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Snakes across the Strait: trans-Torresian phylogeographic relationships in three genera of Australasian snakes (Serpentes: Elapidae: *Acanthophis*, *Oxyuranus*, and *Pseudechis*)

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Abstract

We analyze the phylogeny of three genera of Australasian elapid snakes (*Acanthophis*—death adders; *Oxyuranus*—taipans; *Pseudechis*—blacksnakes), using parsimony, maximum likelihood, and Bayesian analysis of sequences of the mitochondrial cytochrome *b* and ND4 genes. In *Acanthophis* and *Pseudechis*, we find evidence of multiple trans-Torresian sister-group relationships. Analyses of the timing of cladogenic events suggest crossings of the Torres Strait on several occasions between the late Miocene and the Pleistocene. These results support a hypothesis of repeated land connections between Australia and New Guinea in the late Cenozoic. Additionally, our results reveal undocumented genetic diversity in *Acanthophis* and *Pseudechis*, supporting the existence of more species than previously believed, and provide a phylogenetic framework for a reinterpretation of the systematics of these genera. In contrast, our *Oxyuranus scutellatus* samples from Queensland and two localities in New Guinea share a single haplotype, suggesting very recent (late Pleistocene) genetic exchange between New Guinean and Australian populations.

Keywords: Phylogeography; Mitochondrial DNA; Australia; New Guinea; Arafura Shelf; *Acanthophis*; *Pseudechis*; *Oxyuranus*

1. Introduction

The biogeographical relationships between the faunas of New Guinea and Australia have been the subject of extensive research, particularly on the mammalian fauna (e.g., Flannery, 1989; Murray, 1992; Aplin et al., 1993; Pacey et al., 2001). Two recent and competing hypotheses of the interrelationships between these landmasses include: (i) continuous separation from the early Miocene to the Pleistocene (Flannery, 1989); and (ii) separation since the late Oligocene/early Miocene, with multiple instances of temporary land connection, supported by some geological evidence and mammalian immunological clock studies (Aplin et al., 1993). Aplin et al. (1993), in particular, attempted to identify whether faunal exchanges between Australia and New Guinea occurred randomly through the late Cenozoic, or whether there were specific bouts of faunal exchange between the two landmasses, and concluded that mammalian faunal exchanges occurred at three specific times since the early Miocene.

Although the tectonic history of Australia and Wallacea is becoming better understood (see recent reviews by Hall, 1998, 2001), the history of land connections

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between these two land masses remains poorly documented, except for those due to Pleistocene sea level changes (Hall, 1998). In such circumstances, biogeographical patterns can provide important additional evidence not only on the biogeographical but also the physical history of such areas. Similar patterns of relationship across multiple, unrelated lineages can provide strong evidence of a common cause, which may corroborate or contradict a geological hypothesis. Studies using molecular evidence can be particularly powerful, since molecular sequence data can provide not only the sequence of cladogenic events, but also a measure of the absolute timing of these events, which can test whether common patterns of phylogeny and distribution are likely to be due to a common cause (e.g., Vences et al., 2001; Nagy et al., 2003). The development of methods that allow times of divergence to be estimated in the presence of rate inequality among clades have greatly expanded the possibilities offered by this approach (e.g., Sanderson, 1997).

Whereas the relationships between Australian and New Guinea mammals have been researched extensively, this is not the case for squamates. Two phylogeographic studies of pythons (Harvey et al., 2000; Rawlings and Donnellan, 2003) have provided evidence for recent (Pleistocene) faunal exchanges, but there are no similar data for trans-Torresian relationships at higher taxonomic levels.

Elapid snakes form a conspicuous component of the herpetofauna of Australasia, representing the majority of all snakes found in Australia, and a substantial percentage of those found on the island of New Guinea. In addition to their contribution to the herpetological biodiversity of the region, they are of special importance as the sole clade of venomous snakes capable of inflicting medically significant bites in the region (Currie et al., 1991; Lalloo et al., 1995; White, 1995).

Recent studies have shown that the Australasian elapid snakes, including the marine elapids, form a monophyletic group, either constituting the sister taxon of the Old World elapids, or placed within a paraphyletic Old World elapid assemblage (Slowinski et al., 1997; Keogh, 1998; Slowinski and Keogh, 2000). However, the detailed phylogenetic relationships among the Australasian elapids remain inadequately resolved, despite considerable research (Keogh et al., 1998; Keogh, 1999).

An aspect of particular interest in the context of the phylogeny of the Australasian elapids is the relationship between the elapid faunas of New Guinea and Australia. Two major, contrasting patterns are recognized. First, many elapid genera widespread in Australia do not have close relatives in New Guinea, and similarly, three genera are endemic to New Guinea and nearby islands. Second, six genera of elapids found in Australia also have representatives in New Guinea. In New Guinea, these genera are restricted to the southern coastal plains of New Guinea, with the exception of *Acanthophis*, which is widespread across New Guinea, and extends west of the Sahul Shelf onto several Moluccan islands, including Tanimbar, Aru, Seram, and Ambon.

In most of these Trans-Torresian genera, the New Guinea representatives are regarded as conspecific with Australian populations (*Demansia*, *Oxyuranus*, *Pseudechis australis*, *Furina*, and *Rhinoplocephalus*; O'Shea, 1996; Shea, 1998; David and Ineich, 1999). Only one New Guinean form (*Pseudechis papuanus*) is widely regarded as an endemic species, and the New Guinea population of the taipan (*Oxyuranus scutellatus*) has been described as an endemic subspecies, *O. s. canni* (Slater, 1956). In the case of *Acanthophis*, most recent workers have regarded the New Guinea populations as conspecific with one or other of the Australian forms, but the species limits within this genus remain unclear and insufficiently understood (e.g., McDowell, 1984; O'Shea, 1996; Aplin and Donnellan, 1999; Cogger, 2000).

In this paper, we use phylogenetic analysis of mitochondrial DNA sequences to infer the phylogenetic relationships of three genera of elapid snakes found on both sides of the Torres Strait, *Acanthophis*, *Oxyuranus*, and *Pseudechis*, with the aim of testing for the presence of common patterns of trans-Torresian sister-group relationships and their timing. *Acanthophis* (death adders) is a genus containing four recognized species from Australia (Aplin and Donnellan, 1999), New Guinea, and the Moluccas, but the systematics of the genus, and particularly the question of species limits, remain unresolved. *Oxyuranus* (taipans) contains two species, *O. scutellatus* being found in northern Australia and the southern coastal plains of New Guinea. The genus *Pseudechis* (blacksnakes) consists of six widely recognized species (Mengden et al., 1986; Cogger, 2000), one of which (*P. papuanus*) is endemic to New Guinea, and a second (*P. australis*) is found in both Australia and New Guinea. In both *Acanthophis* and *Pseudechis*, recent amateur revisions have resulted in the description of multiple new species, and Hoser (1998) described a new genus associated with *Pseudechis*. An additional aim of this paper is to provide a robust phylogenetic framework for a systematic revision of these taxa.

2. Materials and methods

2.1. Sampling and laboratory methods

We obtained blood or tissue samples from specimens of *Acanthophis*, *Oxyuranus*, and *Pseudechis* of known geographical origin maintained in captive collections in Australia and Europe (Fig. 1, Table 1). DNA extraction was performed following standard protocols (Sambrook et al., 1989). For the polymerase chain reaction (PCR; Saiki et al., 1988), we used primers ND4 and

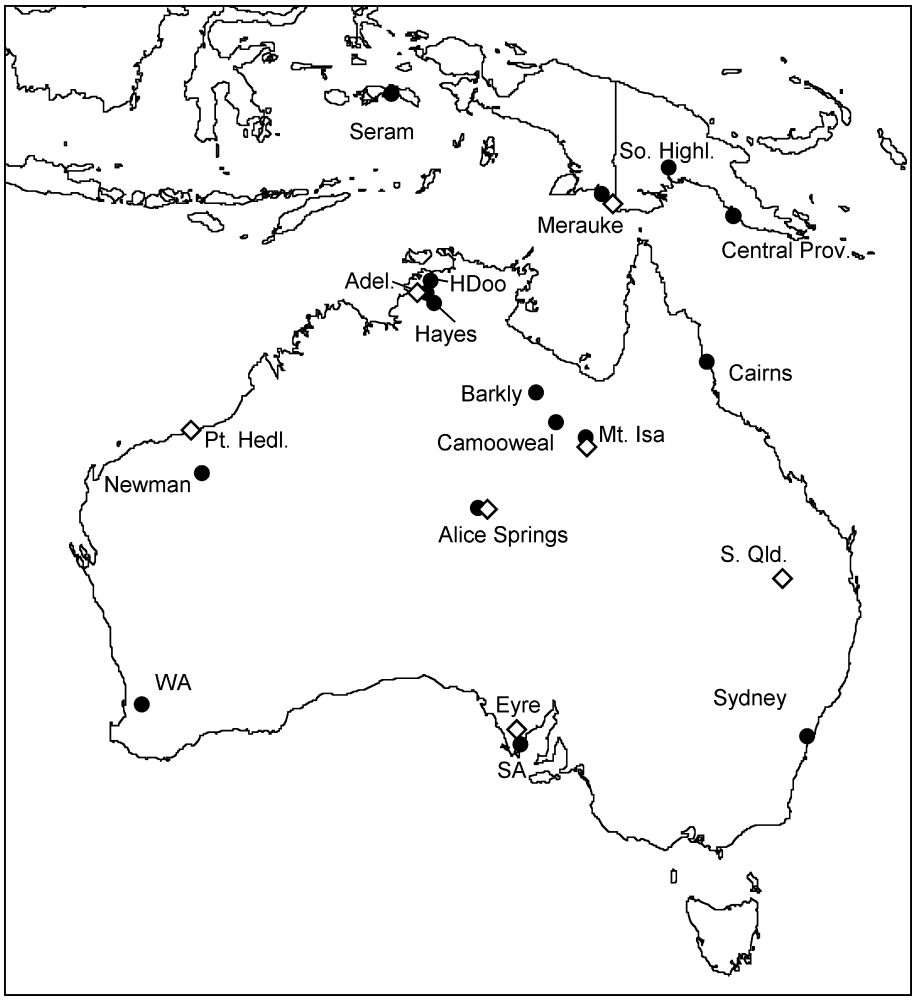

Fig. 1. Sampling localities for *Acanthophis* (circles) and *Pseudechis* (diamonds). *Oxyuranus scutellatus* samples originated from Cairns, Merauke, and Central Province, PNG.

Leu (Arévalo et al., 1994) to amplify a section of the ND4 gene and adjoining tRNAs. In the case of cytochrome *b*, we used the primers mtA (5′-CTC CCA GCC CCA TCC AAC ATC TCA GCA TGA TGA AAC TTC G-3′) and mtF (5′-AGG GTG GAG TCT TCT GTT TTT GGT TTA CAA GAC CAA TG-3′) to amplify a ~ 800 bp section of the cytochrome *b* gene for *Acanthophis* and *Pseudechis*, and primers L 14910 and H 16064 (de Queiroz et al., 2002) to amplify a ~ 1100 bp section of the cytochrome *b* gene in *Oxyuranus*, for which primers mtA and mtF did not work. The same primers were used for sequencing.

Typical conditions for PCR amplification were 50 μl volumes, containing 50 ng template, 0.52 μM primers, 20 mM Tris–HCl, 50 mM KCl, 0.5 mM $MgCl_2$, 0.4 μM dNTP, 2 units *Taq* DNA polymerase, and 0.5% DMSO. Typical amplification conditions involved denaturation for 4 min at 94 °C, followed by 35 cycles 1 min at 94 °C, 1 min at 50 °C, and 2 min at 72 °C, and a final extension step of 3 min at 72 °C. Automated single-stranded sequencing was performed using BigDye Terminator Ready Reaction Mix (ABI), followed by analysis on an ABI 377 DNA Sequencer according to the manufacturer's instructions.

2.2. Phylogenetic analysis

The identification of a suitable outgroup for the analysis of the three genera included in this study is complicated by the fact that the phylogenetic relationships within the Australian elapids remain poorly resolved, or at least poorly supported, even in recent and comprehensive analyses (e.g., Keogh et al., 1998; Scanlon and Lee, 2004), although the monophyly of the Australo-Papuan and marine elapids as a whole is strongly supported by a number of studies (Slowinski et al., 1997; Keogh, 1998; Slowinski and Keogh, 2000). To ensure the monophyly of the ingroup relative to the outgroup, we therefore selected two non-Australasian elapids, the cobra *Naja kaouthia* and the coral snake *Micrurus fulvius*, as outgroups in this study.

Table 1
Taxa and samples used in this study, and GenBank accession numbers

Taxon	Locality	Voucher / Sample no.	Haplotype	GenBank Accession nos.: ND4, cyt*b*
Acanthophis antarcticus	Darling Range, WA	WW 1137	*A. antarcticus* WA	AY340133, AY340162
Acanthophis antarcticus	Sydney area, NSW	WW 1192	*A. antarcticus* Sydney	AY340134, AY340163
Acanthophis praelongus	Cairns, Qld.	WW 1159	*A. praelongus*	AY340135, AY340164
Acanthophis sp.	30 km N. Dajarra, Mt. Isa, Qld.	WW 1120	Isa1	AY340123, AY340152
Acanthophis sp.	Mt. Isa—Cloncurry, Qld.	WW 1121	Isa4	AY340124, AY340153
Acanthophis sp.	30 km N. Dajarra, Mt. Isa, Qld.	WW 1122	Isa2	AY340125, AY340154
Acanthophis sp.	Mt. Isa—Cloncurry, Qld.	WW 1123	Isa3	AY340126, AY340155
Acanthophis sp.	Camooweal, Qld.	WW 1124	Camoo	AY340127, AY340156
Acanthophis sp.	Hayes Creek, NT	NTM R.27146-7	Hay	AY340128, AY340157
Acanthophis sp.	Adelaide River, NT	NTM R. R.27149	Hay	AY340128, AY340157
Acanthophis sp.	Arnhem Highway, NT	WW 1125	Arnhem	AY340131, AY340160
Acanthophis sp.	Anthony Lagoon, Barkly Tableland, NT	NTM R.27150, WW 1127	Barkly	AY340132, AY340161
Acanthophis sp.	Fogg Dam, Humpty Doo, NT	WW 1134	Barkly	AY340132, AY340161
Acanthophis wellsi	Newman, WA	WW 1138	*A. wellsi*	AY340140, AY340169
Acanthophis pyrrhus	Alice Springs, NT	WW 1133	*A. pyrrhus*	AY340139, AY340168
Acanthophis sp.	Central Province, Papua New Guinea	LSTM Aa 4	*A.* sp. PNG1	AY340136, AY340165
Acanthophis sp.	Central Province, New Guinea	WW 1252	*A.* sp. PNG2	AY340137, AY340166
Acanthophis sp.	Seram, Indonesia	R. Mastenbroek, private collection	Seram	AY340138, AY340167
Acanthophis rugosus	Merauke, Irian Jaya, Indonesia	R. Mastenbroek, private collection	Merauke	AY340130, AY340159
Acanthophis rugosus	Unknown	LSTM Ap2 and Ap3	*A. rugosus*	AY340129, AY340158
Pseudechis porphyriacus	Barossa Valley, South Australia	VS Pp 21	*P. porphyriacus*	AY340141, AY340170
Pseudechis guttatus	Qld.	VS Pg 2	*P. guttatus*	AY340143, AY340172
Pseudechis colletti	Qld.	VS Pc9	*P. colletti*	AY340142, AY340171
Pseudechis australis	St. George, S. Qld.	VS Pa 25	*P. australis* S. Qld	AY340145, AY340174
Pseudechis australis	Eyre Peninsula, South Australia	VS Pa 6	*P. australis* Eyre	AY340146, AY340175
Pseudechis australis	Mount Isa, Qld.	WW 1237	*P. australis* Isa1	AY343090, AY343092
Pseudechis australis	Mount Isa, Qld.	WW 1238	*P. australis* Isa2	AY343091, AY343093
Pseudechis papuanus	Bamustu, Aramia River, Western Province, PNG	LSTM Pp1	*P. papuanus*	AY340144, AY340173
Pseudechis australis	Alice Springs, NT	WW 1139	*P. australis* Alice	AY340148, AY340177
Pseudechis australis	Port Hedland, WA	WW 1149	*P. australis* Pt. Hedl.	AY340147, AY340176
Pseudechis cf. australis	Mount Isa, Qld.	(paratype of *P. pailsi*) QM J 80747	*P.* cf. *australis* Isa	AY340150, AY340179
Pseudechis cf. *australis*	Adelaide River, NT	NTM R.27151	*P.* cf. *australis* Adel.	AY342359, AY342360
Pseudechis cf. *australis*	Merauke, Irian Jaya, Indonesia	R. Mastenbroek, private collection; WW 275	*P.* cf. *australis* IJ	AY340149, AY340178
Pseudechis butleri	Leonora, WA	WW 1148	*P. butleri*	AY340151, AY340180
Oxyuranus scutellatus scutellatus	Cairns, Qld.	WW 1199, WW 1132	*Oxyuranus scutellatus*	AY340787, AY340788
Oxyuranus scutellatus canni	Merauke, Irian Jaya, Indonesia	R. Mastenbroek, private collection; WW 274	*Oxyuranus scutellatus*	AY340787, AY340788
Oxyuranus scutellatus canni	Central Province, Papua New Guinea	WW 1256	*Oxyuranus scutellatus*	AY340787, AY340788
Naja kaouthia	Ayeyarwady Division, Burma	CAS 206602	*Naja kaouthia*	AY058982, AF217835
Micrurus fulvius	Florida, USA	CAS 195959	*Micrurus fulvius*	AY058980, AF217839
Porthidium lansbergii arcosae	Salango, Manabí, Ecuador	FHGO 738		AF292613, AF292575
Porthidium nasutum	Zapallo Grande, Esmeraldas, Ecuador	FHGO 517		AF292612, AF292574
Acrochordus granulatus	Asia	No voucher (Slowinski and Keogh, 2000)		U49296, AF217841

"Haplotype" corresponds to the label in Fig. 2. Abbreviations for Australian states: NSW, New South Wales; NT, Northern Territory; and Qld., Queensland. Abbreviations for vouchers/samples: WW, W. Wüster, personal collection; LSTM, Liverpool School of Tropical Medicine, live collection (to be vouchered in Natural History Museum, London, upon death); QM, Queensland; NTM, Northern Territory Museum; SAM, South Australian Museum; VS, Venom supplies, Tanunda, South Australia, live collection (to be vouchered in South Australian Museum, Adelaide, upon death); CAS = California Academy of Science; and FHGO, Fundación Herpetológica Gustavo Orcés, Quito, Ecuador.

Sequences were aligned by eye against the published sequence of *Dinodon semicarinatus* (Kumazawa et al., 1998). Relative rates of evolution of cytochrome *b* and ND4 in these taxa were calculated by plotting the pairwise *p*-distance matrix of ND4 against that for cytochrome *b*, and calculating the slope of the regression line. To test for saturation of certain categories of substitution, we calculated maximum likelihood (ML) distances between all samples, using the ML model and parameters as estimated below. We then plotted pairwise *p*-distances (transitions only) for each codon position against the equivalent ML distance (for all codons). A decline in the rate of accumulation of individual categories of substitution with increased ML distances indicates saturation of that substitution category.

We checked all sequences for unexpected insertions, deletions, frameshifts, or the presence of stop codons. Any of these would have indicated that the sequences represented nuclear insertions of the mitochondrial genes (Zhang and Hewitt, 1996). The presence of a significant phylogenetic signal was tested by means of the g1 tree skewness statistic (Hillis and Huelsenbeck, 1992), calculated from 100,000 trees randomly generated by PAUP* 4.0b10 (Swofford, 2002). To determine whether any saturation effects differentially affected the phylogenetic signal in the three codon positions, we also generated 100,000 random trees for first, second, and third codon positions independently.

The phylogenetic analysis of DNA sequences has received a considerable amount of attention in recent years. Maximum parsimony (MP) and maximum likelihood (ML) approaches have long dominated this field, but in recent years, Bayesian approaches using Markov chain-Monte Carlo (MCMC) methods (Yang and Rannala, 1997) have become increasingly prominent, as they allow the assessment of posterior probability distributions for nodes of ML trees. Using different analytical approaches on the same data allows the evaluation of the dependence of different nodes on the assumptions and properties of the relevant methods (e.g., MP is especially prone to the phenomenon of "long-branch-attraction" Felsenstein, 1978). In this study, we used MP, ML, and Bayesian methods to infer the phylogeny of the genera *Acanthophis* and *Pseudechis*. MP and ML analyses were performed using PAUP*4.0b10, and Bayesian analysis using MrBayes, version 3.0 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003).

For MP analysis, we used heuristic searching and tree bisection–reconnection (TBR) branch swapping, with 1000 random addition sequence replicates to test for the presence of tree islands. Support for the internal nodes of the tree was assessed by means of branch support (Bremer, 1994) and non-parametric bootstrapping (Felsenstein, 1985), using 1000 pseudoreplicates, heuristic searching with TBR branch swapping, and five random addition sequence replicates per bootstrap replicate. We did not use differential weighting of any particular category of base pair substitution for reasons described in Section 3.

For ML analyses, we used a successive approximation approach (Funk et al., 1995; Voelker and Edwards, 1998) to identify the optimal model of sequence evolution and its parameter values. We estimated the appropriate model of sequence evolution using Modeltest 3.0 (Posada and Crandall, 1998). We used this model and the parameter values estimated by Modeltest 3.0 in an initial heuristic ML search, using an NJ starting tree, and TBR branch swapping. We re-estimated the parameters from the resulting tree, and ran a further heuristic search using the new parameter settings. The search was then repeated with 50 random addition sequence replicates and TBR branch swapping, to test for the presence of tree islands. ML bootstrap involved 100 replicates, heuristic searching using NJ starting trees, and NNI branch swapping.

For Bayesian analysis, we used the model of sequence evolution estimated by Modeltest, while allowing the analysis to estimate the relevant parameter values. Burn-in, the time taken for the parameters to reach stationarity, was estimated by plotting tree log-likelihood score against generation number, and visually determining the number of generations after which the values reached an asymptote. Searches were run using four chains, over five million generations, sampling every 250th tree. Trees generated prior to completion of burn-in were discarded.

In any hypothesis-testing scenario, the most important test is whether the data supporting one hypothesis have the power to reject alternative hypotheses with statistical significance. Here, we built constraint trees designed to be compatible with the alternative phylogenetic hypothesis, using PAUP*4.0, and the same settings as in the previous searches for the optimal trees. For MP analyses, we used the Wilcoxon signed-ranks test (Templeton, 1983); for ML, we compared the constrained and unconstrained trees by means of the Shimodaira–Hasegawa test (Shimodaira and Hasegawa, 1999), run in PAUP* using the full option and 1000 bootstrap replicates. These tests ask whether differences in tree optimality are due to chance alone, or whether the optimal tree is significantly better than the alternative.

2.3. Timing of divergence events

One of the aims of this study was to estimate the timing of instances of faunal exchange between Australia and New Guinea. In order to account for rate heterogeneity among lineages, divergence times together with confidence limits were calculated using clock-based and penalized likelihood (PL) methods, implemented in the program r8s (Sanderson, 2003). To provide a

calibration point in these analyses, we re-estimated the ML tree while including sequences of the South American populations of the pitviper genus *Porthidium*, which diverged 3.5 Mybp, following the emergence of the Isthmus of Panamá (Wüster et al., 2002), and used sequences of *Acrochordus granulatus* to root the resulting tree.

In the clock-based method, divergence times were calculated for the optimal ML tree using the Langley–Fitch method (Langley and Fitch, 1974) with the truncated Newton (TN) algorithm, and calibrating the tree by fixing the *Porthidium* node to 3.5 My.

The non-clock based method involved "profiling" a set of 100 ML trees in r8s, using the PL method (Sanderson, 2002) with the Powell (Gill et al., 1981; Press et al., 1992) algorithm, and fixing as calibration points the *Porthidium* node at 3.5 My and the divergence between elapids and viperids at 95 My, based on the earliest colubroid fossils from the Cenomanian (Rage et al., 2003). The profiled ML trees had been generated from pseudoreplicate data sets generated from the topology of the original ML tree in SeqGen (Rambaut and Grassly, 1997), under the optimal evolutionary model in PAUP whilst using the original unscaled ML tree as a topological constraint (in order to resample branch lengths, but not topology).

To test for any generality of biogeographical patterns across other squamate groups, we also assessed divergence (*p*-distances) between trans-Torresian sister clades in the two other groups of snakes for which data are available. We aligned 715 bp of cytochrome *b* sequence of the *Morelia amethistina* group (Harvey et al., 2000), and 291 bp of cytochrome *b* sequence of *Morelia viridis* (Rawlings and Donnellan, 2003), and calculated the divergence between the Australian haplotypes and their New Guinean sister clades using PHYLTEST.

3. Results

3.1. Sequence data

We aligned a total of 1320 bp for the three genera, 648 for ND4 and 671 for cytochrome *b*. Sequences were deposited with GenBank (accession numbers in Table 1). Of these, 607 were variable, and 491 informative under the parsimony criterion.

The 100,000 random trees displayed a length distribution skewness statistic of $g1 = -0.447705$. The corresponding figures for first, second and third codon positions are −0.382976, −0.336462, and −0.468380, respectively, demonstrating that the entire dataset and all codon positions individually contain significant phylogenetic signal ($P < 0.01$; Hillis and Huelsenbeck, 1992).

As previously documented in South American pitvipers (Wüster et al., 2002), the two genes showed similar levels of sequence divergence: when pairwise *p*-distances for ND4 are plotted against the corresponding cytb distances, the regression slope is 1.07. The plots of pairwise *p*-distances (transitions only) against ML distances revealed considerable saturation of transitions at higher levels of overall sequence divergence. Only third codon position transitions were entirely saturated at higher divergence levels, but only at ML distances corresponding to intergeneric comparisons. This, together with the significantly skewed distribution of lengths of random trees generated from third codon positions, suggests that these contain considerable phylogenetic information at intrageneric level, which is where the nodes of biogeographical interest in this study are located.

3.2. Phylogenetic analyses and timing of divergence events

The unweighted MP analysis resulted in 25 equally most parsimonious trees of 1824 steps (CI excluding uninformative characters = 0.4272, retention index = 0.7885). To test whether the MP analysis was affected by the apparent saturation of transitions, we repeated the analysis under exclusion of third codon position transitions and under exclusion of all transitions. The resulting trees were largely congruent with the tree resulting from the unweighted analysis. The only topological differences were loss of resolution among very similar sequences in *Acanthophis* and among the *P. australis* haplotypes, and that *Oxyuranus* was recovered as the sister group of a clade consisting of *Acanthophis* and *Pseudechis*, instead of as the sister group of *Pseudechis*, as found in the unweighted analysis. None of the nodes of biogeographical interest discussed below were affected.

The Modeltest software identified the GTR+I+G model, a variant of the general time-reversible model (Yang et al., 1994), as optimal for our data. The parameter values estimated from a tree obtained using the Modeltest parameters are given in Table 2. The ML

Table 2
Parameter values for the GTR + I + G model estimated for maximum likelihood analysis

Base frequencies	A	0.350279
	C	0.321630
	G	0.083139
	T	0.244952
Rates	AC	1.59710
	AG	28.57954
	AT	1.37449
	CG	1.04954
	CT	19.24092
	GT	1.00000
Proportion of invariable sites		0.454325
Gamma shape parameter		1.059031

analysis resulted in a single tree each, with a −ln(L) score of 9536.71047 (Fig. 2).

The MP, ML, and Bayesian trees for *Acanthophis* and *Pseudechis* were largely congruent in topology, with few exceptions. The numbering of clades is as in Fig. 2.

A number of haplotype clades are strongly supported within *Acanthophis* (Figs. 2 and 3):

(i) Clade A1 consists of populations of *Acanthophis* in northwestern Queensland (Mt. Isa area, Camooweal), the Northern Territory (Humpty Doo, Hayes Creek, Adelaide River, Barkly Tableland), and Irian Jaya (Merauke). Additional phylogenetic structure is evident within this clade: clade A1b contains the haplotypes from Humpty Doo and the Barkly Tableland, whereas clade A1a contains the remaining populations.

(ii) Haplotype clade A2 contains specimens of *Acanthophis antarcticus* and *Acanthophis praelongus* from northern Queensland, New South Wales and Western Australia and forms the sister clade to clade A1.

(iii) Clade A3 contains the well-differentiated species *Acanthophis wellsi* and *Acanthophis pyrrhus*, which form the sister group to clades A1 and A2. However, this latter relationship is poorly supported.

(iv) A strongly supported clade A4 contains specimens from Papua New Guinea, and Seram, Indonesia, forming the sister clade to the remainder of *Acanthophis*, although that latter relationship is not robustly supported.

In the *Pseudechis* tree, several clades of interest are strongly supported by high bootstrap and branch support values:

(i) Clade P1a contains *P. australis* sensu stricto haplotypes, which form a highly supported clade with little variation or phylogeographic structure within it.

(ii) Clade P1b contains haplotypes belonging to four specimens associated with *P. australis*, corresponding to Hoser's (1998, 2000) "*Pailsus pailsei*" from Mt. Isa and "*Pailsus rossignolii* " from Irian Jaya,

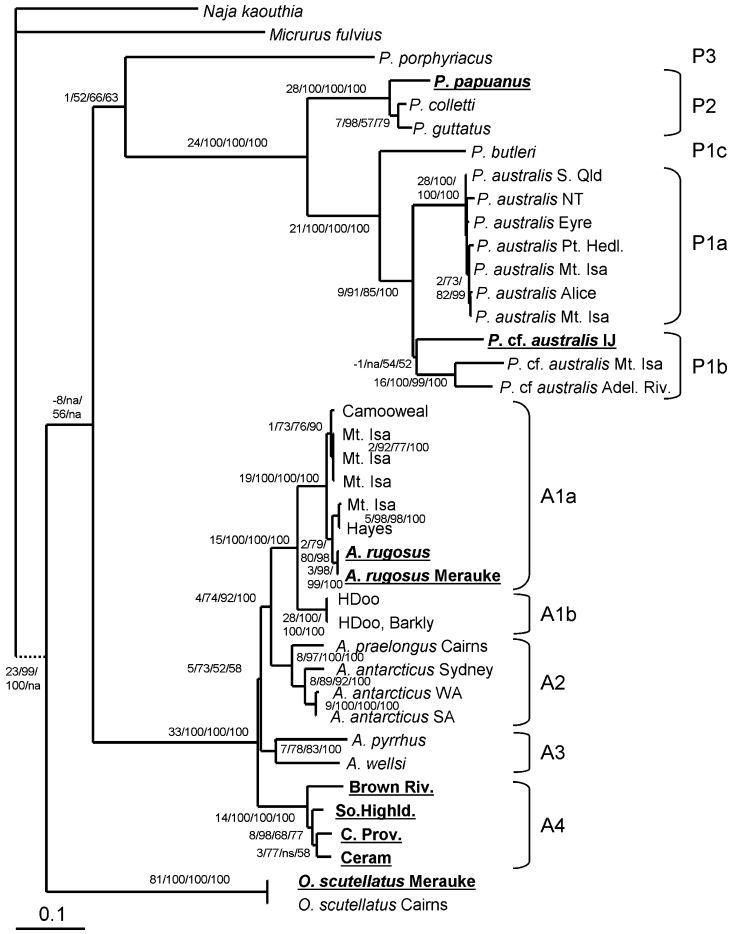

Fig. 2. ML phylogram of the Australian elapids included in this study. Bold type and underlining indicates taxa from New Guinea, all others are from Australia. Locality abbreviations correspond to those in Fig. 1. Numbers on nodes refer to Branch support (parsimony—unweighted analysis)/% MP bootstrap support (unweighted analysis)/% ML bootstrap support/% posterior probability from Bayesian analysis. Negative branch support indicates the number of extra steps required by a particular clade not included in the MP tree. ns = <50%, na = clade not found in MP or Bayesian tree.

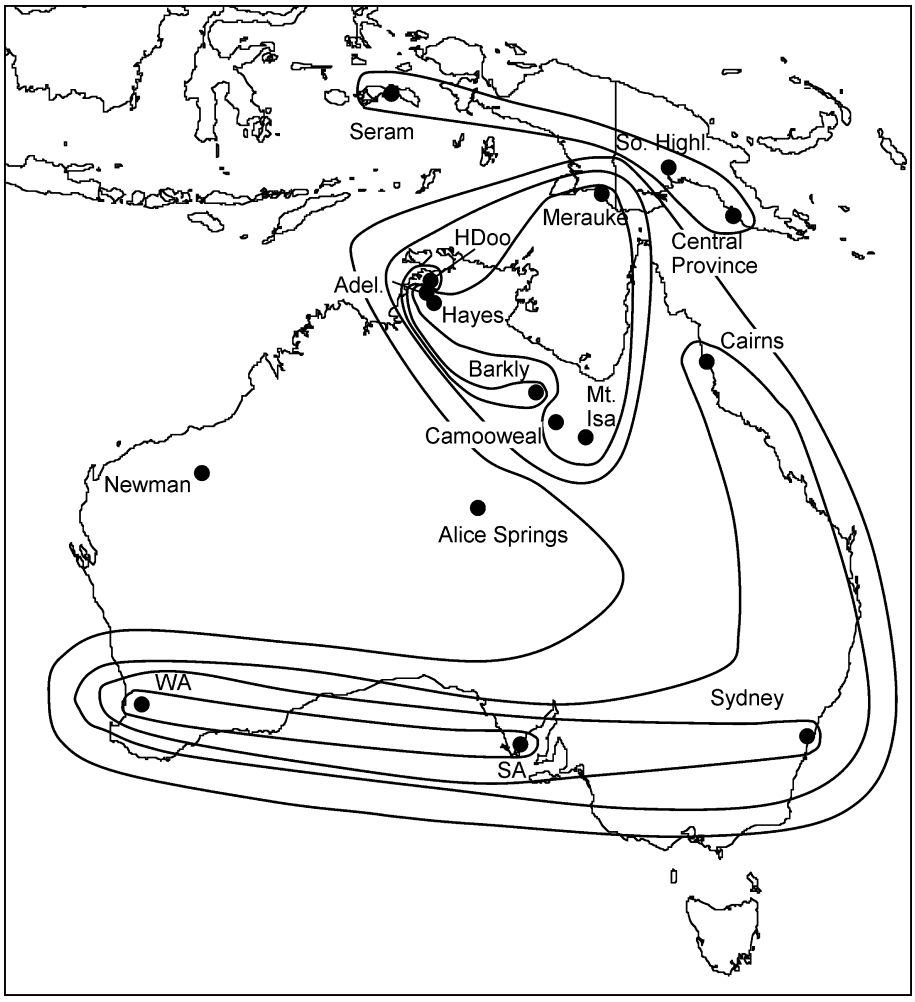

Fig. 3. Distribution of clades of *Acanthophis*. Concentric lines indicate nested sets of clades. Detailed relationships within clade A1a are not shown, and the relationships between the most basal clades are not shown either.

as well as an additional specimen from Adelaide River, Northern Territory. These are clearly distinct and separated by substantial distances from each other and from the *P. australis* sensu stricto haplotypes (clade P1a). The monophyly of clade P1b is weakly supported by our ML and Bayesian analysis, and weakly opposed by MP analysis, but the monophyly of all *P. australis* and *P.* cf. *australis* haplotypes is strongly supported.

(iii) *Pseudechis butleri* (P1c) forms the sister group to clades P1a and P1b, and this robustly supported clade constitutes the *P. australis* group.

(iv) *Pseudechis papuanus*, *Pseudechis guttatus*, and *Pseudechis colletti* form the strongly supported *P. papuanus* group (clade P2), the latter two being monophyletic within it.

(v) The *P. australis* and *P. papuanus* groups form a strongly supported monophyletic group to the exclusion of *P. porphyriacus* (P3). The position of the latter as the sister taxon of all other *Pseudechis* is only weakly supported.

In both *Acanthophis* and *Pseudechis*, the monophyly of either all the New Guinean or all the Australian forms of each genus was rejected with statistical significance by both Wilcoxon signed-ranks and Shimodaira–Hasegawa tests (Table 3). The divergence time estimates for New Guinean and Australian sister groups are indicated in Table 4. The estimates derived from PL and the Langley–Fitch method were broadly similar, but the confidence limits were much wider in the case of the former method.

The sequences of all samples of *Oxyuranus scutellatus* from Australia and New Guinea were identical in both genes

4. Discussion

4.1. Phylogenetic relationships

Both in *Acanthophis* and in *Pseudechis*, our analyses revealed clear and strongly supported phylogenetic rela-

Table 3
Results of Wilcoxon signed-ranks and Shimodaira-Hasegawa tests comparing optimal trees and trees constrained to be consistent with alternative biogeographical hypotheses

	Wilcoxon signed-ranks test			Shimodaira-Hasegawa test	
	d (steps)	$-z$	*p*	*d* ($-\ln(L)$)	*p*
Acanthophis					
Monophyly of New Guinea populations	44	4.8007–5.5880	<0.0001	53.54091	<0.001
Monophyly of Australian populations	33	4.5329–4.8135	<0.0001	57.17280	<0.001
Pseudechis					
Monophyly of New Guinea populations	85	7.7273–7.9263	<0.0001	162.72618	<0.001
Monophyly of Australian populations	110	8.7451–8.8603	<0.0001	233.98181	<0.001

Significant results ($P < 0.05$) indicate rejection of the stated alternative hypothesis.

Table 4
Estimated age of lineage splits between New Guinean and Australian sister clades, calculated with the Langley-Fitch method and the penalised likelihood method

	Estimated age of divergence (My)	95% confidence limits
Langley–Fitch method with truncated Newton algorithm		
A. rugosus vs. Northern Territory sister clade	0.60	0.37–0.93
A. laevis group vs. other *Acanthophis*	7.82	6.34–9.79
P. papuanus vs. *P. guttatus* + *P. colletti*	2.95	2.00–3.94
P. cf. *australis* (Irian Jaya) vs. *P.* cf. *australis* (Adelaide River) + *P.* cf. *australis* (Mt. Isa)	5.89	4.77–7.48
Penalized likelihood method		
A. rugosus vs. Northern Territory sister clade	0.43	0.17–4.04
A. laevis group vs. other *Acanthophis*	5.60	3.65–54.17
P. papuanus vs. *P. guttatus* + *P. colletti*	1.95	1.07–21.59
P. cf. *australis* (Irian Jaya) vs. *P.* cf. *australis* (Adelaide River) + *P.* cf. *australis* (Mt. Isa)	3.57	1.07–17.42

tionships demonstrating multiple trans-Torresian sister-group relationships in both genera (Figs. 2 and 3). Our phylogeny of *Pseudechis* is broadly congruent with that of Mengden et al. (1986), except that here, *Pseudechis colletti* is the sister species of *P. guttatus*, not of *P. papuanus*. The incomplete dataset for *P. papuanus* used by Mengden et al. (1986) may partly explain this inconsistency. In the case of *Acanthophis*, there have been no previous comprehensive phylogenetic studies. Our results revealed a robustly supported phylogeographic pattern that is largely incompatible with currently accepted taxonomic arrangements. In contrast, our sequences of *O. scutellatus* from Queensland and two localities at opposite ends of the New Guinean range of the species revealed a total lack of variation, which is remarkable in view of the considerable geographical (800–1000 km) and physiographical separation of the localities, and the fact that the two populations differ sufficiently in morphology for the New Guinea taipan to have been described and widely recognized as a separate subspecies, *O. s. canni* (Slater, 1956).

4.2. Biogeography

Our phylogenetic trees strongly suggest an Australian origin for both *Acanthophis* and *Pseudechis*, with two separate dispersal events each from Australia to New Guinea. The hypothesis that the distribution of both genera on either side of the Torres Strait may be due to a single vicariance event caused by rising sea levels is robustly rejected by highly significant tree topology tests.

In *Acanthophis*, an Australian origin is most parsimonious as all previous studies have placed *Acanthophis* within the viviparous elapid clade endemic to Australia (Keogh et al., 1998; Keogh, 1999), and its most likely sister genus, *Echiopsis* (Greer, 1997; Scanlon and Lee, 2004), occurs in the southern half of Australia. Two *Acanthophis* lineages occupy New Guinea, but differ radically in their estimated time of divergence from their Australian sister clade (Table 4). The widespread New Guinea-Seram clade A4 diverged from its Australian sister clade in the late Miocene. On the other hand, *Acanthophis rugosus* appears to have diverged from its Australian sister clade in the late Pleistocene. The most parsimonious scenario to account for the phylogeographic pattern observed in *Acanthophis* would appear to be an Australian origin, followed by early range expansion into New Guinea, then followed by vicariance due to the separation of the two landmasses. This was then followed in the late Pleistocene by a second colonization of southern New Guinea by some populations

belonging to clade A1, which would presumably have coincided with times of low eustatic sea levels in the Pleistocene.

In *Pseudechis*, an Australian origin is also most parsimonious, as both New Guinea lineages are placed deep within the tree among Australian taxa. An Australian origin would require two independent dispersals to New Guinea, whereas a New Guinea origin would require five impendent dispersals to Australia, or three dispersals to Australia and a secondary dispersal back to New Guinea by *P.* cf. *australis*. The *P.* cf. *australis* population from Irian Jaya appears to have diverged from its Australian sister clade in the late Miocene, whereas *P. papuanus* appears to have diverged from the ancestor of *P. colletti* and *P. guttatus* in the Pliocene (Table 4).

In the case of *Oxyuranus*, the presence of shared haplotypes between New Guinea and Australia suggests that, at the very least, there was genetic exchange between New Guinea and Australia in the late Pleistocene, most probably during the most recent episode of lowered eustatic sea levels. The direction of any dispersal can be ascertained only by further sampling, although the lack of variation between two specimens from opposite parts of the New Guinean range leads to the suspicion that an Australian origin is more likely.

An interpretation of the phylogeography of these snake genera in the general context of biogeographical relations between Australia and New Guinea remains problematic due to our hitherto incomplete understanding of the geology of the area, especially of the history of land connections between the continents (Hall, 1998).

Flannery (1989) proposed that, after continuous connections from the late Paleocene to the Oligocene, New Guinea and Australia were separated by a marine passage from the early Miocene onwards, until eustatic sea-level fluctuations during the Pleistocene created renewed opportunities for the exchange of terrestrial faunal elements. However, more recent reconstructions of Cenozoic land connections (e.g., Hall, 1998) suggest that most of present-day New Guinea remained submerged for much of the late Tertiary, that only small parts of northern New Guinea have been above sea level continuously since the early Miocene, approximately 20 Mybp, and that these remained separate from Australia throughout the remainder of the Tertiary until the late Pliocene/Pleistocene.

Hall (1998, 2001) also emphasized the difficulties involved in estimating land boundaries from the geological record. While noting the tentative nature of any such reconstructions, Hall (1998, 2001) showed much of the Arafura Shelf as exposed at least episodically between the early Miocene and in the late Miocene and Pliocene, at 20, 10, and 5 Mybp, but not 15 Mybp. However, in his reconstructions, these emergences simply constituted extensions of the Australian landmass that did not extend north to the continuously emerged parts of New Guinea, and thus did not constitute landbridges between Australia and any New Guinean land mass that remained emergent after renewed flooding of the Arafura Shelf.

Both our results and previous molecular studies of Australian mammals are difficult to reconcile with a history of continuous separation between Australia and any continuously emergent New Guinean landmass since the early Miocene. In particular, an analysis of immunological distances in mammals (Aplin et al., 1993) suggested four periods of faunal exchange: approximately 20 Mybp, 10–12 Mybp, 2.7–4.7 Mybp, and in the Pleistocene. Aplin et al. (1993) noted the relatively close correspondence of these periods to apparent perturbations of the sedimentary process in a series of sedimentary sequences described by Davies (1990), which Aplin et al. (1993) tentatively interpreted as being potential indicators of aerial exposure. Similarly, the DNA–DNA hybridization study of Kirsch and Springer (1993) also revealed mammalian trans-Torresian divergences dated at approximately 8–12.5 Mybp, and Krajewski et al. (1993) found trans-Torresian sister-group relationships dating back to 5–10 Mybp. Fossil evidence from some marsupial groups, such as the zygomaturines (Murray, 1992), also suggests Pliocene faunal exchanges.

Our own results are also inconsistent with a history of continuous separation between Australia and New Guinea from the early Miocene to the Pleistocene, as suggested by Flannery (1989): the events leading to the presence of *Pseudechis* and *Acanthophis* on both sides of the Torres Strait (with the exception of the distribution of the *A. rugosus* group) almost certainly occurred between the middle Miocene and the Pliocene. Moreover, the presence of two *Pseudechis* lineages endemic to southern New Guinea, but dating back to the late Miocene/early Pliocene, is difficult to reconcile with Hall's (1998, 2001) scenario that no southern part of New Guinea has been continuously emergent since before the late Pliocene/Pleistocene. Instead, our results suggest either the existence of a separate, southern New Guinea landmass since at least the late Miocene, or that there were episodic land connections between Australia and the continuously emergent parts of New Guinea since the late Miocene.

Comparable data are lacking for other groups of squamate reptiles. The pythons *M. viridis* (Rawlings and Donnellan, 2003) and the *M. amethistina* complex (Harvey et al., 2000) both display phylogeographic patterns consistent with radiation in New Guinea and neighboring islands, followed by a recent single invasion of northeastern Australia. In both, sequence divergences between Australian and New Guinean sister clades are low (around 1.5% *p*-distance in both), and consistent with dispersal from New Guinea to Australia during the Pleistocene. Phylogeographic studies of other species complexes will be required to ascertain whether a

pattern of both older and more recent Australia–New Guinea dispersal events, contrasted with only recent New Guinea–Australia dispersals, is a general pattern among squamates and/or other vertebrates.

4.3. Systematics

Our data have uncovered hitherto unsuspected patterns of genetic diversity in all three genera, which suggest that radical taxonomic revision will be required, particularly for *Acanthophis* and the *P. australis* group. Although the aims of this study were primarily biogeographical rather than systematic, our results do provide, for the first time, a phylogenetic framework for the systematics of these genera, on which further revisions can be built.

4.3.1. Acanthophis

The systematics of the genus *Acanthophis* have long been a subject of debate, supported only by limited data. For much of the latter part of the 20th century, only three taxa were recognized: *A. antarcticus* (eastern and southern Australia), *A. praelongus* (northern Queensland, northern Northern Territory, and Kimberley region of Western Australia, and *A. pyrrhus* (deserts of central and western Australia). Although the affinities of the New Guinea forms were acknowledged to be problematical (e.g., McDowell, 1984; O'Shea, 1996; Cogger, 2000), most authors shoehorned them into either *A. antarcticus* or *A. praelongus*. In Australia, a number of divergent populations from the northern parts of the country have been flagged as distinct (e.g., Mirtschin and Davis, 1992), but no thorough study of the entire complex has been undertaken. Aplin and Donnellan (1999) demonstrated the Pilbara death adder to be a valid species, *A. wellsi*.

Our data reveal strongly supported, hitherto unsuspected patterns of genetic diversity within the death adders (Figs. 2 and 3). These patterns differ radically from all previous interpretations of species limits within this medically important genus. This study thus provides a first phylogenetic framework for a thorough and much-needed revision of the systematics of the genus, although a phylogeographic study based on mitochondrial DNA may not be able to resolve species limits without additional evidence, especially where the suspected species are parapatric or sympatric (Puorto et al., 2001). Nevertheless, our results allow several conclusions on the systematics of the genus *Acanthophis* :

(i) The populations of *Acanthophis* from New Guinea cannot be regarded as conspecific with either *A. antarcticus* or *A. praelongus*. Two phylogenetically distinct sets of populations are present in New Guinea. The older (clade A4, Fig. 2) differs from its sister clade by an average *p*-distance of 9.0%, and deserves recognition as separate species. This set of populations consists of relatively smooth-scaled snakes with conspicuous supraocular horns. The oldest available name for any population of this complex is *Acanthophis laevis* Macleay, 1878. We suggest referring to this clade as the *A. laevis* complex pending a fuller review of the systematics of the New Guinea death adders. The other New Guinea lineage (part of clade A1a) consists of rough-scaled death adders from the Merauke region of Irian Jaya, which are closely related to northern Australian populations. It must be emphasized that there is considerable variation in scalation and pattern in death adders throughout New Guinea (e.g., McDowell, 1984; O'Shea, 1996), and the presence of additional lineages cannot be excluded at this point, especially as we did not sample any specimens north of the central mountain range, a known genetic barrier in other New Guinea organisms (e.g., Rawlings and Donnellan, 2003).

(ii) The populations of clade A1 (Fig. 2), from the Northern Territory, northwestern Queensland, and southeastern Irian Jaya, are neither *A. praelongus* nor *A. antarcticus*. The subclades A1a and A1b differ from each other by 5.3%, and overlap in geographical distribution. This level of differentiation is often associated with interspecific rather than intraspecific comparisons (Johns and Avise, 1998; Harris, 2002), including in many studies of snakes (Zamudio and Greene, 1997; Slowinski and Wüster, 2000; Wüster and Broadley, 2003), and the pattern seen is thus consistent with the sympatric occurrence of two separate species. However, we refrain from attempting to diagnose species limits on the basis of mtDNA phylogeography alone (Puorto et al., 2001). More detailed and rigorous studies combining an analysis of morphology (particularly multivariate morphometrics—e.g., Wüster and Broadley, 2003; Wüster et al., 2001b) and nuclear markers are required to resolve the population systematics of these snakes. The Merauke death adders were described as *Acanthophis antarcticus rugosus* by Loveridge (1948). We suggest referring to the rough-scaled Irian Jaya death adder and the Northern Territory and northwestern Queensland death adders of clade A1 as the *A. rugosus* complex, pending further revisions. The oldest available name for clade A1b, should it eventually be found to characterize a separate species from clade A1a, is *Acanthophis hawkei* Wells and Wellington, 1985.

(iii) It is important to emphasize that *A. praelongus* is restricted to northeastern Queensland, and is most closely related to *A. antarcticus*. None of the other populations of northern Australia have any affinities with the northern Queensland populations.

Irrespective of arguments over species status, the identification of genetically distinct lineages is of importance not only for academic purposes, but also for conservation management (Moritz, 1995). This is particularly pertinent in the case of *Acanthophis*, as some taxa in the genus are already deemed to be of conservation concern (Reed and Shine, 2002), and many are vulnerable to the spread of the introduced cane toad, *Bufo marinus* (Phillips et al., 2003).

4.3.2. Oxyuranus

Although the lack of mtDNA differentiation between New Guinea and Australian taipans makes the synonymization of the New Guinea subspecies *O. s. canni* with *O. s. scutellatus* very tempting, we refrain from this course of action due to the obvious and consistent differences in color pattern between the two forms, as well as our very limited sampling regime. The name *O. s. barringeri*, proposed for the populations from the Kimberley area of Western Australia by Hoser (2002), is a *nomen nudum*, as the description does not provide a diagnosis compliant with Article 13.1 of the International Code of Zoological Nomenclature.

4.3.3. Pseudechis

The systematics of the genus *Pseudechis* has been generally less controversial than that of *Acanthophis*. However, Hoser (1998, 2000) described a new genus, *Pailsus*, into which he placed the forms referred in this paper to *P.* cf. *australis* from Mt. Isa and Irian Jaya. He described these as two new species, *Pailsus pailsei* and *Pailsus rossignolii*, respectively, in both cases with inadequate levels of character evidence and sampling (Wüster et al., 2001a). Both populations had previously been regarded as *P. australis*. We have found considerable sequence divergence (*p*-distances of 8.8–9.5%) between these two forms, and between them and *P. australis* sensu stricto, which suggests that they represent two valid species, especially when this divergence is compared to the low levels of genetic variability within *P. australis* sensu stricto across its vast Australian range (pairwise *p*-distances under 1% throughout). An additional haplotype, from Adelaide River, Northern Territory, forms a clade with the haplotype of *P.* cf. *australis* from Mt. Isa, but differs from this by a *p*-distance of 6.2%. The Adelaide River specimen may represent the taxon *Cannia weigeli*, described by Wells and Wellington (1987), or an additional hitherto unsuspected taxon within the *Pseudechis australis* group. Further studies will be required to resolve the status of these forms. At the generic level, the monophyly of Hoser's genus *Pailsus* is not significantly supported by our data, and moreover, all three forms are clearly placed within the genus *Pseudechis*, and closest to *P. australis*. We therefore consider *Pailsus* a synonym of *Pseudechis*.

As a general systematic comment, the present study illustrates how an uncritical acceptance of untested conventional taxonomic arrangements can be misleading in biogeographical studies. Whereas the New Guinean and Australian populations of *P.* cf. *australis* were not, until now, recognized as taxonomically differentiated, they are in fact one of the oldest trans-Torresian sister-group relationships. On the other hand, *P. papuanus*, always regarded as an unambiguously distinct species, differentiated from its sister clade much more recently, and *O. s. canni*, long recognized as a separate subspecies, diverged only in the latest Pleistocene. This result should serve as a warning against assumptions of times or sequences of divergence based solely on the supposed conspecificity of populations.

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