostril and rostral; cleft not visible from above. Nostrils situated almost halfway between rostral and preocular, occasionally closer to rostral. Prefrontal variable but larger than frontal. Interparietal subequal to frontal. Width of prefrontal/rostral contact highly variable. Supraoculars broadly separated by frontal/prefrontal and subequal to parietals. Parietals larger than postoculars and subequal to supraoculars. An uncommon variant involves splitting of parietal and fusion of lower portion with upper postocular. Two postoculars.

Four supralabials ranked 4–3–2–1 from largest to smallest. Second supralabial bisected by preocular/nasal suture. Third supralabial forms angular contact with preocular and ocular. Fourth supralabial large, extending past ocular to posterior margin of postocular scale row. Mental smaller than or subequal to postmental. Third infralabial showing the following relationships with supralabial scale row: broad overlap with third supralabial, minimal contact with fourth supralabial (6.8%; $n = 44$); bisected by third/fourth supralabial suture (38.6%); broad overlap with fourth supralabial, minimal contact with third supralabial (54.5%). Microtubercles present on head scales.

Tail-spine present but not pronounced. Spine heavily pigmented, distinct from dorsal pigmentation in 17% of individuals examined ($n = 41$); spine heavily pigmented, not distinct from dorsal pigmentation due to overlap of three or more pigmented vertebral

scales in 12.2% of sample; spine distinct but not heavily pigmented or less pigmented than dorsum in 34.1% of sample; spine not distinct and showing reduced pigmentation in 14.6% of sample; spine unpigmented in 22% of sample. Spine diameter, if pigmented, equal to 0.49% of SVL ($n = 34$).

Midbody scale rows 22. Adult SVL averages 187 mm in males (s.d. = 23.2; $n = 34$) and 250.6 mm (s.d. = 55.6; $n = 14$) in females. Male tails average 4.54% total length (s.d. = 0.78; $n = 34$) and female tails average 2.40% SVL (s.d. = 0.33; $n = 9$). Vertebral scale rows range from 305–374 for males, with a mean of 327.1 (s.d. = 18.9; $n = 31$). Female vertebral counts range from 338–401, with a mean of 351.7 (s.d. = 16; $n = 15$). Midbody diameter equal to 2.76% of SVL ($n = 47$; s.d. = 0.29) for males and females. Number of midbody pigmented scale rows ranges from 0 to 14.

Description of lectotype: Midbody scale rows 22; 320 vertebral scales. Total length 195.8 mm, tail length 6.2 mm. Midbody diameter measured at 25%, 50% and 75% of total length equal to 5.75 mm, 5.83 mm and 5.4 mm, respectively. Distance between eyes is 3.24 mm; distance between nares is 2.4 mm. Thirteen post-anal scales, not including tail-spine. Tail-spine present, but weakly developed.

Snout rounded in all aspects, not trilobed. No indication of edge along anterior margin of rostral. Rostral oval in dorsal aspect, width approximately equal to length. Nasal cleft joining second supralabial and extending above nostril ~1/3 of the distance between nostril and rostral. Nostril and nasal cleft not visible from above. Second supralabial bisected by preocular/nasal suture. Third infralabial overlaps fourth supralabial exclusively. Mental subequal to postmental. Frontal subequal to prefrontal. Prefrontal contact margin with rostral equal to half of total width of prefrontal.

Eyes small and darkly pigmented, equidistant from anterior and posterior margins of ocular scale.

Colouration in alcohol: Dorsum showing wide range of pigmentation, ranging from unpigmented to red-brown to grey to grey-brown. Venter cream or almost white. Boundary between pigmented dorsum and venter may or may not be distinct.

Range: *R. australis* is distributed along the southern portion of the Western Australian coastline, from the Shark Bay region in the north to the southern coast (Fig. 1). The species occurs within several hundred kilometres of the coast in the northern range, but extends far inland to Kalgoorlie in the south. No records have been identified from east of Kalgoorlie/Norseman/Cape Arid (123° East). A single individual from the Pilbara region (WAM R70727) might be a questionable locality record, because extensive collecting in that region has not revealed additional *R. australis*.

Remarks: Considerable geographic variation exists in populations of *R. australis*. Pigmentation is highly variable; specimens from the south-western coast of Western Australia tend to have the heavy dorsal pigmentation characteristic of *R. bicolor*. Several individuals examined from Perth appear almost uniformly coloured. Individuals from the Shark Bay/Kalbarri region are notable for both low vertebral scale counts and distinctive pigmentation. Specimens from these regions often have a distinctive ‘collared’ appearance caused by loss of dorsal pigmentation for 5–10 scale rows immediately posterior to the oculars.

The type specimen of *Typhlops preissi* (Jan 1864) was examined by staff at the Rijksmuseum van Natuurlijke Histoire (RMNH) in Leiden, The Netherlands, for this study. The following attributes suggest that this specimen is correctly synonymised with *R. australis*: (1) snout rounded in all aspects (not angular); (2) tail-spine diameter = 0.59 mm (SVL = 142.6); and (3) specimen nearly colourless, with no indication of loss of pigmentation from dorsal surface.

The type locality of *R. australis* is 'W. Australia' (Gray 1845), and this presumably refers to Perth or adjacent regions (G. Shea, personal communication). Because individuals from these regions are contained in the south–central clade, the northern clade would require a new name should the two be found to be distinct species in the future.

Ramphotyphlops bicolor, Peters (1857) (Fig. 6*b*)

Synonymy: *Onychocephalus (Ophthalmidion) bicolor*, (Peters 1857: 508); *Onychocephalus bicolor*, (Peters 1860: 81); *Typhlops bicolor*, (Boulenger 1893: 35).

Syntypes: (Probable) ZMB 4721–4722 presumed to be from Adelaide, South Australia, although original description lists 'Melbourne, Victoria' (Cogger *et al*. 1983).

Material examined: See Appendix.

Diagnosis: A moderately elongate, stout blindsnake with 22 midbody scale rows, 300–380 vertebral scales, and nasal cleft usually extending from second supralabial and terminating less than halfway between nostril and rostral. It is distinguished from *R. australis* by the presence of a weak to moderate transverse 'edge' along the anterior margin of the snout, extending to the preocular-nasal suture and shielding nostrils from above (snout lacking 'edge' in *R. australis*), whereas the rostral in *R. hamatus* is moderately to strongly keeled with a distinct cutting edge. *R. bicolor* is further separated from *R. australis* and *R. hamatus* by the presence of a large, heavily pigmented tail-spine, distinct from dorsal colouration; *R. australis* and *R. hamatus* have reduced tail-spines with light to moderate pigmentation, often continuous with and inseparable from dorsal pigmentation.

Description: Head shape moderately elongate, not markedly depressed. Rostral with distinct edge, weakly keeled or not keeled; edge particularly visible in frontal aspect. Slight to moderate bilateral indentations at rostral/nasal and nasal/preocular sutures, giving head a weak to moderate trilobed appearance. Rostral slightly longer than wide (ratio of rostral width : length equal to 0.99; $n = 16$), extending 4/5 of distance to eye midpoint (ratio of rostral length to distance between snout and eye midpoint equal to 0.83; $n = 16$).

Nostrils inferior, shielded from above by lateral extension of rostronasal edge. Nostrils widely separate, internasal distance occupying 77% of total head width at nostrils ($n = 16$). Nasal cleft typically joining second supralabial at 1/4 of distance between first and third supralabials. Nasal cleft extends slightly upward from nostrils, curving anteriorly before terminating. Cleft does not extend above rostronasal edge, is not visible from above, and terminates midway or slightly less than half the distance between rostronasal suture and nostril.

Eye moderate, usually not touching preocular/ocular suture. Prefrontal variable in size, but larger than frontal. Prefrontal contact with posterior margin of rostral ranges from a single point to half total prefrontal width. Frontal subequal to interparietal. Supraoculars broadly separated by frontal/prefrontal and subequal to parietals. Parietals larger than postoculars. Two or three postoculars. Upper postocular often long and narrow, as though resulting from fusion of two small cycloid scales. Frequently encountered variants involve splitting of parietal and fusion of lower portion with upper postocular; fusions and splittings may or may not show bilateral symmetry.

First supralabial smallest. Second supralabial larger, bisected by preocular/nasal suture. Third supralabial larger, bounded above by preocular and ocular. Fourth supralabial largest, broadly overlapping ocular. Mental subequal to or much smaller than postmental. Third infralabial shows the following relationships to supralabial scale row ($n = 45$): broad

overlap with third supralabial and minimal overlap with second supralabial (6.7% of specimens examined); broad overlap with third supralabial, minimal overlap with fourth supralabial (55.6%); bisected by third/fourth supralabial suture (33.3%); broad overlap with fourth supralabial and minimal or no overlap with third supralabial (4.4%). Microtubercles present on head scales.

Tail-spine very pronounced and formidable. Spine heavily pigmented, often appearing almost black; spine invariably as dark or darker than dorsum. Vertebral scales overlapping spine typically lack pigmentation, leaving spine distinct from dorsal pigmentation (96% of specimens examined; $n = 47$). Spine diameter equal to 0.79% of total snout–vent length ($n = 44$).

Midbody scale rows 22. Adult snout–vent length (SVL) averages 224.5 mm (s.d. = 40.1; $n = 28$) in males and 269.7 mm (s.d. = 66.6; $n = 19$) in females. Maximum SVL in specimens examined was 418.5 mm. Tail length is significantly longer in males than females ($t = 5.26$; df = 31; $P < 0.0001$), with tails averaging 4.54% total length (s.d. = 0.78) in males and 2.4% (s.d. = 0.32) in females. Males and females are stout; midbody diameter averages 3.29% of SVL (s.d. = 0.28; $n = 25$) in males and 3.45% (s.d. = 0.40; $n = 19$) in females. Vertebral scale counts range from 308 to 349 for males, with a mean of 330.0 (s.d. = 13.8; $n = 22$). Female vertebral scale counts range from 309 to 363, with a mean of 344.6 (s.d. = 12.5; $n = 17$). Midbody pigmented scale rows average 12, but range from 9.5 to 15 ($n = 20$).

Colouration in alcohol: Dorsum is heavily pigmented, dark brown to grey-brown. Venter is cream or almost white in colour. Ventral surfaces of rostral and nasal pigmented, although not so dark as dorsum. Individual scale papillae typically bicolor, with anterior third black and posterior portion rich brown or grey-brown. Boundary between pigmented dorsal scale rows and ventrals distinct.

Range: *R. bicolor* is broadly distributed across the southern third of Australia (Fig. 1). The species ranges from near Mt Jackson, Western Australia, 250 km north-west of Kalgoorlie, in the north-west, to Ravensthorpe, Western Australia, in the south-west, and eastward to the western slope of the Great Dividing Range in Victoria and New South Wales. Waite (1918) listed several records of '*R. australis*' from south–central Northern Territory, but Shea (1999) has recently shown these records to be in error.

Nomenclature: *R. bicolor* has been regarded as a junior synonym of *R. australis* by Waite (1918) and more recent authors (although allocated to other genera). The syntypes of *R. bicolor* (ZMB4721–2) from Adelaide, South Australia, have not been examined, but their collection locations are far from any locality records of *R. australis*, as recognised in the present study, and are presumed by virtue of geography to represent *R. bicolor*.

Discussion

Status of R. bicolor

Combined morphological and molecular analysis of *R. australis* and *R. bicolor* indicates that these forms comprise phylogenetically distinct and reproductively isolated species. Phylogenetic analysis of mitochondrial 12S rRNA and 16S rRNA sequences showed high levels of divergence between these species. Despite moderate support, results of both the NJ and MP/ML/Bayesian analyses strongly suggest that *R. australis* does not have a particularly close relationship with *R. bicolor*. Furthermore, these species can be distinguished by several morphological characters, including snout morphology, midbody diameter and tail-spine diameter. Sympatry at two sites in Western Australia (Ravensthorpe and Kalgoorlie) strongly suggests that these species are reproductively isolated.

The recognition of this cryptic species of *Ramphotyphlops* is consistent with previous molecular phylogenetic analyses of typhlopid snakes, where other cryptic species were uncovered (Hedges and Thomas 1991; Aplin and Donnellan 1993). Progress in typhlopid systematics is hindered by several factors, including minimal interspecific morphological differentiation and lack of ecological, behavioural and life-history data for most species. Many species are known from one or few individuals (e.g. Aplin 1998) and this has contributed to poor knowledge of intraspecific morphological variation.

Systematics of Australian Ramphotyphlops

We examined only 11 of ~40 species of Australian *Ramphotyphlops*, and most of these species are found primarily in Western Australia (with the exception of *R. bicolor* and *R. bituberculatus*). Therefore, we cannot address the higher-level relationships or biogeography of Australian typhlopids. However, if the phylogenetic relationships suggested by this analysis are supported by additional sequence data, the phylogenetic utility of several characters used in *Ramphotyphlops* taxonomy will need to be re-evaluated (McDowell 1974).

For example, the position of the nasal cleft has occupied a central place in the identification of Australian typhlopids (Waite 1918), and the results of this study suggest that this character might not be a general predictor of species relationships. For example, *R. pilbarensis* and *R. ammodytes* are unique among the *Ramphotyphlops* examined in this study in that the nasal cleft originates from the preocular scale, despite no particular close relationship between these species. Snout morphology in *Ramphotyphlops* is highly variable, and the proposed phylogenetic relationships would indicate that rounded or keeled snouts have arisen independently in multiple lineages. It is noteworthy that, despite a moderate number of nucleotides (1064), phylogenetic resolution among all *Ramphotyphlops* species examined is generally poor. Therefore, we caution against strong conclusions concerning higher-level phylogenetics and biogeography until additional species and sequence data are available for analysis.

Historical biogeography of R. australis

The coastal margin and arid interior of western Australia has been noted for its distinctive herpetofauna, anomalous range disjunctions and morphologically distinctive populations suggestive of ongoing or recent speciation processes (Storr and Harold 1980; K. P. Aplin, M. A. Cowan, M. Adams, unpublished). Of the two major clades within *R. australis* (Fig. 4), one appears to contain the northernmost populations of *R. australis* (Shark Bay/Kalbarri regions), although an individual was found nearly 100 km south at Geraldton, with an identical mtDNA sequence. The northern clade occupies a small fraction of the range of *R. australis* and may reflect a past vicariant event.

The molecular phylogenetic results of this analysis raise the possibility that the northern clade of *R. australis* is specifically distinct from the southern clade. However, the lack of complete concordance with morphological variation (collared pigmentation and vertebral scales) instead suggests that gene flow might be occurring between these clades, or alternatively, that convergence underlies the observed patterns. In the absence of additional morphological and genetic data for *R. australis* from the central-coast region of Western Australia, we recommend that the traditional taxonomy be retained.

Distributional records (Cogger 1996) and systematic treatments of reptiles from coastal regions of western Australia (Storr and Harold 1978; Storr and Harold 1980) suggest that speciation in this region might be driven by an unidentified historical factor. These

observations include (1) range disjunctions along coastline between Shark Bay and Geraldton (e.g. *Acylys concinna* and *Pletholax gracilis*), (2) restricted distributions in the vicinity of Shark Bay and the greater Carnarvon Basin (e.g. *Ctenotus youngsoni*, *C. zastictus*; *Lerista connivens*, *L. gascoynensis*, *L. uniduo*; *Menetia amaura*; *Aprasia haroldi*) or restricted distributions further north along the Pilbara coast (e.g. *Diplodactylus rankini*; *Aprasia rostrata*; *Lerista allochira*, *L. onsloviana*) and (3) restricted, morphologically variable populations of widespread nominate species within the Carnarvon basin region (*Aprasia fusca*, *Delma australis*, *Egernia stokesii*, *Lerista elegans*, *L. muelleri*, *L. nicholsii*, *L. lineopunctulata*, *L. praepedita*; *Menetia greyi*, *M. surda*).

At least three major environmental and historical factors can be proposed to explain patterns of differentiation in the herpetofauna of the central western Australian coastlands: (1) sea-level fluctuations, with corresponding expansion/contraction of coastal plain habitat; (2) spatial environmental heterogeneity, including such variables as precipitation, vegetative communities, and substrate type; and (3) climatic variation in western Australia from the Pliocene to present. Discussion of these factors is speculative at this point and evidence supporting their role in promoting diversification in the western Australian herpetofauna remains largely anecdotal.

Substrate composition may have played a role in the isolation and differentiation of the western Australian terrestrial biota. There have been no comprehensive treatments of this topic, but large regions of western Australia consist of mosaic landscapes of sand plains and alluvial soils (Newsome 2000). For example, well-developed sand plains exist in the Kalbarri region, and these extend north to Shark Bay and the greater Carnarvon Basin. However, these northern sand plains are isolated from coastal sand plains near Geraldton by a large zone of alluvial soils lacking sand communities, the Northampton block (Newsome 2000; Fig. 1). This corresponds to putative disjunctions for several pygopodid species (*Acylys concinna* and *Pletholax gracilis*; Cogger 1996) and the northern and south–central populations of *R. australis*.

Mid- to late- Cenozoic sea-level changes might have played an important role in structuring herpetofaunal diversity along Australia's continental margins. Evidence supports a long history of sea-level fluctuations along the western Australian coastal margin (Veeh *et al*. 1979; Kendrick *et al*. 1991; Chappell 1994). Glacial–interglacial cycles appear to have occurred with a periodicity of 0.1 million years from 0.7 million years ago through the present, and interglacial cycling occurred to 2.8 million years ago, although with longer periodicity (Webb and Bartlein 1992). Owing to the buildup of continental and polar ice sheets during glacial maxima, sea levels may have been at least 100 m lower than the present day (Siddall *et al*. 2003). The consequence of these periodic drops in sea level (current sea levels are possibly as high as they have been since the late Pliocene (3 million years ago: Vail *et al*. 1977; Collins and Baxter 1984)) would have been to expove occupied only a small fraction of the past 2 million se a large region of continental shelf, fluctuating in area between 100 000 and 200 000 km^2, along the western coastal margin alone (Chappell 1994). Periods of high sea level (as currently exist) would hayears; therefore, ecological communities might have evolved under conditions very different from those now present.

During interglacial periods, large areas of coastal plain habitat would have been submerged by rising sea levels. As coastal habitats decreased in area, some species might have been blocked from eastward range expansion by unfavourable substrate composition or other antagonistic ecological interactions. There is some evidence that the coastal plain habitats exposed during glacial maxima would have supported dune complexes, whereas regions along the present coast are typically skeletal or clay-rich soils (K. P. Aplin, M. A.

Cowan, M. Adams, unpublished data). Some species with broad coastal distributions might have undergone population fragmentation, as suitable habitat was inundated. The large numbers of western Australian species with restricted or disjunct coastal distributions provide anecdotal support for this model.

Acknowledgments

We thank B. Maryan for preparing tissue samples from Western Australian *Ramphotyphlops*, examining specimens and coordinating loans from the Western Australian Museum. J. Marden, S. Schaeffer and D. Pisani provided useful comments on methods presented herein. A. Wynn assisted DLR during several visits to the United States National Museum of Natural History (NMNH). R. Thomas and V. Wallach provided general comments on typhlopid systematics. M. Hutchinson assisted in identifying the distribution of *R. bicolor* in South Australia, and C. Secombe and M. Hutchinson coordinated loans from the South Australian Museum. T. Bertozzi prepared tissue samples of South Australian *R. bicolor*. M. Hoogmoed examined the holotype of '*T. preissi*' for this study. The lectotype of *R. australis* was examined at the NMNH courtesy of C. McCarthy. G. Shea graciously provided his thorough notes on the type-locality of *R. australis*. We thank the Western Australian Department of Conservation and Land Management and the South Australian Department for the Environment and Heritage for permission to collect specimens and for animal ethics clearances.

References

Aplin, K. P. (1998). Three new blindsnakes (Squamata : Typhlopidae) from northwestern Australia. *Records of the Western Australian Museum* **19**, 1–12.

Aplin, K. P., and Donnellan, S. C. (1993). A new species of blindsnake, genus *Ramphotyphlops*, from northwestern Western Australia, with a redescription of *R. hamatus*, Storr 1981. *Records of the Western Australian Museum* **16**, 243–256.

Avise, J. C., and Ball, R. M. (1990). Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Surveys in Evolutionary Biology* **7**, 45–67.

Boulenger, G. A. (1893). 'Catalogue of the Snakes in the British Museum (Natural History). Vol. 1.' (Trustees of the British Museum: London, UK.)

Cabot, E. L., and Beckenbach, A. T. (1998). Simultaneous editing of nucleic acid and protein sequences with EESE. *Computer Applications in the Biosciences* **5**, 233–234.

Chappell, J. (1994). Upper Quaternary sea levels, coral terraces, oxygen isotopes and deep-sea temperatures. *The Journal of Geography* **103**, 828–840.

Clement, M., Posada, D., and Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**, 1657–1659. doi:10.1046/J.1365-294X.2000.01020.X

Cogger, H. G. (1996).'Reptiles and Amphibians of Australia.' (Reed Books: Sydney.)

Cogger, H. G., Cameron, E. E., and Cogger, H. M. (1983). 'Zoological Catalogue of Australia. Volume 1. Amphibian and Reptilia.' (Australian Government Publishing Service: Canberra.)

Collins, L. B., and Baxter, J. L. (1984). Heavy mineral-bearing strand line deposits, associated with high-energy beach environments, southern Perth Basin, Western Australia. *Australian Journal of Earth Sciences* **31**, 292–298.

Gray, J. E. (1845). 'Catalogue of the Specimens of Lizards in the Collection of the British Museum (Natural History).' (Trustees of the British Museum: London.)

Hedges, S. B. (1994). Molecular evidence for the origin of birds. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 2621–2624.

Hedges, S. B., and Thomas, R. (1991). Cryptic species of snakes (Typhlopidae : Typhlops) from the Puerto Rico Bank detected by protein electrophoresis. *Herpetologica* **47**, 448–459.

Hedges, S. B., Bezy, R. L., and Maxson, L. R. (1991). Phylogenetic relationships and biogeography of xantusiid lizards, inferred from mitochondrial DNA sequences. *Molecular Biology and Evolution* **8**, 767–780.

Higgins, D. G., Thompson, J. D., and Gibson, T. J. (1996). Using CLUSTAL for multiple sequence alignments. *Methods in Enzymology* **266**, 383–402. doi:10.1016/S0076-6879(96)66024-8

Huelsenbeck, J. P., and Ronquist, R. (2001). MR-BAYES: Bayesian inference of phylogeny. *Bioinformatics (Oxford, England)* **17**, 754–755. doi:10.1093/BIOINFORMATICS/17.8.754

Jan, G. (1864). 'Iconographie Generale des Ophidiens. Vol. 1.' (G. Jan: Milan.)

Kendrick, G. W., Wyrwoll, K. H., and Szabo, B. J. (1991). Pliocene–Pleistocene coastal events and history along the western margin of Australia. *Quaternary Science Reviews* **10**, 419–439. doi:10.1016/0277-3791(91)90005-F

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120.

Kumar, S., Tamura, K., Jakobsen, I. B., and Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics (Oxford, England)* **17**, 1244–1245. doi:10.1093/BIOINFORMATICS/17.12.1244

Leviton, A. E., Gibbs, R. H., Jr, Heal, E., and Dawson, C. E. (1985). Standards in herpetology and ichthyology. Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. *Copeia* **1985**, 802–832.

McDowell, S. B. (1974). A catalogue of the snakes of New Guinea and the Solomons, with special reference to those in the Bernice P. Bishop Museum, Part I. Scolecophidia. *Journal of Herpetology* **8**, 1–57.

Nei, M., and Kumar, S. (2000). 'Molecular Evolution and Phylogenetics.' (Oxford University Press: Oxford.)

Newsome, D. (2000). Origin of sandplains in Western Australia: a review of the debate and some recent findings. *Australian Journal of Earth Sciences* **47**, 695–706. doi:10.1046/J.1440-0952.2000.00802.X

Peters, W. (1857). Abhandlung über die Typhlopinen oder blodsichtigen Schlangen und über neue dahin gehörige Arten. *Monatsbericht der Königlich Preussischen Akademie der Wissenschaften zu Berlin* **1857**, 508–509.

Peters, W. (1860). Eine neue zu der Gattung Onycocephalus gehörige Wurmschlange, *Onychocephalus macrurus*, und verglich sie mit den bisher von dieser Gattung bekannt gewordenen Arten. *Monatsbericht der Königlich Preussischen Akademie der Wissenschaften zu Berlin* **1860**, 77–83.

Posada, D., and Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818. doi:10.1093/BIOINFORMATICS/14.9.817

Shea, G. M. (1999). Waite's blind snakes (Squamata : Scolecophidia : Typhlopidae): identification of sources and correction of errors. *Records of the Western Australian Museum* **51**, 43–56.

Shea, G. M., and Horner, P. (1996). A new species of *Ramphotyphlops* (Squamata : Typhlopidae) from the Darwin area, with notes on two similar species from northern Australia. *Beagle* **13**, 53–60.

Siddall, M., Rohling, E. J., Almogi-Labin, A., Hemleben, C., Meischner, D., Schmelzer, I., and Smeed, D. A. (2003). Sea-level fluctuations during the last glacial cycle. *Nature* **423**, 853–858. doi:10.1038/NATURE01690

Storr, G. M. (1981). The genus *Ramphotyphlops* (Serpentes : Typhlopidae) in Western Australia. *Records of the Western Australian Museum* **9**, 235–271.

Storr, G. M., and Harold, G. (1978). Herpetofauna of the Shark Bay region, Western Australia. *Records of the Western Australian Museum* **6**, 449–467.

Storr, G. M., and Harold, G. (1980). Herpetofauna of the Zuytdorp Coast and hinterland, Western Australia. *Records of the Western Australian Museum* **8**, 359–375.

Takahashi, N., and Nei, M. (2000). Efficiencies of fast algorithms of phylogenetic inference under the criteria of maximum parsimony, minimum evolution, and maximum likelihood when a large number of sequences is used. *Molecular Biology and Evolution* **17**, 1251–1258.

Templeton, A. R., Crandall, K. A., and Sing, C. F. (1992). A cladistic-analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA-sequence data. 3. Cladogram estimation. *Genetics* **132**, 619–633.

Vail, P. R., Mitchum, R. M., Jr, and Thompson, S., III (1977). Global cycles of relative changes of sea level. *American Association of Petroleum Geologists, Memoirs* **26**, 83–97.

Veeh, H. H., Schwebel, D., Van Der Graaff, J. E., and Denman, P. D. (1979). Uranium-series ages of coralline terrace deposits in Western Australia. *Journal of the Geological Society of Australia* **26**, 285–292.

Waite, E. R. (1897). Notes on Australian Typhlopidae. *Transactions of the Royal Society of South Australia* **21**, 25–27.

Waite, E. R. (1918). Review of the Australian blindsnakes (Family Typhlopidae). *Records of the South Australian Museum* **1**, 1–34.

Webb, T. I., and Bartlein, P. J. (1992). Global changes during the last 3 million years: climatic controls and biotic response. *Annual Review of Ecology and Systematics* **23**, 141–173. doi:10.1146/ANNUREV.ES.23.110192.001041

Webb, J. K., and Shine, R. (1993). Dietary habits of Australian blindsnakes (Typhlopidae). *Copeia* **1993**, 762–770.

Manuscript received 8 June 2004, accepted 16 September 2004

Appendix. Specimens examined

Museum catalogue numbers, GenBank accession numbers and locality information for specimens examined. Acronyms for museum collections follow standardised use (Leviton *et al*. 1985). SBH, S. Blair Hedges field series. Square brackets give sequence identity, with numbers corresponding to localities from Fig. 1, followed by GenBank accession numbers for 12S (if available) and 16S partial sequences, respectively. Locality abbreviations: SA, South Australia; WA, Western Australia.

Ramphotyplops australis

BMNH 1946.1.10.61 (lectotype, 'W. Australia'); SAMA R00859 (Bunbury); SAMA R926A, R926B (Fremantle); SAMA R26901 (Lake Joondalup); WAM R49111 (Padbury); WAM R49164 (Mt Peron); WAM R70727 (Burrup Peninsula, Pilbara; locality may be in error); WAM R100805, R127402 [16A, AY442866] (Esperance); WAM R114375 [15B, AY442860] (Mount McMahon); WAM R129707 [15C, AY442861], R146903 [15F, AY442876], R146904 [15E, AY442845], R146905 [15D, AY442857], R119300 [15A, AY442859], R119301 (Ravensthorpe); WAM R119345 [14A, AY442858] (Lake Magenta Reserve); WAM R108289–91 (Cape Arid National Park); WAM R135228 [10B, AY442864], R135244 [10A, AY442862] (Norseman); WAM R129073 [11B, AY442868], R129650 [11A, AY442856] (Lake Jasper); WAM R129501 [13A, AY442855] (Margaret River); WAM R113105 [9A, AY442843], R114691, R114692 [9B, AY442875], R114693 (Kalgoorlie); WAM R97265 [8A, AY442839], R115067, R119083, R119449 [8B, AY442833] (Bold Park, Perth); WAM R119062, R119082 [8F, AY442874], R119450 (Swanview, Perth); WAM R119357 [8E, AY442863], R119360, R121160 [8C, AY442851], R121161 [8G, AY442852], R121162, R121188, R121189 (Bungendore Park, Perth); WAM R113304 [8D, AY442844] (Mount Helena, Perth); WAM R127577 (Stoneville); WAM R140860 [5A, AY442849] (Marchagee); WAM R141543 (Hult River); WAM R141887 (Gutha); WAM R144892 [5B, AY442873] (Marchagee); WAM R3774 (Koorda); WAM R151270–1 (Cervantes); WAM R146570 [7A, AY442846] (Dongara); WAM R121286 [12A, AY442872] (Black Point); WAM R121952 [6A, AY442867] (Mt Lesueur); WAM R125985 [4A, AY442865] (East Yuna Nature Reserve); WAM R114673 [3B, AY442870] (Spalding Park, Geraldton); WAM R116282 [3A, AY442869] (Cape Burney, near Geraldton); WAM R120304 [3C, AY442878] (Wicherina Dam, Geraldton); WAM R146401 [2D, AY442853], R146402 [2A, AY442871], R146452 [2C, AY442850], R146453 [2B, AY442847] (Kalbarri); WAM R115848 (Dorre Island, Shark Bay); WAM R135521 [1A, AY442848] (False Entrance Well, Edel Land); WAM R103968 (Bernier Island, Shark Bay); WAM R115859 [1B, AY442854] (Edel Land).

Ramphotyhlops bicolor

WAM R113883 [9A, AY442900, AY442836], R125641 (Kalgoorlie, WA); WAM R144589 [17A, AY442881] (Mt Jackson, WA); SAMA R29465 [15A, AY442883] (Ravensthorpe, WA); SAMA R22906 (Coomalbidgup, WA); SAMA R54590 [20A, AY442888] (Blancheton, SA); SAMA R01367 (Brentwood Yorke Peninsula, SA); SAMA R02769 (Bute, SA); SAMA R50105 [21A, AY442886] (Canopus, SA); SAMA R00480 (Cleve, SA); SAMA R29195 [18A, AY442887] (Coralbignie, SA); SAMA R36502 [19A, AY442880] (Courtabie, SA); SAMA R00820 (Cowell, SA); SAMA R00817 (Denial Bay, SA); SAMA R00809 (Emu Flat, SA); SAMA R03720, R14210, R36738, R56557, R56564, R56608, R56682 (Eyre Peninsula, SA); SAMA R00857A, R00857B (Fowlers Bay, SA); SAMA R28380, R29165 (Gawler Ranges, SA); SAMA R14377, R19043 (Great Victoria Desert, SA); SAMA R39175 [18C, AY442882] (Iron

Chieftain, SA); SAMA R45508 [20B, AY442884], SAMA R45508 [20C, AY442879] (Karte Conservation Park, SA); SAMA R18739 (Keyneton, SA); SAMA R00981, R02299 (Kingoonya, SA); SAMA R02542 (Kirton Point, Port Lincoln SA); SAMA R42472 (Konettra Downs, SA); SAMA R17948 (Lake Gilles Conservation Park, SA); SAMA R14068 (Lake Torrens Basin, SA); SAMA R14505, R45865, R56006 (Middleback Ranges, SA); SAMA R53188 (Mt Cavern, SA); SAMA R02177 (Mt Cooper, SA); SAMA R43160 (Murray River, SA); SAMA R00867A, R00867B (Ooldea, SA); SAMA R46662 (Peebinga Conservation Park, SA); SAMA R31921 [18B, AY442885] (Pinjarra Dam, SA); SAMA R00813, R08950 (Port Lincoln, SA); SAMA R49244 (Pt. Kenny, SA); SAMA R38446 (Tailem Bend, SA); SAMA R11767 (Tickera, SA); SAMA R27471 (Wanilla, SA); SAMA R40981 (Witera, SA); SAMA R41173 (33°17′30″S, 139°33′40″E); SAMA R41567 (33°17′30″S, 140°06′30″E); SAMA R41585, R41588–9 (33°23′60″S, 139°56′10″E); SAMA R41666 (33°43′51″S, 140°35′58″E); SAMA R41667 (33°44′47″S, 140°37′20″E); SAMA R41668 (33°45′20″S, 140°28′19″E).

Ramphotyhlops ammodytes

WAM R108831 (Karratha, WA) AY442890, AY442841.

Ramphotyhlops bituberculatus

WAM R114694 (Kalgoorlie, WA) AY442893, AY442831.

Ramphotyhlops grypus

WAM R116678 (Onslow, WA) AY442898, AY442835.

Ramphotyhlops hamatus

WAM R113176 (Atley Station, WA) AY442894, AY4428342.

Ramphotyhlops leptosoma

WAM R114892 (Geraldton, WA) AY442889, AY442830.

Ramphotyhlops longissimus

WAM R120049 (Barrow Island, WA) AY442901, AY442838.

Ramphotyhlops pilbarensis

WAM R108989 (Karratha, WA) AY442896, AY442834.

Ramphotyhlops unguirostris

WAM R115861 (Lake Argyle, WA) AY442902, AY442837.

Ramphotyhlops waitii

WAM R104279 (Gabalong, WA) AY442892, AY442840.

Typhlops lumbricalis

SHB 172600 (Cuba) AF366700, AF366769.