



## Origin of the eastern brownsnake, *Pseudonaja textilis* (Duméril, Bibron and Duméril) (Serpentes: Elapidae: Hydrophiinae) in New Guinea: evidence of multiple dispersals from Australia, and comments on the status of *Pseudonaja textilis pughii* Hoser 2003

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### Abstract

*Pseudonaja textilis* is a widespread and common snake in eastern parts of Australia, but its distribution in New Guinea is poorly understood, and the origin of the New Guinea populations and its timing have been the subject of much speculation. Phylogenetic analysis of mitochondrial DNA sequences from three New Guinea populations of *P. textilis* indicates that New Guinea was colonised from two independent eastern and western migration routes most likely in the Pleistocene. One dispersal event from northern Queensland led to the populations in eastern New Guinea (Milne Bay, Oro and Central Provinces, Papua New Guinea), whereas another, from Arnhem Land to central southern New Guinea, led to the populations from the Merauke area, Indonesian Papua. The results are consistent with the effects of Pleistocene sea level changes on the physical geography of Australasia, and are thus suggestive of a natural rather than anthropogenic origin of the New Guinea populations. The taxonomic status of the New Guinean populations is discussed.

**Key words:** *Pseudonaja textilis*, New Guinea, Australia, phylogeography, Pleistocene, sea level changes, Lake Carpentaria, taxonomy, mitochondrial DNA

### Introduction

In recent years, the advent of molecular markers, and particularly the use of mitochondrial DNA-based phylogeographical approaches, has revolutionised our understanding of the biogeographical history of animal species (Avice, 2000). One region that has received increasing attention in this regard is the Australasian region, including the faunal relationships between Australia and New Guinea. While earlier studies focussed primarily on mammals (e.g., Aplin *et al.*, 1993; Pacey *et al.*, 2001), reptiles, including snakes, have been studied extensively in recent years (Harvey *et al.* 2000; Rawlings & Donnellan, 2003; Rawlings *et al.*, 2004; Kuch *et al.*, 2005; Wüster *et al.*, 2005). The results have revealed a variety of biogeographical patterns, including older exchanges resulting in endemic New Guinean species that are clearly distinct from their Australian sister taxa (e.g., *Acanthophis laevis*, *Pseudechis papuanus* and *P. rossignolii*—Kuch *et al.*, 2005; Wüster *et al.*, 2005), to

lower levels of divergence, where Australian and New Guinean sister groups are nested within a wider intraspecific phylogeny (e.g., *Acanthophis rugosus*—Wüster *et al.*, 2005; *Morelia viridis*—Rawlings & Donnellan, 2003; *Morelia amethystina* complex—Harvey *et al.*, 2000), and to zero or minimal differentiation (e.g., *Oxyuranus scutellatus*—Wüster *et al.*, 2005; Doughty *et al.*, 2007)

Among the elapid snakes found on both sides of the Torres Strait, the origins of the New Guinea populations of the genus *Pseudonaja* have remained the most contentious. The genus *Pseudonaja* is widespread in Australia, where it is represented by seven species (Cogger, 2000), although several analyses have revealed the likely presence of cryptic species within what is currently referred to as *Pseudonaja nuchalis* Günther (Mengden, 1985; Skinner *et al.*, 2005). A single species, *P. textilis*, inhabits both Australia (where it is widespread and common in eastern parts, but more patchy and sporadic in the centre) and parts of the island of New Guinea.

The earliest records of *P. textilis* in PNG were a female from Menapi, Cape Vogel, and a male from Baiawa, Moi Biri Bay (part of Collingwood Bay), northern Milne Bay District, collected during the 4<sup>th</sup> Archbold Expedition to New Guinea in 1953 (AMNH 73949 & 73959 respectively) (McDowell, 1967). McDowell further remarked that the two specimens resemble Queensland and North Australian *Pseudonaja* in all respects except one, the presence of 12, as opposed to 9–11, solid maxillary teeth.

Eric Worrell (1961) received a specimen at the Australian Reptile Park, Gosford, shipped with *Pseudechis papuanus* from locations in ‘Papua’, which then referred to the southern part of present-day Papua New Guinea (Campbell, 1969). However, in 1961 and 1963 he recorded the species’ New Guinea distribution erroneously as “Highlands” (Worrell, 1961). No records or specimens remain.

Slater (1968) included *P. textilis* in the second edition of his small handbook, referring to specimens being collected around Dogura, Milne Bay District. His source was probably the Dogura specimen which arrived in Port Moresby in 1965 and which was subsequently identified as *P. textilis* by Cogger at the Australian Museum (Campbell, 1969). It is likely that Slater was unaware of the two Archbold specimens. Campbell (1969:26) mentioned the Dogura specimen and also reported a further two specimens from Dogura (AM R25774–5), and the head of a specimen from Embogo Hospital (AM R25880), the first record from Oro (then Northern District), collected around the same time and identified as *P. textilis* by Cogger. The National Museum and Art Gallery of Papua New Guinea also contains a specimen from Dogura (as Dogua) (NMPNG 22295—old acc. no. 10419).

The records from the northern coast of eastern PNG, in Milne Bay and Oro Provinces, remained the only confirmed records until the 1990s. This apparently atypical distribution for a savanna-dwelling elapid with Australian affinities (the remainder of which are restricted to the savannas fringing the southern side of New Guinea—*Oxyuranus scutellatus*, *Pseudechis papuanus*, *P. rossignolii*, *Demansia vestigiata*), and the lack of records of the species in apparently suitable habitat in Papua New Guinea’s Central and Western Provinces, led Slater (1968) to suggest that the species’ presence in New Guinea may have been the result of an accidental introduction either in military equipment imported during or after World War II, or in post-war agricultural supplies, a hypothesis commented on by subsequent workers (e.g., O’Shea, 1990, 1996; Laloo, 1994; Kuch & Yuwono, 2002; Williams & Wüster, 2005).

The presence of *Pseudonaja* in the Central Province savannas has long been suspected. Early clues to its presence came from five positive ELISA results from snakebite victims (Laloo, 1994; O’Shea, 1996) from a number of scattered localities in Central Province and the National Capital District (NCD). The first specimen from the southern savannas, NMPNG 24583 (old acc. no. 18937) from 6-Mile (NCD) was discovered by the authors in 1996, in the NMPNG collection, mislabelled as *Oxyuranus scutellatus*. The head of a large specimen from Hisiu (Hiri Sub-Province, Central Province) is also represented (NMPNG 25305). Four additional cases of envenoming by *P. textilis* were detected using immunological assays among patients admitted to Port Moresby General Hospital from Central Province during 2006 (D.J. Williams, *in prep.*). In combination, these scattered specimens, immunoassay results and snakebite records suggest that *P. textilis* is broadly distributed throughout the southern savannas of Central Province.

*Pseudonaja textilis* remains a rarely collected snake in PNG, but the same cannot be said of the population in West Papua. Currie (2000) believed that *Pseudonaja* sp. could inhabit the region but was unaware that J.S. Keogh had placed two specimens from Merauke in the Australian Museum in 1995 (AM R147652, R147659). Kuch and Yuwono (2002) reported that since 1993 numerous specimens have been captured in the vicinity of Merauke. *Pseudonaja textilis* could be expected to inhabit the neighbouring savannas of Western Province, PNG, but to date no positive record or specimen exists. Based on the recent records suggesting a widespread distribution of the species in New Guinea and the abundance of *P. textilis* in the vicinity of Merauke, Kuch and Yuwono (2002) concluded that the hypothesis of an origin through anthropogenic introduction was an unlikely explanation for the presence of *P. textilis* on the island.

The taxonomic status of the New Guinea populations of *Pseudonaja* has remained unclear. McDowell (1967) assigned his material to *Pseudonaja textilis*, but other authors (e.g., O'Shea, 1996; Kuch & Yuwono, 2002) considered the status of the form as unresolved, referring to the New Guinea populations as "*Pseudonaja* cf. *textilis*". As in the case of many other Australasian snakes, the nomenclature of the genus *Pseudonaja* has been confused by largely evidence-free contributions from a small minority of amateur herpetologists (Williams *et al.*, 2006b; see Skinner *et al.*, 2005, for a discussion of the genus-level classification of *Pseudonaja*). Using the Archbold specimens described by McDowell (1967) as type material, Hoser (2003) described the New Guinea populations of *P. textilis* as a separate subspecies, *P. textilis pughii*, emphasising that this would include all New Guinea populations of *Pseudonaja*, including those from Merauke, West Papua. His diagnosis was based primarily on the dentitional differences between Milne Bay and northern Queensland specimens reported by McDowell (1967), as well as anecdotal evidence of slight differences in colour.

The recent study of the phylogeny and phylogeography of *Pseudonaja* by Skinner *et al.* (2005) included a large number of geographically widespread samples of *P. textilis* from Australia, and Keogh's two specimens from Merauke (Indonesian West Papua), and revealed considerable phylogeographic structuring within *P. textilis*. The specimens from Merauke were found to nest among a set of haplotypes from northern South Australia, western Queensland and the Northern Territory. However, representatives of the apparently allopatric populations from eastern parts of New Guinea were not included in that study.

Understanding the origin and taxonomic status of the New Guinea populations of *P. textilis* is especially important from the point of view of snakebite management in New Guinea (Williams & Wüster, 2005). In Australia, *Pseudonaja* spp. are the elapid snakes that have adapted best to anthropogenic habitat changes, and as a result, they have become the most common cause of snakebites and snakebite fatalities (Sutherland, 1992; White, 1998) in Australia. At present, bites by *P. textilis* are an apparently uncommon cause of snakebite morbidity and mortality in New Guinea (Lalloo *et al.*, 1994; D.J. Williams, in prep.). Understanding the origin and taxonomic status of the New Guinea populations of *P. textilis*, and monitoring their abundance and distribution, is essential to predict likely future trends in the medical importance of this species, and thus antivenom needs for New Guinea, especially as human impacts on vegetation and habitats may create conditions favouring this species over other native elapids. In particular, it is essential to determine whether the late discovery of the species and the currently accumulating records of its presence reflect rarity in a native species restricted to a relict distribution that has been neglected by collectors, or whether this is a reflection of a recent introduction and relatively rapid range expansion. The latter case predicts a rapid future increase in the medical importance of this species in New Guinea, whereas in the former case, studies on the effectiveness of existing antivenoms against *Pseudonaja* for the New Guinea populations may be required (Fry *et al.*, 2003).

During 2006, three *Pseudonaja* were collected by CCA from Wamawamana and environs on the north coast of Milne Bay Province, and one of us (MOS) observed six additional specimens and captured three of them in an oil-palm plantation at Heropa, between Doboduru and Embogo on the Buna road, Oro Province, Papua New Guinea. These recent new specimens, and the combination of the mitochondrial DNA sequences obtained from these specimens with the published data of Skinner *et al.* (2005), provide an opportunity to

investigate the origin and status of these populations, with the aim of resolving several questions, in particular:

- Do all New Guinea *P. textilis* descend from a single colonization event?
- Is the phylogenetic nesting of the New Guinea haplotypes consistent with natural colonisation from Australia, or does it indicate human intervention, or possibly a combination of both?
- Is there evidence that the New Guinea populations of *P. textilis* represent one or more distinct taxa from those in Australia?

## Material and methods

Blood or tissue samples were obtained from five PNG specimens (AVRU-UPNG live collection PT001–PT003 and LSUMZ 90636–37), as well as from an additional specimen from Merauke, Papua, Indonesia (gift from Ulrich Kuch). Total DNA was extracted by standard methods (Sambrook *et al.*, 1989).

Laboratory work was conducted in two independent labs. A ~ 900 base pair fragment of the mitochondrial NADH dehydrogenase subunit 4 (ND4) gene and the adjoining tRNA-His and tRNA-Leu genes was amplified using either the primers ND4 (Arévalo *et al.*, 1994) and HIS12763v (5'–TTC TAT CAC TTG GAT TTG CAC CA–3', Sylvain Ursenbacher, pers. comm.) or the primers used by Skinner *et al.* (2005) (ND4 and M246). The two PCR protocols were used in each lab: (1) 20 µl reactions were carried out using 18 µl of 2x ReddyMix™ PCR Mastermix (Abgene™, catalogue no. AB-0575-DC-LD), consisting of 1.25 units of Thermoprime Plus DNA polymerase, 75mM Tris-HCL (pH 8.8 @ 25°C), 20mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.5 mM of MgCl<sub>2</sub>, and 0.01% (v/v) Tween® 20, 0.2 mM of each dNTP and precipitant red dye for electrophoresis. (2) 25 µl reactions were carried out using 0.2 mmol each dNTP, 5 pmol each primer, 0.5 units of Taq polymerase (New England BioLabs Inc., Ipswich, MA), 2.5 µL of 10X Buffer and 18.9 µL nuclease-free, ultrapure H<sub>2</sub>O in 25 µL.

The amplification protocol comprised denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturing for 45 seconds at 92°C, followed by 1 minute of annealing at 50–55°C, one and a half minutes extension at 72° C. The reaction ended with one longer extension phase at 72° C for 5 minutes. Sequencing was carried out either by Macrogen (Seoul, S. Korea—<http://dna.macrogen.com>), using the ND4 primer or PCR amplicons were purified with ExoSAP-IT® (USB Corp., Cleveland, OH), cycle sequenced for both ND4 and M246 primers using BigDye® v3.1 and run on an ABI-3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Newly generated sequences were aligned with those of Skinner *et al.* (2005), GenBank accession numbers DQ098598–DQ098645. To test for the presence of nuclear pseudogenes (Zhang & Hewitt, 1996), we translated the DNA sequences into amino acid sequences in MEGA 2.1 (Kumar *et al.*, 2001) to check for premature stop or nonsense codons or frameshifts.

Before phylogenetic analysis, only single copies of each haplotype were retained. We noted no differences between the GenBank sequences of haplotypes 78 and 85, and haplotypes 96, 99 and 101 from Skinner *et al.* (2005), and therefore did not include them separately. We tested for the presence of a significant phylogenetic signal by examining the skewness of the distribution of the lengths of 10<sup>6</sup> random trees (Hillis & Huelsenbeck, 1992). For phylogenetic analysis, we used maximum parsimony (MP) and Bayesian inference (BI) methods. MP analysis was carried out using the software PAUP\* 4.0b10 (Swofford, 2002). For MP analysis, we performed an unweighted heuristic search, using TBR swapping and 10,000 random addition sequence replicates. Internal support for different nodes was estimated using non-parametric bootstrap searching (Felsenstein, 1985), using 1000 bootstrap replicates and heuristic searches with 10 random addition sequence replicates each. We also estimated Bremer support (Bremer, 1994) by repeating the heuristic searches while retaining successively longer trees, and determining how many steps were necessary to break up each clade.

Trees were rooted using outgroup sequences from *Pseudonaja affinis* Günther (DQ098468), *P. inframacula* (Waite) (DQ098467) and *P. ingrami* (Boulenger) (DQ098488).

For phylogenetic analysis using BI, we used MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). There is increasing evidence that complex models of sequence evolution can extract additional phylogenetic signal from data, especially where saturation of base pair substitutions is commonplace (Castoe *et al.*, 2004). Therefore, we used different models of sequence evolution for biologically relevant partitions of our data. In the case of protein coding mitochondrial genes, the most relevant partitions are first, second and third codon positions, which are known to display different patterns of sequence evolution. We therefore partitioned our data into four separate data partitions, namely first, second and third codon positions separately for ND4, and the tRNA sequences as a single partition. To identify the most appropriate models of sequence evolution for each data partition, we used MrModeltest 2.2 (Nylander, 2004), and selected the model favoured under the Akaike Information Criterion for each category in our Bayesian analysis. Since MrBayes can only use a single outgroup taxon, *P. ingrami* was specified as the sole outgroup for all BI analyses. We ran the analysis for  $4 \times 10^6$  generations using 4 simultaneous independent runs initiated with different random starting trees, and sampling every 200 generations. Plots of lnL against generation were inspected to determine the burn-in period, and trees generated prior to the completion of burn-in were discarded. As a safety margin, we discarded the first  $10^6$  generations.

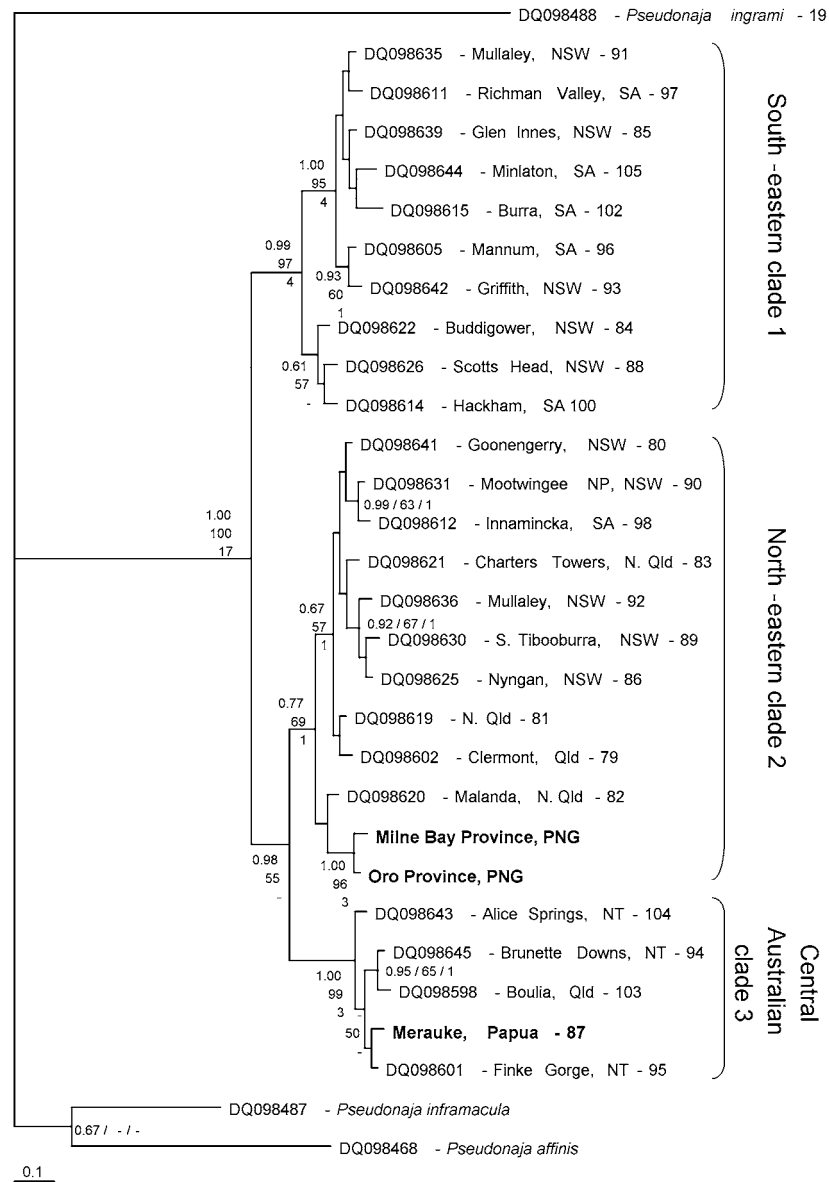
To test whether the data reject the monophyly of the haplotypes found in either the New Guinean or the Australian populations of *P. textilis* with statistical significance, we used PAUP\* to build the most parsimonious trees constrained to be consistent with those hypothesis. We then used Wilcoxon signed-ranks tests (Templeton, 1983, implemented in PAUP\*) to test for the significance of differences in tree length between the most parsimonious trees and the constrained tree. We used the Shimodaira-Hasegawa test (SH test—Shimodaira & Hasegawa, 1999) to perform the analogous analysis under the maximum likelihood (ML) criterion. To do this, we carried out a heuristic ML search in PAUP\*, using TBR branch swapping and 10 random addition sequence replicates, and a model of sequence evolution obtained for the unpartitioned data with MrModeltest. The ML search was then repeated while constraining the New Guinean or the Australian haplotypes to be monophyletic. The constraint trees were compared to the optimal ML tree using the RELL option of the SH test in PAUP\*. Finally, in the case of Bayesian inference analysis, we used PAUP\* to filter all trees obtained after completion of the burn-in phase to retain only those consistent with the alternative constraint trees, and considered the alternative hypothesis as rejected if it was supported by less than 5% of Bayesian trees.

## Results

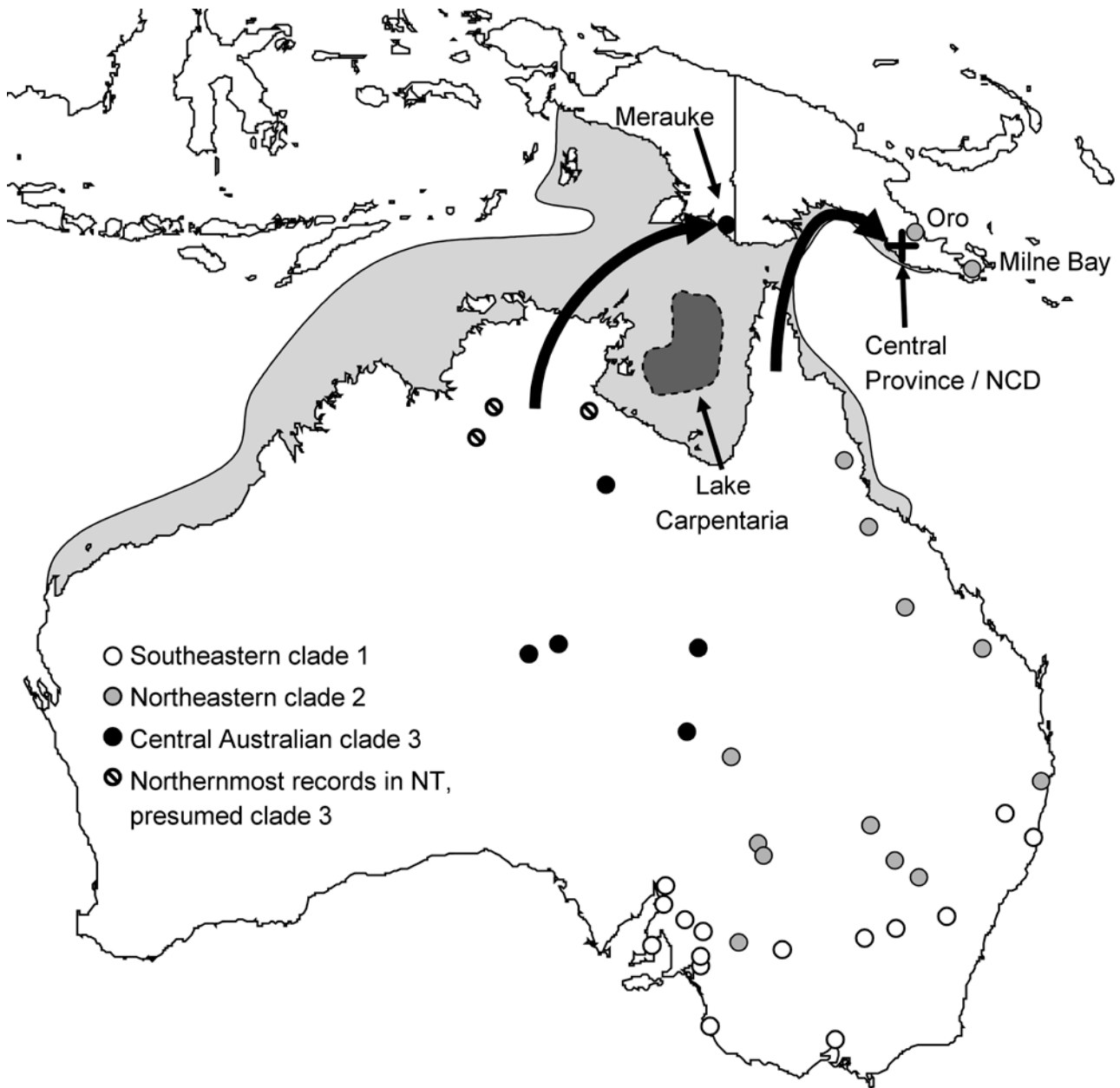
We obtained sequences corresponding to those of Skinner *et al.* (2005) from the new material from New Guinea (GenBank accession numbers for the Milne Bay and Oro specimens: EU258951–EU258952; our additional Merauke sample yielded an identical sequence to Skinner *et al.*'s Merauke haplotype). There were no indels, unexpected stop codons or frameshifts in the protein coding parts of our sequences, leading us to conclude that they represent genuine mitochondrial DNA (Zhang & Hewitt, 1996). Of 767 included base pairs, 148 were variable and 74 parsimony-informative. The  $10^6$  random trees generated a skewness statistic of  $g_1 = -0.662122$ , rejecting the null hypothesis that the data contain no significant phylogenetic signal ( $P < 0.001$ —Hillis & Huelsenbeck, 1992).

Parsimony analysis resulted in 20 equally most parsimonious trees of 202 steps (c.i. = 0.7673, r.i. = 0.823). MP and BI analysis revealed essentially similar phylogenetic trees (Fig. 1). The results are largely identical to those of Skinner *et al.* (2005). Both analyses reveal three distinct clades of haplotypes within *P. textilis* (Fig. 2): a robustly supported southeastern clade 1, with samples from southeastern South Australia, Victoria, and southern and eastern New South Wales, a weakly supported northeastern Australian clade 2,

with samples from eastern South Australia, central and northern New South Wales and eastern Queensland, and a robustly supported central Australian clade 3, with samples from scattered localities in the Northern Territory, northern South Australia and western Queensland. The main difference between our results and those of Skinner *et al.* (2005) is the placement of the haplotype from Malanda, northern Queensland, which clustered with clade 3 in Skinner's Bayesian analysis (with low support), but with clade 2 (and stronger support) in his parsimony tree. Our data consistently place it at the base of clade 2, albeit with low support values. The samples from New Guinea are diphyletic, nesting separately within two of the three clades: the samples from Milne Bay and Oro Provinces, eastern Papua New Guinea, nest within clade 2, whereas the Merauke haplotype nests within clade 3.



**FIGURE 1.** Bayesian inference tree for the included haplotypes of *Pseudonaja textilis*. New Guinea haplotypes are in bold type and enlarged font, all other samples are Australian. Branch support values are Bayesian posterior probabilities, parsimony bootstrap and Bremer support. Only values of over 0.5 (Bayesian) and 50% bootstrap support (MP) are given, dashes indicate support below that level. Taxon label information includes GenBank accession number, locality, state/country (NSW = New South Wales, SA = South Australia, Qld = Queensland, NT = Northern Territory, PNG = Papua New Guinea), and, where applicable, the haplotype number from Skinner *et al.* (2005).



**FIGURE 2.** Distribution of sampling sites, haplotype clades and hypothesised colonisation routes of *Pseudonaja textilis* into New Guinea. + indicates populations from Central Province, PNG, that were not sampled in this study, but are predicted to nest with the eastern New Guinea populations. Light grey shading designates likely areas of dry land at times with sea levels more than 75 metres below current levels (modified from Voris, 2000). This is only shown for the Sahul Shelf, not for other parts of the Indonesian islands or Australia. Dark grey shading indicates the approximate location of Lake Carpentaria (Keenan, 1994; Voris, 2000). The northernmost records of *P. textilis* in the Northern Territory are also indicated.

Mean between-clade p-distances ( $\pm 1$  SE) were  $0.0236 \pm 0.0046$  between clade 1 and 2,  $0.0290 \pm 0.0055$  between clade 1 and 3, and  $0.0190 \pm 0.0045$  between clade 2 and 3. The Milne Bay and Oro haplotypes differed from each other by a single nucleotide substitution, and from other haplotypes in clade 2 by 4–8 substitutions (p-distance 0.0065–0.0104), and the Merauke haplotype from other clade 3 haplotypes by 1–3 substitutions (p-distance 0.0013–0.0039).

Pairwise Wilcoxon signed-ranks tests largely rejected the alternative tree topologies of monophyly of either the New Guinea or the Australian populations of *P. textilis*, although a minority of pairwise compari-

sons were insignificant (Table 1). The Shimodaira-Hasegawa tests convincingly rejected the alternative topologies, and none of the 60004 post-burn-in Bayesian trees was consistent with either of the two alternative hypotheses.

**TABLE 1.** Tests of alternative phylogenetic scenarios.

|                                    | Wilcoxon signed ranks test |               |               | Shimodaira-Hasegawa test |         | Bayesian inference<br>proportion of Bayesian trees consistent with alternative hypothesis |
|------------------------------------|----------------------------|---------------|---------------|--------------------------|---------|---|
|                                    | d(steps)                   | -Z            | P             | d(lnL)                   | P       |   |
| Monophyly of New Guinea haplotypes | 10                         | 1.6389–3.1623 | 0.0016–0.1012 | 38.90171                 | < 0.001 | 0/60004   |
| Monophyly of Australian haplotypes | 10                         | 1.8898–2.8868 | 0.0039–0.0588 | 42.57724                 | < 0.001 | 0/60004   |

## Discussion

### Biogeography

The phylogeographic pattern we have identified in this study clearly indicates that the populations of *P. textilis* in New Guinea are the result of two separate colonisation events. The populations from the Merauke area of Indonesian Papua group with populations of *P. textilis* from central and central northern Australia, whereas the eastern populations from Milne Bay and Oro group with populations from northern Queensland. The levels of sequence divergence between the New Guinean haplotypes and their Australian sister groups are low in both cases (< 1% p-distance). This suggests that a divergence between the New Guinea populations and their Australian sister groups is recent, most likely dating back to the Pleistocene, given most current estimates rates of sequence evolution in squamates (Zamudio & Greene, 1997; Wüster *et al.*, 2002).

Although there has been much speculation that the New Guinea populations of *P. textilis* were introduced by humans (Slater, 1968; discussed in O'Shea, 1996, Kuch & Yuwono, 2002, and Williams & Wüster, 2005), our results are entirely congruent with natural colonisation. We do not consider the abundance or broad distribution of a species to be any indicator of its native or introduced status (Kuch & Yuwono, 2002). In fact, successfully introduced species are often found in great abundance across a broad geographic range (e.g. rabbits in Australia or the cane toad *Bufo marinus* in Australia and New Guinea). Indeed, the combination of wide but scattered distribution, coupled with apparent rarity, of *P. textilis* in Central Province and the NCD are more suggestive of native status than its abundance elsewhere. Instead, we think strong evidence of the native status of *P. textilis* would be genetic data showing haplotype diversity and divergence: the fact that the haplotypes from Oro and Milne Bay Provinces differ by one base pair substitution thus also supports the hypothesis of native status of the PNG populations.

Our data show that the two New Guinea populations possess haplotypes that are nested within the geographically closest Australian haplotype clades, a pattern that would not necessarily be expected from a human introduction. Instead, the pattern observed in *P. textilis* is consistent with a hypothesis of dispersal during times of lowered sea levels during the Pleistocene, which resulted in repeated land connections between Australia and New Guinea (Voris, 2000). At various times through the Pleistocene, much of the Sahul Shelf was above sea level, leading to more or less wide land bridges between Australia and New Guinea. Voris (2000) estimated that a 10 m lowering of sea levels would be sufficient to lead to a narrow land connection between Cape York (northern Queensland) and the central southern coast of New Guinea, whereas a lowering of 75 m or more would result in extensive emergence of the Sahul Shelf, exposing most of the current sea



floor from between Arnhem Land and Aru Island in the west to Cape York and the Gulf of Papua in the east. However, during at least part of the Pleistocene, the centre of this shelf was occupied by a large lake, Lake Carpentaria, situated within the present-day Gulf of Carpentaria (Torgersen *et al.*, 1983; Keenan, 1994; Voris, 2000). The presence of this lake essentially meant that there were two dispersal corridors between Australia and New Guinea, one along the western side of the Sahul Shelf, from Arnhem Land to southern New Guinea, and the other along the eastern side from present-day northern Queensland to the present-day Trans-Fly area or the Gulf of Papua, part of which would also have been exposed due to lowered sea levels. Voris (2000) estimated that sea levels dropped to 75m or more below their present level on at least two separate occasions, for a total duration of approximately 42,000 years, during the last 250,000 years. Extensive global climatic fluctuations with high-latitude glaciation started occurring at least 2.4 Mya (Shackleton *et al.* 1984; Hooghiemstra & Cleef, 1995), and it therefore seems likely that similar episodes of lowered sea levels have occurred throughout the late Pliocene and the Pleistocene, although their timing and extent remain poorly known, and are likely to be confounded by tectonic changes in this region.

Recent genetic studies of several snake taxa indicate that both corridors may have been important in shaping the snake faunas of New Guinea and northern Australia: the *Acanthophis rugosus* complex clearly used the western route, with closely related haplotypes being found in the Merauke area and in the Top End (Wüster *et al.*, 2005). On the other hand, water pythons (*Liasis fuscus-mackloti* complex—Rawlings *et al.*, 2004), green tree pythons (*Morelia viridis*—Rawlings & Donnellan, 2003) and scrub pythons (*Morelia amethystina*—Harvey *et al.*, 2000) appear to have dispersed solely along the eastern route, although the *Liasis fuscus-mackloti* complex also appears to have undergone separate overwater dispersal from the Northern Territory to the lesser Sunda Islands (Rawlings *et al.*, 2004).

Our analysis of *P. textilis* reveals the first apparent instance of a species that used both dispersal routes to colonise New Guinea from an Australian origin. The close phylogenetic relationship between the eastern PNG populations and those from northern Queensland (clade 2) very strongly indicates dispersal along the eastern route. Torgersen *et al.* (1983) suggested that the Fly River drained into Lake Carpentaria rather than the Gulf of Papua until the uplift of the Oriomo Plateau during the Pleistocene. As a result, it seems likely that the present-day swamplands of the Gulf of Papua coast, where *P. textilis* and other savanna species such as *Oxyuranus scutellatus* and *Pseudechis papuanus* do not occur, would have been much drier than is now the case. If this hydrographic pattern was still prevailing at the time of the eastern dispersal of *P. textilis*, then it may have facilitated dispersal along the southeastern coast of New Guinea to the eastern tip of the island.

The Merauke population, on the other hand, is most parsimoniously interpreted as the result of dispersal along the western route (Fig. 2). Although *P. textilis* currently appears to be lacking in the Top End, the northernmost populations from the Northern Territory, presumably assignable to clade 3, are from the base of the Peninsula, at Nathan River Station (NT Museum R22316) in the east and at Victoria River Downs (NT Museum R3714; see also Gillam, 1979) and “Delamere Road, 2 km S. of Victoria Highway” (15° 20' S, 131° 37' E—NT Museum R17151) in the west (Fig. 2). This, plus the fact that the available sampling indicates that all the northeastern Queensland populations are assignable to clade 2, suggests that dispersal to the Merauke region via the eastern route by clade 3 populations would be an unparsimonious assumption, as it would imply repeated exchanges of populations in northern Queensland. More extensive sampling of *P. textilis* in the Northern Territory and northwestern Queensland would provide additional evidence for the most likely dispersal routes.

Within New Guinea, the populations from Central Province and the NCD are situated along the likely dispersal route along the southern coast of New Guinea towards the eastern tip of the island. This hypothesis therefore predicts that these populations should have haplotypes that group together with those of the Milne Bay and Oro specimens. An analysis of additional taxa with wider distributions in northern Australia and southern New Guinea (e.g., *Morelia spilota*, *Demansia vestigiata*, *Furina tristis*) would reveal the relative importance of the two dispersal corridors in shaping the snake faunas of Australia and New Guinea.

The confirmation of the native status of *P. textilis* in New Guinea suggests that the paucity of records of the species, coupled with a scattered distribution, is most likely a natural phenomenon, and gives little reason to predict any rapid change in the relative importance of this species as a snakebite hazard, which would have been the case if the species was present as a result of an introduction (Williams & Wüster, 2005). Nevertheless, given the adaptability of different species of *Pseudonaja* in Australia to human-impacted habitats, it is possible that the abundance of the species and the frequency of humans being bitten by *Pseudonaja* in New Guinea may increase over coming decades. Indeed, recent fieldwork in New Guinea by the authors suggests that both *P. textilis* and *Oxyuranus scutellatus* may occur in greater densities where savanna has been converted into oil palm monoculture. Similar increased concentrations have also been observed for *Micropechis ikaheka* in New Guinean copra and cocoa plantations (O’Shea, 1994).

A recent cost-benefit trial of CSL Snake Venom Detection Kits at Port Moresby General Hospital identified *P. textilis* as the culprit in four cases from a series of forty envenomed patients (Williams *et al.*, 2006, in prep.). In two of these four cases the patients presented 18–24 hours post-snakebite with coagulopathy and profound irreversible neurotoxicity and both subsequently died. CSL Brown Snake Antivenom is not presently registered for clinical use in Papua New Guinea. Continued epidemiological surveillance in New Guinea, preferably using immunoassay methods such as the CSL Snake Venom Detection Kits, are therefore essential to monitor the situation and ensure that antivenom acquisition and distribution reflects changes in the frequency of bites by different species, and thus the needs of bitten patients. Inadequate knowledge and misidentification of biting snakes has led to medical problems in PNG in the past, when *Pseudechis papuanus* antivenom was stocked and administered to numerous patients, when in reality the vast majority were bitten by taipans (*Oxyuranus scutellatus*) (Williams, 2005).

## Taxonomy

In his description of the first two specimens of *P. textilis* from New Guinea, McDowell (1967) provided basic scale and dentition counts for the two specimens, and commented briefly that they “resemble Queensland and North Australian material examined, except in having 12, rather than 9 to 11, solid maxillary teeth”. However, no indication of the sample size or provenance of the Australian material was provided at the time. Hoser (2003) based his description of *Pseudonaja textilis pughii* primarily on McDowell’s brief description, designating the specimens described by McDowell as his types, and used the maxillary tooth counts as his main diagnostic character. In addition, he briefly described the coloration of living specimens examined by him personally (without indicating sample size or origin), and explicitly stated that all New Guinea *Pseudonaja* are assignable to his new subspecies.

Our data reveal that the situation of the New Guinean *Pseudonaja* is more complex than suggested by Hoser (2003). First, the New Guinean *P. textilis* are diphyletic, with populations from Merauke being descended from a different set of Australian populations than the Milne Bay/Oro populations from eastern Papua New Guinea. Second, it is also clear that the two New Guinea populations of *P. textilis* are recent colonisers of New Guinea and share a recent common ancestry with their respective Australian sister populations.

The extent to which the two New Guinea populations are diagnosably distinct from each other and their Australian sister populations remains unclear: McDowell’s (1967) sample size for tooth counts for New Guinea was only two, and not stated for Australia (He later stated that his Queensland sample size was “no more than eight” [McDowell, pers. comm. 2007 to MOS]). Greer (1997) provided data for an additional 14 specimens (origin not stated), with a range of 9–11 solid maxillary teeth. However, two of the new specimens acquired by the authors (LSUMZ 90636–37) were examined and found to have only 11 solid maxillary teeth, demonstrating that the character is not diagnostic for all New Guinea specimens.

Hoser's comments on coloration are also based on an unknown sample size of unknown geographic origin, and are thus difficult to interpret in view of the documented variability in the coloration and pattern of *P. textilis* from Australia (see Sutherland, 1981, for an example).

In Table 2, we provide scale counts for most of the known specimens so far documented from Papua New Guinea, and for three specimens from Merauke. The scale counts are well within published limits for *P. textilis* as a whole (Gillam, 1979; Annable, 1985; Cogger, 2000), and there are no clear differences between specimens from eastern PNG and the Merauke area, as far as can be told from our very limited sample. However based on the material in Table 2 as well as photographs of four additional Merauke specimens, there appear to be consistent differences in colour between the *P. textilis* populations from eastern PNG and from Merauke: the Merauke specimens imported to Europe through the Indonesian pet trade are olive or tan to warm mid-brown, whereas all the PNG specimens examined (including the preserved material in the NMPNG) are dark grey-brown to almost black (Fig. 3).



**FIGURE 3.** Specimens of the common brownsnake, *Pseudonaja textilis*, from New Guinea, illustrating colour variation. A. AVRU-UPNG PT004, from Heropa plantation, Doboduru, Oro Province, Papua New Guinea. B. AVRU-UPNG PT001) from Wamawamana, Milne Bay Province, Papua New Guinea. C. Captive specimen from Merauke, Papua, Indonesia (Klaus Römer, personal collection; photograph by Klaus Römer). Note the warmer colour tone and lighter head compared to the PNG specimens. D. NMPNG R 24583, from Port Moresby, NCD, Papua New Guinea.

**TABLE 2.** Scale counts and measurements for known specimens of *Pseudonaja textilis* from New Guinea.

| Specimen/locality  | Sex | Dorsals<br>(neck, mid-<br>body, vent) | Ven-<br>trals | Subcaudals                | SVL        | TL     | Colour  |
|--|-----|---------------------------------------|---------------|---------------------------|------------|--------|---|
| LSUMZ 90636:<br>Wamawamana, Milne Bay<br>Province, PNG                         | M   | 18–17–15                              | 205           | >25 divided               | 939 mm     | n/a    | (tail incomplete) dark grey-brown dorsally with whitish chin and dark grey ventral surfaces                     |
| LSUMZ 90637:<br>Wamawamana, Milne Bay<br>Province, PNG                         | M   | 18–17–15                              | n/a           | 62 divided                | 764 mm     | 152    | (damage to portion of ventral region) dark grey-brown dorsally with whitish chin and dark grey ventral surfaces |
| AVRU-UPNG PT001:<br>Wamawamana, Milne Bay<br>Province, PNG                     | M   | 18–17–15                              | 202           | 60 divided                | 954 mm     | 173 mm | dark grey-brown dorsally with whitish chin and dark grey ventral surfaces                                       |
| AVRU-UPNG PT002:<br>Heropa, Oro Province, PNG                                  | M   | 17–17–15                              | 199           | 62, divided               | 968 mm     | 191 mm | Chocolate brown dorsally, whitish chin, grey ventrally  |
| AVRU-UPNG PT003:<br>Heropa, Oro Province, PNG                                  | M   | 17–17–15                              | 198           | 2 single, ><br>43 divided | 981 mm     | n/a    | (tail incomplete) Chocolate brown dorsally, whitish chin, grey ventrally  |
| AVRU-UPNG PT004:<br>Heropa, Oro Province, PNG                                  | F   | 17–17–15                              | 201           | 63, all<br>divided        | 977 mm     | 180 mm | Uniformly chocolate brown dorsally, whitish chin, grey ventrally  |
| AMNH 73949 Menapi, Cape<br>Vogel, Milne Bay Province,<br>PNG                   | F   | 17–17–15                              | ~203          | > 45<br>divided           | ~765<br>mm | n/a    | (specimen damaged, tail incomplete, no colouration data)  |
| AMNH 73959: Baiawa,<br>Milne Bay Province, PNG                                 | M   | 17–18–15                              | 205           | > 25                      | 959 mm     | n/a    | (tail incomplete, no colouration data)  |
| NMPNG 24583:<br>Port Moresby, National Capi-<br>tal District, PNG              | M   | 17–17–15                              | 207           | 56, all<br>divided        | 1195<br>mm | 195 mm | Dark greyish-brown dorsals, chin whitish, ventral surfaces grey   |
| NMPNG 22295:<br>Dogura, Milne Bay Province,<br>PNG                             | M   | 17–17–15                              | 200           | 61, all<br>divided        | 1240<br>mm | 212 mm | Significantly faded   |
| WW Pt1:<br>Merauke, Papua, Indonesia<br>(shed skin, courtesy I. Hen-<br>drikk) | M   | 17–17–13                              | 201           | ≥58, 1–6<br>undivided     | n/a        | n/a    | Warm medium brown   |
| WW Pt2:<br>Merauke, Papua, Indonesia<br>(shed skin, courtesy Klaus<br>Römer)   | M   | 17–17–14                              | 204           | 62, 3–4<br>undivided      | n/a        | n/a    | Warm medium brown   |
| Freek Vonk (personal collec-<br>tion):<br>Merauke, Papua, Indonesia            | F   | 17–17–15                              | 206           | 63                        | 960        | 193    | Medium brown  |

The taxonomic treatment of slightly differentiated allopatric populations has been contentious for many years, with different workers using different criteria for the recognition of such isolates as separate species. Some have argued that any diagnosably differentiated isolated populations should be regarded as separate species, even if in terms of phylogeography they are nested deeply within a widespread species and phylogeographically complex species, irrespective of the nature of the difference (e.g., Grismer, 1999). Others have retained minimally differentiated recent island populations as part of a single species if phylogeographic patterns place them within more widespread mainland clades (e.g., Pook *et al.*, 2000; Keogh *et al.*, 2005; Castoe *et al.*, 2007). Although parphyly of the mainland populations in gene trees does not preclude recognition of the island population as a separate species (Funk & Omland, 2003; Graziottin *et al.*, 2006), it should stimulate a synthetic approach based on a thorough assessment of the systematics of the entire complex. We therefore suggest that an assessment of the taxonomic status of the New Guinean populations of *P. textilis* should

involve a comprehensive revision of the entire species complex in both Australia and New Guinea, based, for instance, on morphological methods such as multivariate analysis (e.g., Wüster & Thorpe, 1992) in conjunction with molecular data (e.g., Puerto *et al.*, 2001), rather than a piecemeal approach based on small samples and few characters.

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## References

- Annable, T. (1985) Subcaudal scalation analysis of *Pseudonaja textilis* (Duméril and Bibron) in the eastern Riverina region. *Herpetofauna*, 16, 40–42.
- Aplin, K.P., Baverstock, P.R. & Donnellan, S.C. (1993) Albumin immunological evidence for the time and mode of origin of the New Guinean terrestrial mammal fauna. *Science in New Guinea*, 19, 131–145.
- Arévalo, E., Davis, S.K. & Sites, J.W. (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. *Systematic Biology*, 43, 387–418.
- Avise, J.C. (2000) *Phylogeography*. Harvard University Press, Cambridge, Massachusetts.
- Bremer, K. (1994). Branch support and tree stability. *Cladistics*, 10, 295–304.
- Campbell, C.H. (1969) *A clinical study of venomous snake bite in Papua*. PhD thesis, Univ. Sydney. 208 pp.
- Castoe, T.C., Doan, T.M. & Parkinson, C.L. (2004) Data partitions and complex models in Bayesian analysis: the phylogeny of gymnophthalmid lizards. *Systematic Biology*, 53, 448–469.
- Castoe, T.A., Spencer, C.L. & Parkinson, C.L. (2007) Phylogeographic structure and historical demography of the western diamondback rattlesnake (*Crotalus atrox*): a perspective on North American desert biogeography. *Molecular Phylogenetics and Evolution*, 42, 193–212.
- Cogger, H. (2000) *Reptiles and Amphibians of Australia, sixth edition*. Reed Books, Sydney, 775 pp.
- Currie, B.J. (2000) Snakebite in tropical Australia, Papua New Guinea and Irian Jaya. *Emergency Medicine*, 12, 285–294.
- Doughty, P., Maryan, B., Donnellan, S.C. & Hutchinson, M.N. (2007) A new species of taipan (Elapidae: *Oxyuranus*) from central Australia. *Zootaxa*, 1422, 45–58.
- Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791.
- Fry, B.G., Winkel, K.D., Wickramaratna, J.C., Hodgson, W.C. & Wüster, W. (2003) Effectiveness of snake antivenom: species and regional venom variation and its clinical impact. *Journal of Toxicology—Toxin Reviews*, 22, 23–34.
- Funk, D.J. & Omland, K.E. (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution and Systematics*, 34, 397–423.
- Gillam, M.W. (1979) The genus *Pseudonaja* (Serpentes: Elapidae) in the Northern Territory. *Territory Parks and Wildlife Commission Research Bulletin*, 1, 1–28.

- Grazziotin, F.G., Monzel, M., Echeverrigaray, S. & Bonatto, S.L. (2006) Phylogeography of the *Bothrops jararaca* complex (Serpentes: Viperidae): past fragmentation and island colonization in the Brazilian Atlantic Forest. *Molecular Ecology*, 15, 3969–3982.
- Greer, A.E. (1997) *The Biology and Evolution of Australian Snakes*. Surrey Beatty & Sons, Chipping Norton, 358 pp.
- Grismer, L.L. (1999) An evolutionary classification of reptiles on islands in the Gulf of California, Mexico. *Herpetologica*, 55, 446–469.
- Harvey, M.B., Barker, D.G., Ammerman, L.K. & Chippindale, P.T. (2000) Systematics of pythons of the *Morelia amethystina* complex (Serpentes: Boidae) with the description of three new species. *Herpetological Monographs*, 14, 139–185.
- Hillis, D.M. & Huelsenbeck, J.P. (1992) Signal, noise and reliability in phylogenetic analyses. *Journal of Heredity*, 83, 189–195.
- Hooghiemstra, H. & Cleef, A.M. (1995) Pleistocene climatic change and environmental and generic dynamics in the north Andean montane forest and paramo. In: Churchill, S.P., Balslev, H., Forero, E., Luyeyn, J.L. (Eds.), *Biodiversity and Conservation of Neotropical Montane Forests*. New York Botanical Garden, New York, pp 35–49.
- Hoser, R. (2003) A new subspecies of elapid from New Guinea. *Boydii*, 2003, 2–4.
- Keenan, C.P. (1994) Recent evolution of population structure in Australian barramundi, *Lates calcarifer* (Bloch): an example of isolation by distance in one dimension. *Australian Journal of Marine and Freshwater Research*, 45, 1123–1148.
- Keogh, J.S., Scott, I.A.W. & Hayes, C. (2005) Rapid and repeated origin of insular gigantism and dwarfism in Australian tiger snakes. *Evolution*, 59, 226–233.
- Kuch, U., Keogh, J.S., Weigel, J., Smith, L.A. & Mebs, D. (2005) Phylogeography of Australia's king brown snake (*Pseudechis australis*) reveals Pliocene divergence and Pleistocene dispersal of a top predator. *Naturwissenschaften*, 92, 121–127.
- Kuch, U. & Yuwono, F.B. (2002) First record of brown snakes *Pseudonaja* cf. *textilis* (Duméril, Bibron & Duméril, 1854) from Papua, Indonesia. *Herpetozoa*, 15, 75–78.
- Kumar, S., Tamura, K., Jakobsen, I.B. & Nei, M. (2001) MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics*, 17, 1244–1245
- Laloo, D. (1994) *The Epidemiological, Clinical and Laboratory Features of Snakebite in the Central Province and National Capital District of Papua New Guinea*. DM Thesis. University of Newcastle upon Tyne, United Kingdom.
- Laloo, D., Trevett, A., Black, J., Mapao, J., Naraqi, S., Owens, D., Hutton, R., Theakston, R.D.G. & Warrell, D.A. (1994) Neurotoxicity and haemostatic disturbances in patients envenomed by the Papuan black snake (*Pseudechis papuanus*). *Toxicon*, 32, 927–936.
- McDowell, S.B. (1967) *Aspidomorphus*, a genus of New Guinea snakes of the family Elapidae, with notes on related genera. *Journal of Zoology, London*, 151, 497–543.
- Mengden, G.A. (1985) A chromosomal and electrophoretic analysis of the genus *Pseudonaja*. In: Grigg, G.C., Shine, R. & Ehmann, H. (Eds.), *Biology of Australasian Frogs and Reptiles*. Royal Zoological Society of New South Wales, Sydney, pp. 193–208.
- Nylander, J.A.A. (2004) *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.
- O'Shea, M.T. (1990) The highly and potentially dangerous elapids of Papua New Guinea. In: Gopalakrishnakone, P. & Chou, L.M. (Eds.), *Snakes of Medical Importance, Asia-Pacific Region*. Venom and Toxin Research Group, National University of Singapore, Singapore, pp. 585–640.
- O'Shea, M. (1994) The herpetofauna of coconut husk piles on Kar Kar Island, Madang Province, Papua New Guinea: The initial surveys. *ASRA Journal*, 1994, 51–72.
- O'Shea, M. (1996) *A Guide to the Snakes of Papua New Guinea*. Independent Publishing, Port Moresby, 239 pp.
- Pacey, T.L., Baverstock, P.R. & Jerry, D.R. (2001) The phylogenetic relationships of the bilby, *Macrotis lagotis* (Peramelimorphia: Thylacomyidae), to the bandicoots—DNA sequence evidence. *Molecular Phylogenetics and Evolution*, 21, 26–31.
- Pook, C.E., Wüster, W. & Thorpe, R.S. (2000) Historical biogeography of the western rattlesnake (Serpentes: Viperidae: *Crotalus viridis*), inferred from mitochondrial DNA sequence information. *Molecular Phylogenetics and Evolution*, 15, 269–282.
- Puerto, G., Salomão, M.G., Theakston, R.D.G., Thorpe, R.S., Warrell, D.A. & Wüster, W. (2001) Combining mitochondrial DNA sequences and morphological data to infer species boundaries: phylogeography of lanceheaded pitvipers in the Brazilian Atlantic forest, and the status of *Bothrops pradoi* (Squamata: Serpentes: Viperidae). *Journal of Evolutionary Biology*, 14, 527–538.
- Rawlings, L.H. & Donnellan, S.C. (2003) Phylogeographic analysis of the green python, *Morelia viridis*, reveals cryptic diversity. *Molecular Phylogenetics and Evolution*, 27, 36–44.
- Rawlings, L.H., Barker, D. & Donnellan, S.C. (2004) Phylogenetic relationships of the Australo-Papuan *Liasis* pythons (Reptilia: Macrostromata), based on mitochondrial DNA. *Australian Journal of Zoology*, 52, 215–227.

- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Sambrook, F., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Shackleton, N.J., Backman, J., Zimmerman, H. Kent D.V., Hall, M.A., Roberts, D.G., Schnitker, D., Baldauf, J.G., Desprairies, A., Homrighausen, R., Huddlestun, P., Keene, J.B., Kaltenback, A.J., Krumsiek, K.A.O., Morton, A.C., Murray, J.W. & Westberg-Smith, J. (1984) Oxygen isotope calibration of the onset of ice-rafting and history of glaciation of the North Atlantic region. *Nature*, 307, 620–623.
- Shimodaira, H. & Hasegawa, M. (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, 16, 1114–1116.
- Skinner, A., Donnellan, S. C., Hutchinson, M. N. & Hutchinson, R. G. (2005) A phylogenetic analysis of *Pseudonaja* (Hydrophiinae, Elapidae, Serpentes) based on mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 37, 558–571.
- Slater, K.R. (1968) *A Guide to the Venomous Snakes of Papua*. 2<sup>nd</sup> Edition PNG Government Printer, Port Moresby, 18 pp.
- Sutherland, S.K. (1981) *Venomous Creatures of Australia. A Field Guide with Notes on First Aid*. Oxford University Press, Melbourne, 128 pp.
- Sutherland, S.K. (1992) Deaths from snake bite in Australia, 1981–1991. *Medical Journal of Australia*, 157, 740–746.
- Swofford, D.L. (2002) PAUP\*—*Phylogenetic Analysis Using Parsimony (\*and Other Methods)*. Beta version 4.0b10. Sinauer, Sunderland.
- Templeton, A.R. (1983) Phylogenetic inference from restriction endonuclease cleavage sites maps with particular reference to the evolution of humans and the apes. *Evolution*, 37, 221–244.
- Torgersen, T., Hutchinson, M. F., Searle, D.E. & Nix, H.A. (1983) General bathymetry of the Gulf of Carpentaria and the Quaternary physiography of Lake Carpentaria. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 41, 207–225.
- Voris, H.K. (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography*, 27, 1153–1167.
- White, J. (1998) Envenoming and antivenom use in Australia. *Toxicon*, 36, 1483–1492.
- Williams, D.J. (2005) *Snakebite in southern Papua New Guinea*. Dissertation thesis School of Public Health & Tropical Medicine, James Cook University.
- Williams, D. & Wüster, W. (2005) Snakes of Papua New Guinea. In: Williams, D., Jensen, S., Nimorakiotakis, B. & Winkel, K.D. (Eds.), *Venomous Bites and Stings in Papua New Guinea*. Australian Venom Research Unit, Melbourne, pp. 33–64.
- Williams, D.J., Jensen, S.D., Winkel, K.D. (2006a) CSL Snake Venom Detection Kits: Analysis of clinical outcomes and cost benefits in the management of snakebite at PMGH. Proceedings of the 42<sup>nd</sup> Annual Symposium of the PNG Medical Society. Madang, PNG, pp. 27–28.
- Williams, D., Wüster, W. Fry, B.G. (2006b) The good, the bad and the ugly: Australian snake taxonomists and a history of the taxonomy of Australia's venomous snakes. *Toxicon*, 48, 919–930.
- Worrell, E. (1961) *Dangerous Snakes of Australia and New Guinea*. 4<sup>th</sup> Edition Angus & Robertson, Sydney, 66 pp.
- Wüster, W., Dumbrell, A.J., Hay, C., Pook, C.E., Williams, D.J. & Fry, B.G. (2005) Snakes across the Strait: Trans-Torresian phylogeographic relationships in three genera of Australasian snakes (Serpentes: Elapidae: *Acanthophis*, *Oxyuranus* and *Pseudechis*). *Molecular Phylogenetics and Evolution*, 34, 1–14.
- Wüster, W., Salomão, M.G., Quijada-Mascareñas, J.A., Thorpe, R.S. & B.B.B.S.P. (2002) Origin and evolution of the South American pitviper fauna: evidence from mitochondrial DNA sequence analysis. In: Schuett, G.W., Höggren, M., Douglas, M.E. & Greene, H.W. (Eds.), *Biology of the Vipers*. Eagle Mountain Publishing, Eagle Mountain, Utah, pp. 111–128.
- Wüster, W. & Thorpe, R.S. (1992) Asiatic cobras: population systematics of the *Naja naja* species complex (Serpentes: Elapidae) in India and Central Asia. *Herpetologica*, 48, 69–85.
- Zamudio, K.R. & Greene, H.W. (1997) Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for neotropical biogeography, systematics and conservation. *Biological Journal of the Linnean Society*, 62, 421–442.
- Zhang, D.-X. & Hewitt, G.M. (1996) Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution*, 11, 247–251.