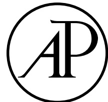

ACADEMIC PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Molecular Phylogenetics and Evolution 27 (2003) 36–44

MOLECULAR PHYLOGENETICS AND EVOLUTION

www.elsevier.com/locate/ympev

Phylogeographic analysis of the green python, *Morelia viridis*, reveals cryptic diversity

Lesley H. Rawlings[a,b] and Stephen C. Donnellan[a,*]

[a] *Evolutionary Biology Unit and Centre for Evolutionary Biology and Biodiversity, South Australian Museum, North Terrace, Adelaide, SA 5000, Australia*

[b] *Genetics Department, University of Adelaide, Adelaide, SA 5000, Australia*

Received 19 December 2001; revised 19 September 2002

Abstract

Green pythons, which are regionally variable in colour patterns, are found throughout the lowland rainforest of New Guinea and adjacent far northeastern Australia. The species is popular in commercial trade and management of this trade and its impacts on natural populations could be assisted by molecular identification tools. We used mitochondrial nucleotide sequences and a limited allozyme data to test whether significantly differentiated populations occur within the species range. Phylogenetic analysis of mtDNA sequences revealed hierarchal phylogeographic structure both within New Guinea and between New Guinea and Australia. Strongly supported reciprocally monophyletic mitochondrial lineages, northern and southern, were found either side of the central mountain range that runs nearly the length of New Guinea. Limited allozyme data suggest that population differentiation is reflected in the nuclear as well as the mitochondrial genome. A previous morphological analysis did not find any phenotypic concordance with the pattern of differentiation observed in the molecular data. The southern mitochondrial lineage includes all of the Australian haplotypes, which form a single lineage, nested among the southern New Guinean haplotypes.

© 2002 Elsevier Science (USA). All rights reserved.

Keywords: Mitochondrial DNA; Control region; Snake; Python; Phylogeography; New Guinea

1. Introduction

Pythons are a family of non-venomous constricting snakes found from Australia, through New Guinea, Indonesia, southern Asia to Africa. The family reaches its greatest generic level diversity in Australia and New Guinea. Many of the species are spectacularly coloured and patterned. In some species these attributes show regional variation indicating that the taxon maybe polytypic. The green python, *Morelia viridis* (Schlegel, 1872), is found throughout the island of New Guinea and its offshore islands (with the exception of the Bismarck Archipelago) and in a small rainforest block in northeastern Australia (Barker and Barker, 1994; McDowell, 1975; O'Shea, 1996) (Fig. 1). It has an altitudinal range from 0 to 2000 m above sea level, inhabiting lowland and lower montane forests (O'Shea, 1996). McDowell (1975) found no significant distinguishing morphological features between populations, with the possible exception that juveniles from the Sandaun Province, Papua New Guinea are brick red rather than the more common yellow or orange (Parker, 1982). Anecdotal evidence suggests that colour variation and markings may be diagnostic for some island populations, e.g., Aru and Biak Islands (F. Yuwono and D. McCrae, pers. comm). Furthermore, the species' wide geographic and altitudinal distribution are attributes often indicative of cryptic species level diversity (Donnellan et al., 1993).

Aside from the taxonomic interest, understanding evolutionary relationships among green python populations also has applications in two other areas. Firstly, the striking appearance of green pythons makes them popular exhibits in zoos and in the pet trade. Much of

* Corresponding author. Fax: +61-8-8207-7222.
E-mail address: donnellan.steve@saugov.sa.gov.au (S.C. Donnellan).

1055-7903/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
doi:10.1016/S1055-7903(02)00396-2

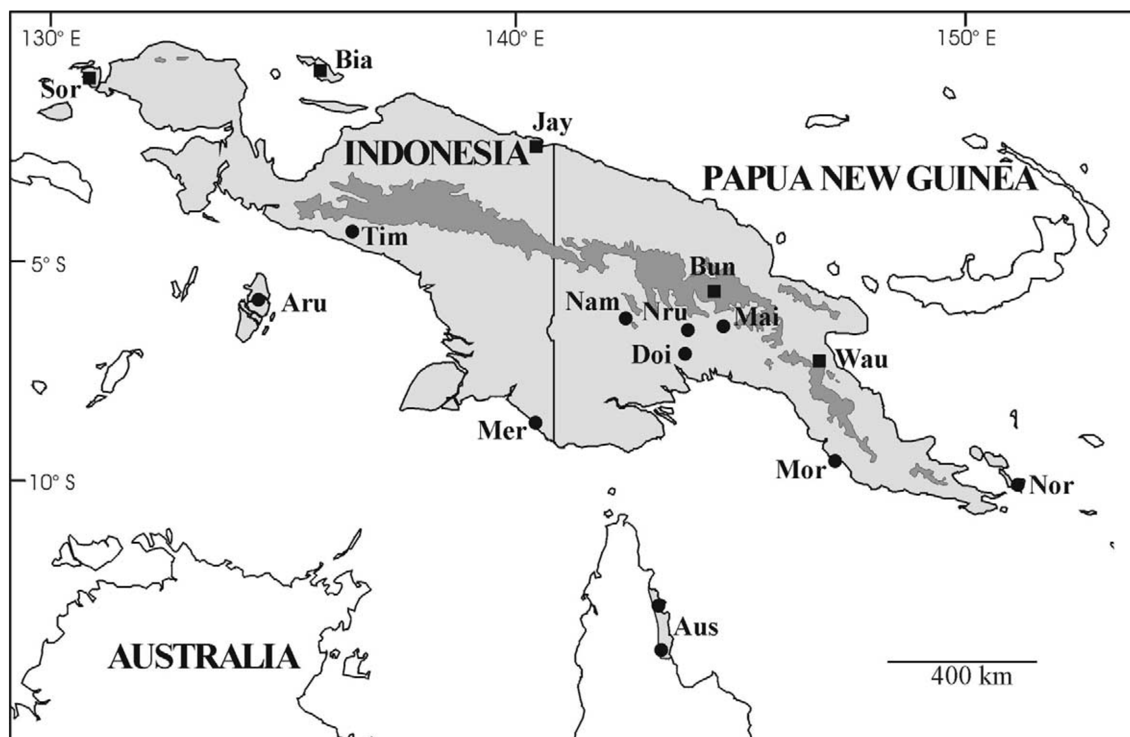

Fig. 1. Map of northern Australia and New Guinea showing the range of *M. viridis* (light shading) and sample locations. The locality code is: Aru, Aru Island; Aus, Queensland; Bia, Biak Island; Bun, Bundi; Doi, Doido; Jay, Jayapura; Mai, Maimufu; Mer, Merauke; Mor, Port Moresby; Nam, Namasado; Nor, Normanby Island; Nru, Noru; Tim, Timika; and Wau, Wau. ●, Southern populations; ■, northern populations; dark shading shows land above 2000 m elevation.

the pet trade is supplied from Irian Jaya, although there is now considerable success with captive breeding in the USA and Europe from Irian Jayan stock (D. Barker, pers. comm; D. MacCrae, pers. comm). In contrast there has been limited captive breeding success with Australian green pythons (Barker and Barker, 1994) and Australian law prohibits the unregulated capture of wild snakes. These factors combining with its very limited Australian range, have led to the green python being given special status for conservation management (Banks, 1999). In a global effort to conserve biodiversity, zoological parks of the Australasian region established the Australasian Species Management Program (ASMP) to determine conservation selection criteria and appropriate management strategies for species at risk (Banks, 1999). These management programs are overseen by Taxon Advisory Groups (TAGs). Two of the main goals for the green python TAG are to clarify suggested differences between Australian and New Guinean snakes and to achieve captive breeding of Australian specimens. In earlier years, before quarantine restrictions closed off legal importation, green pythons for exhibit in Australian zoos were imported from New Guinea as well as collected from Queensland. Therefore, to establish a breeding program in accordance with TAG guidelines, the origin of green pythons already in zoos needs to be determined.

Secondly, the rarity of the green python in Australian herpetologists' collections, and consequent high market price, and the strict regulations on importing exotic specimens into Australia have encouraged the continued importation of green pythons, now an illegal activity, into Australia (McDowell, 1997). Currently, these illegal imports can easily be absorbed into the Australian pet trade undetected. The development of molecular makers that can be used to provenance and identify individual animals would ensure better quarantine management.

In the present study, we investigated the genetic population structure of green pythons by examining samples from representative localities in Australian and New Guinea. We used nucleotide sequences of two mitochondrial genes and incorporated limited allozyme electrophoretic data to examine whether the species shows strong phylogeographic structuring and in particular whether the Australian population could be distinguished from New Guinean populations. Phylogeographic analyses of other snake species have provided powerful insights into their population history and systematics (e.g., Ashton and de Queiroz, 2001; Burbrink et al., 2001; Creer et al., 2001; Henderson and Hedges, 1995; Keogh et al., 2001; Rodriguez-Robles and De Jesus-Escobar, 2000; Zamudio and Greene, 1997).

2. Materials and methods

2.1. Specimens examined

Fifty-two *M. viridis* were collected from 16 locations from across the species range in New Guinea and northern Queensland, Australia (Fig. 1). Tissue samples used in this study were (*—samples used for allozyme electrophoresis; ABTC, Australian Biological; Tissue Collection; AMS, Australian Museum Sydney; BPBM, Bernice P. Bishop Museum, Hawaii; MV, Museum Victoria, Melbourne; QM, Queensland Museum Brisbane; SAMA, South Australian Museum Adelaide): *Australia*—Aus 1–18: Iron Range, Queensland ABTC 65592, ABTC 65605, ABTC 67593-4, ABTC 67596, ABTC 67627-34, QM J66805, MV T888, Lockhart River, Queensland ABTC 51497-8*, McIlwraith Range, Queensland QM CJS919; *Southern PNG*—Simbu Province Doi 1–3: AMS R115348-50*, Nru 1–2: AMS R115355-6*, Southern Highlands Province Nam 1–2: AMS R122363-4*; Milne Bay Province Nor 1: AMS R129716*; Central District Mor 1: ABTC 68320; Eastern Highlands Province Mai 1: ABTC 67151; *Northern PNG*—Madang Province Bun 1: AMS R124531*; Morobe Province Wau 1–2: BPBM 11617*, BPBM 13798*; *Southern Irian Jaya*—Aru Island Aru 1–4: ABTC 66380-1, ABTC 68312-3; Merauke Mer 1–6: ABTC 66384-5, ABTC 67170, ABTC 67172, ABTC 68315-6; Timika Tim 1: ABTC 66387; *Northern Irian Jaya*—Biak Island Bia 1–5: ABTC 66388-9, ABTC 67175-6, ABTC 67182; Jayapura Jay 1–2: ABTC 66383, ABTC 66386; Sorong Sor 1–5: ABTC 67173-4, ABTC 67183, ABTC 68318-9. *Morelia spilota* SAMA R26878, was used as the outgroup.

2.2. Mitochondrial DNA

DNA was extracted from liver, body scales or shed skin of snakes using a salting out method (Miller et al., 1988). For skin and scales, the Proteinase-K digestion at 37 °C step was extended to overnight. PCR primers L14841 and H15149 (Kocher et al., 1989) were used to amplify a 350 bp *cytochrome b* (*cytb*) fragment. To avoid the amplification of a *control region-like* gene that is present in snakes between the *ND1* and *ND2* genes (Kumazawa and Nishida, 1996), nested PCR was used to amplify the *control region* (*CR*). An initial amplification used the primers L15926 and H690 (Kumazawa et al., 1996) situated in the $tRNA^{Thr}$ and *12S rRNA* genes, respectively, with an eLONGase PCR protocol. This product was then used as a template for a hemi-nested amplification with primers L15926 and snake 17 (Kumazawa, pers. comm.—5′-TATGTCTAACAAGCATT AAG-3′) situated in the Conserved Sequence Block I of the *CR* to amplify a ~850 bp product that was used for sequence analysis. Sequencing reactions in both directions of the *CR* fragment tended to stall at a C-rich region [described by Kumazawa et al. (1998)]. Additional nested primers (Kumazawa, pers. comm.) were used to sequence across this region in each direction. These primers were Snake 1 (5′-CCT ATG TAT AAT AAT ACA TTA A-3′), Snake 6 (5′-ACC CTT CCC GTG AAA TCC-3′), and Snake 7 (5′-TGA AAG GAT AGA GGA TTT CAC G-3′). Both strands of the PCR amplified gene fragment were sequenced using the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit on a FTS-1 thermal sequencer (Corbett Research). The reaction products were electrophoresed on an Applied Biosystems 373A DNA sequencer.

Sequences were aligned by eye and only 12 insertion/deletion (indel) events were inferred in the *CR* alignment. Sequences are deposited with GenBank, Accession Nos. are AY169826–99.

The potential for the *CR* primers to amplify mitochondrial genes rather than nuclear paralogues (Zhang and Hewitt, 1996) was tested using the parallel titration protocol of Donnellan et al. (1999) in which the control nuclear locus was *18S rRNA* amplified with the primers 18e (Hillis and Dixon, 1991) and G59 (S. Cooper pers. comm. 5′-GCT GGC ACC AGA CTT GCC CTC C-3′). Aligned sequences were phylogenetically analysed with maximum parsimony (MP) and maximum likelihood (ML) and distance methods via neighbour-joining (NJ) in PAUP* 4.0b2a (Swofford, 1999). To determine whether the two data sets should be combined, the ILD test (Farris et al., 1995) (the Partition Homogeneity Test in PAUP) was performed in PAUP* 4.0b2a. The most appropriate model of nucleotide substitution for ML and distance analyses was found with using Modeltest3 (Posada and Crandell, 1998). Because of the large number of haplotypes analysed, values for parameters of the nucleotide substitution model were estimated using quartet puzzling by successive approximations (Strimmer and Vonhaeseler, 1996). The one of the most parsimonious trees was used as a starting point to estimate the parameters. These parameters were then used in a puzzling analysis to generate a tree topology for the next round of parameter estimation. When the values of estimated parameters no longer changed, it was concluded that the best estimate of the parameters had been reached. The estimated parameter values were then used in a full ML analysis under the specified model.

2.3. Allozyme electrophoresis

Frozen tissues suitable for allozyme electrophoresis were available for only 12 *M. viridis* due to the constraint of collecting many of the samples from captive specimens. Allozyme electrophoresis was done according to the methods of Richardson et al. (1986). Enzymes stained are listed in Table 1 and their Enzyme Commission Numbers and locus abbreviations are in

Table 1
Allele frequencies expressed as a percentage, in seven OTUs of *Morelia* at 36 allozyme loci

OTU	Wau	Nor	Nam	Doi	Nru	Aus	*M. spilota*
N	2	1	2	3	2	1	1
Aat-2	a	c	c	c	c	–	b
Acoh-2	c(75) a(25)	b	b	b	b(75) a(25)	–	b
Ada	c	d	d	d	d	d	b(50) a(50)
Eno	b	b	b	b(17) a(83)	b(75) a(25)	b	b
Gpi	b	b	b	b	c(25) b(75)	b	a
Gr	b(50)^{1} a(50)	b(50) a(50)	b	b(17) a(83)	a	b	b
Idh-1	c(25) b(75)	b(50) a(50)	a	a^{1}	a^{1}	a	a
PepA	b(75) a(25)	b	b	b	b	–	b
PepB1	c	b(50) a(50)	b(50) a(50)	b(83) a(17)	b(50)^{1} a(50)	–	c
PepB2	b	a	a	a	a	a	–
Pgm-1	b(25) a(75)	a	b(25) a(75)	b(83) a(17)	b(25) a(75)	a	b
Pgm-2	b	a	a	a	a	–	b

Note. Alleles are designated alphabetically, with 'a' being the most cathodally migrating allele. Where enzymes are encoded by more than one locus, the loci are designated numerically in order of increasing electrophoretic mobility. Where the allele frequencies are not given, the frequency is 100. The number of individuals sampled from each population (*N*) is given at the head of each column, except where except when fewer individuals were successfully typed, in which case *N* is indicated by the number in superscript beside the first allelic frequency entry for a locus. The following loci were invariant: *Acoh-1*, *Acp*, *Ak-1*, *Ak-2*, *Ca*, *Est*, *Fbp*, *Fumh*, *Gda*, *Iddh*, *Idh-2*, *Lap*, *Ldh-1*, *Ldh-2*, *Mdh-1*, *Mdh-2*, *Ndpk*, *PepD*, *Pgam*, *Pgdh*, and *Pgk*. For *Aat-1*, *Lgl*, and *Mpi*, OTUs Wau-Aus had the *b*, *a* or *a* alleles, respectively and *M. spilota* had the *a*, *b* or *b* alleles, respectively. OTU codes are Wau, Nor, Normanby Island; Nam, Namosado; Doi, Doido; Nru, Noru; Aus, Queensland.

Murphy et al. (1996). Phylogenetic analysis was done using MP criterion of optimality, with loci as characters and alleles as unordered character states. Polymorphisms were treated as uncertainties following the recommendations of Kornet and Turner (1999). Trees were also constructed with NJ based on Cavalli-Sforza and Edwards (CSE) chord distances between OTUs (Cavalli-Sforza and Edwards, 1967).

3. Results

3.1. Mitochondrial DNA

Partial *cytb* and *CR* sequences from purified mitochondrial DNA were compared with sequences amplified from total cellular DNA for *M. viridis* AMS R115348. The two sequences for each gene were indistinguishable.

Partial sequences of the *cytb* and *CR* genes were obtained from one *M. spilota* and 36 *M. viridis* samples. In addition, four specimens, Aus15, Nru1, Mer1, and Jay1, were typed only for *cytb* and 14 specimens, Aus 3-14, Sor2/6, were typed only for the *CR*. Among the 18 individuals sequenced only for *cytb* or *CR*, there were no additional haplotypes that were not already observed among the 36 individuals that had been sequenced for both genes. From 40 individuals sequenced for *cytb* there were 14 haplotypes and from 50 individuals sequenced for *CR* there were 33 haplotypes indicating the higher level of variation observed in the *CR* data. Unique haplotypes were represented in the combined data that were used for the final analyses (Fig. 2).

Of 292 nucleotide sites of aligned *cytb* sequence, 64 were variable, and 42 were parsimony informative, while 786 nucleotide sites of *CR* sequence (47 bp of $tRNA^{Pro}$ and 739 bp of *control region*) had 243 variable sites, with 87 parsimony informative. The partition homogeneity test failed to detect significant incongruence between the two data partitions ($P = 0.61$), indicating that the two data sets could be combined for analysis. The combined data sets of 1078 aligned sites (307 variable, 129 parsimony informative) were analyzed by MP, NJ, and ML. For MP analysis, gaps were treated as a fifth character state. Of a total of 12 indels that were inferred in the alignment of the CR, all but one was a single site indel. Single site gaps can be treated as the equivalent of a fifth character state (Simmons and Ochoterena, 2000) while the only multi-site indel, two sites long, was autapomorphic. In the MP analysis, a heuristic search found 208 equally most parsimonious trees of length 342 steps. The model of nucleotide substitution found for the

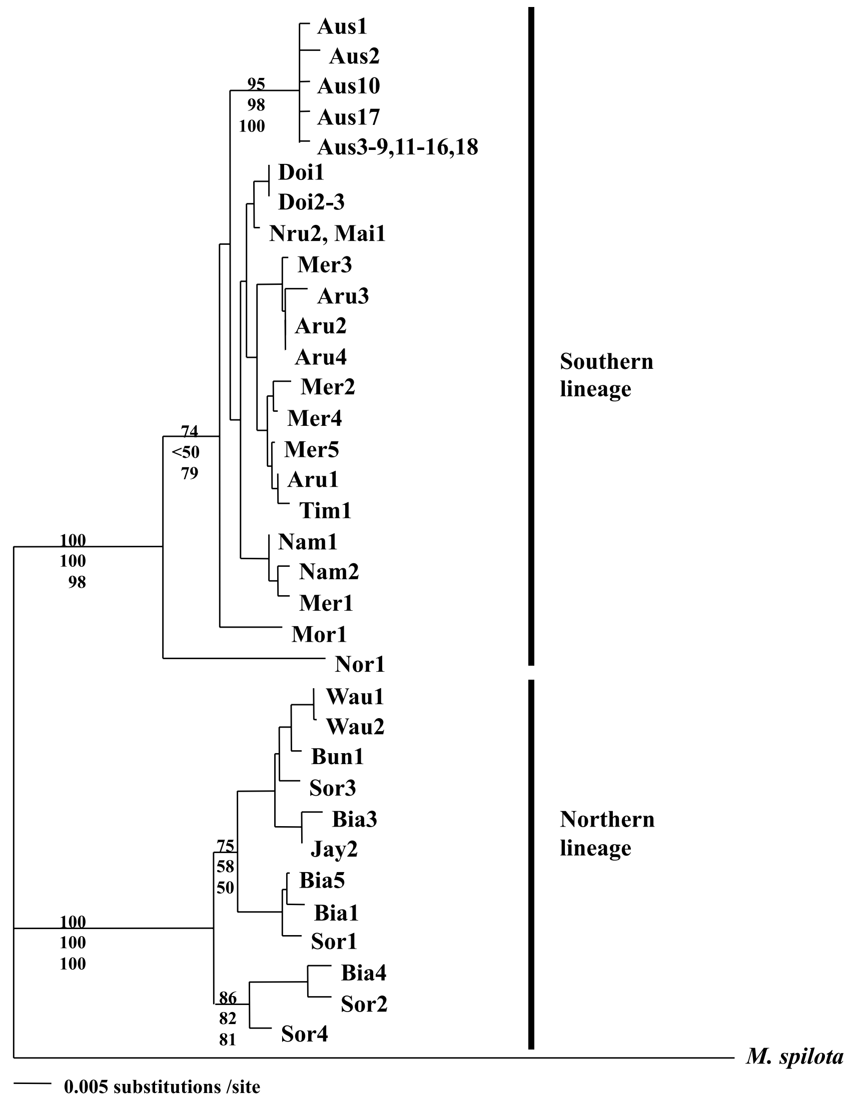

Fig. 2. Maximum likelihood phylogram of evolutionary relationships of Australian and New Guinea populations of *M. viridis*. Bootstrap pseudoreplicates proportions are indicated for MP, ML, and NJ analyses in descending order respectively.

combined data set was HKY85 + Γ (Hasegawa et al., 1985). Parameters, used in the ML analyses, estimated using successive approximations for this model for the combined data set were: nucleotide frequencies A = 0.271227, C = 0.287991, G = 0.131905, and T = 0.308877, ts/tv ratio = 2.3341, and $\Gamma = 0.2929$. Pairwise distances between haplotypes estimated with the HKY85 + Γ model were used for NJ analysis.

The ML tree is shown in Fig. 2 with bootstrap pseudoreplicate proportions for ML, MP, and NJ analyses indicated. Phylogenetic reconstructions for all three methods of analysis of mtDNA showed two monophyletic lineages supported by bootstrap pseudoreplicate proportions greater than 98%. One lineage, designated the southern lineage, comprised the southern New Guinea and Australian localities and the other, designated the northern lineage, comprised the northern localities. All three analyses also had the Australian specimens forming a monophyletic clade within the southern lineage with bootstrap pseudoreplicate support greater than 95%.

There was polyphyly of populations within the northern and southern lineages. Some of the western Sorong and Biak samples clustered with the eastern Wau and Bundi samples in the northern clade, and amongst the southern localities some samples from Merauke clustered with those from Aru Is., whilst other samples from Merauke grouped with individuals from Namasado. Relationships among haplotypes within the northern localities were well supported, but much of the population structure amongst the southern localities was not supported by bootstrapping in MP and ML analyses.

For the combined sequence data, there were four synapomorphies for the Australian clade—three transitions and an indel. One of the transitions was found in

the *cytb*, and one was in *tRNA^Pro^*. There was a C $\Longleftrightarrow$ T transition unique to the southern New Guinea individuals. There was also an A $\Longleftrightarrow$ G transition that was found in all the southern New Guinea animals except for the individual from Normanby Island (Nor1) which had an A at this site. Nor1 is the most divergent individual in the southern lineage, averaging more than 3% sequence divergence from all the other southern animals. Whilst this sequence has a character state that is synapomorphic for the southern lineage, it also shares five nucleotide substitutions that would otherwise be synapomorphies of the northern lineage, one of which is an indel. There were 57 synapomorphies for the northern lineage, (31 transitions, 23 transversions, and 3 indels) 16 of the substitutions were in *cytb* and one in the *tRNA^Pro^*. There was an average of 7.6% uncorrected sequence divergence between the northern and southern lineages (range 7.0–8.9%) and 1.5% divergence between Australia and the southern New Guinean haplotypes. In comparisons among haplotypes within each region, maximum uncorrected sequence divergence was 0.2% for Australia, 1.0% for southern New Guinea, and 1.4% for northern New Guinea.

3.2. Allozymes

Allele distributions at the 36 loci resolved are shown in Table 1. These data were converted into a matrix of CSE chord distances between OTUs and used to construct a tree by NJ. Twelve equally most-parsimonious trees were found using the branch and bound search method under the MP criterion of optimality. A strict consensus of these trees and the NJ tree both showed a dichotomy of OTU's representing the northern and southern mitochondrial lineages. Strong MP bootstrap support (84%) was evident for monophyly of the southern OTU's. Five loci, *Aat-2*, *Ada*, *PepB1*, *PepB2*, and *Pgm-2*, contribute most to the differentiation of the northern and southern lineages (Table 1). There was little differentiation within the southern lineage, with the Australian sample sharing alleles with the southern New Guinean localities at the 31 loci where the Australian OTU could be typed.

4. Discussion

4.1. Phylogeographical patterns and taxonomic implications

The discovery of two very distinct genetic lineages of green pythons from New Guinea and northeastern Australia begs the question as to whether the lineages represent two species of green pythons. The evolutionary species concept (Simpson, 1951; Wiley, 1978) has become a popular choice of species concept among investigators that use gene genealogies as part of the evidence to examine whether a group of organisms is "a phyletic lineage, i.e., an ancestral-descendent sequence of interbreeding populations, evolving independently of others, with its own separate and unitary evolutionary role and tendencies." Indeed de Queiroz's (1998) further development of this concept to the "generalised lineage concept" appears to offer the best combination of theoretical underpinning and operational criteria for delineating species at present. Herein, we utilise information from gene genealogies (monophyly of haplotypes representing the group) and the degree of sequence differentiation relative to other well accepted pairs of snake species to indicate the presence of evolutionary species. It is minimally desirable that a measure of differentiation of characters other than the mtDNA sequences be available, as even deeply bifurcated single gene genealogy may not represent population divergence but rather retention of ancestral gene lineages within a single population (e.g., Thomaz et al., 1996). We are unable to infer reliably the geographic distribution of the taxa that we have found as the green python has an apparently continuous distribution through New Guinea and our sampling in likely areas of contact is not adequate.

The pattern of relationships found for mitochondrial and nuclear genes suggests the presence of two species of *M. viridis*, one present north of the central cordillera and the other present in southern New Guinea and Australia. Phylogenetic analyses of the mitochondrial data shows that into two reciprocally monophyletic clades each with very strong bootstrap support. The minimum uncorrected sequence divergence between the northern and southern lineages (7.0%) is within the range of values for minimum mitochondrial sequence divergence between other closely related snake, 1.6–5.3% (Ashton and de Queiroz, 2001; Burbrink et al., 2001; Keogh et al., 2001; Zamudio and Greene, 1997) and reptile species, 2.5–18%, mean = 12% (Johns and Avise, 1998). The allozyme allelic data suggest that the divergence apparent between the two mitochondrial lineages is indicative of genome wide divergence. However the small sample size of the allozyme data from the northern lineage limits the strength of this indication. Morphological analyses do not provide any more substantive evidence of population differentiation. McDowell (1975) carried out a thorough assessment of variation in body meristics and pattern, maxillary tooth counts, and hemipenile morphology of green pythons from throughout their geographic range. Only body scale row counts were subject to geographic variation, but the pattern of variation was not partitioned across the central cordillera, indeed the full range of variation was present along the north of the island. Moreover McDowell (1975) did not identify any broad concordant geographic patterns across a number of characters that would suggest morphological differentiation between the northern and southern lineages. Simi-

larly variation in colour patterns described from living specimens due to the presence/absence of a vertebral stripe and/or scattered light coloured spots shows variation within and between localities without any clear geographical pattern (McDowell, 1975; O'Shea, 1996). A determination of the species status of the northern and southern lineages awaits a more thorough assessment of divergence at nuclear genes based on wider geographic sampling than we could achieve herein with allozymes.

Because our sampling of the eastern and western tips of New Guinea is sparse, we are not able to define the geographic limits of the northern and southern lineages. There are no obvious contemporary barriers that would maintain separate distributions for the northern and southern lineages. The lowland to mid-montane rainforest habitat of green pythons apparently forms a continuous "ring" around the central mountain range or cordillera of New Guinea (Johns, 1982; Pratt, 1982). The lowland rainforests at the western end of the central cordillera are extensive and apparently contiguous with similar tracts to the north and south of the central cordillera and indeed would be contiguous with similar habitats on the Vogelkop Peninsula. In the east, the lowland to mid-montane habitats of the green python are substantially constricted by the narrowing of the island and the presence of the high mountainous spine, but there is no evidence for exclusion of the species from this area as there are several records based on museum vouchers from the Milne Bay Province, the most easterly part of mainland New Guinea (McDowell, 1975). However, the east/west limits of the distribution of the two lineages may not necessarily be at the extreme ends of the central cordillera or the island. In the east, the Huon Peninsula region could be a candidate barrier to gene flow. Colgan et al. (1993) reported a possible zoogeographic barrier for lowland mammal species in the Huon Peninsula region and many lowland bird species which occur throughout the rest of New Guinea are not found in the lowlands of the Huon Gulf and Markham Valley even though rainforest habitat is present (Pratt, 1982). In the absence of physical barriers, competitive ecological exclusion may maintain separate contemporary distributions of evolutionary species that had their origins in the distant past.

Given the relatively high sequence divergence between the northern and southern lineages it is unlikely that the latest episodes of climate cycling of the Plio-Pleistocene would have been responsible for initiating the divergence. In any biogeographic analysis of the New Guinean biota throughout the Tertiary and Quaternary, the influence of two factors cannot be ignored: the substantial changes in the geomorphology of the area due to complex tectonism over the entire period (Dow, 1977; Pigram and Davies, 1987), and the dramatic climate oscillations of the late Tertiary and the Quaternary (Axelrod and Raven, 1982; Haig and Medd, 1996; Read and Hope, 1996). During this period, tectonic movements due to continental and island arc collisions have seen the formation and infilling of several major sedimentary basins to produce new lowland habitats and the rapid uplift of the central cordillera, the latter beginning approximately 5.8 MYA (Dow, 1977; Hill and Gleadow, 1989; Hill et al., 1993; Haig and Medd, 1996). The uplift of the cordillera has been episodic rather than a continuously gradual event, with an initial major burst of uplift at 5.8–5.3 MYA and less intense episodes of folding and thrusting spanning the period 5.3–4.7 MYA. The degree of sequence divergence is compatible with the uplift of the central mountain range through the Pliocene being the causative factor, but any further inference will require more accurate determinations of genetic divergence and also lineage specific calibration of a local molecular clock. Consistent with our observations, a species of bird (pitohui) from lowland New Guinean rainforests also shows a north–south divergence that is estimated from molecular clock methods at 3.5 MYA (Dumbacher and Fleischer, 2001).

The low magnitude of sequence divergence between haplotypes from southern New Guinea and Australia and indeed among the southern lineage haplotypes overall is consistent with their diversification during the late Tertiary or Quaternary. Through this period during times of lowered sea-level, a land bridge was exposed across the Torres Strait between Australia and New Guinea. Because of the shallow depth of the Torres Strait, a land bridge would have been periodically in existence over at the least the last 500,000 years (Galloway and Löffler, 1972) with the most recent marine incursion severing the connection starting approximately 8000 years ago. However, opportunities for rainforest dependent species to disperse south onto Cape York may have existed only sporadically during the late Tertiary and Quaternary, as the high rainfall/high humidity conditions of the Miocene gave way to an increasingly drier atmospheric regime (Nix and Kalma, 1972) resulting in the present-day distribution of remnant rainforest patches and intervening sclerophyll forests (Truswell, 1993). However fluctuating climatic conditions may have periodically modulated the extent of suitable rainforest on the land bridge and intervening Cape York region allowing dispersal (Nix and Kalma, 1972; Read and Hope, 1996). Moreover, extant Australian rainforests are similar structurally to the lower montane forests of New Guinea not to the complex lowland humid rainforests for which there is presently no northern Australian equivalent (Nix and Kalma, 1972). Whilst some rainforest species with New Guinean origins dispersed into northern Australia and continued southwards to the Atherton Tableland region (Winter,

1997), other rainforest dependent species, including *M. viridis,* extended their distributions no further south than the McIlwraith Range (~13°30′S) strengthening the argument that their present distribution was achieved during the latter part of the Tertiary or Quaternary when rainforest would not have been continuously distributed as far south as the Atherton Tableland rainforest block (~16°S).

4.2. Management issues

It is clear from the present study that the distribution of the green python should be considered to encompass at least three management units. Whilst it has not been directly tested, it is possible that the anecdotal reports of frequent lack of breeding success in captive green pythons could be due to pairing of individuals from the northern and southern lineages. In Australia, there are already many specimens in captivity that are descendants of New Guinean specimens and as there has also been interbreeding with Australian individuals, the captive population represents a mixed gene pool. Therefore, in order for Australian zoos to conform to the ZooTag recommendations to breed only Australian stock, the management of the captive breeding program will require genotyping of the present captive stock with biparentally inherited markers to detect individuals derived from crosses between Australian and New Guinean individuals. Mitochondrial markers would provide only a one-way test of the genetic origin of such individuals.

As poaching green pythons from the wild and illegal importation of specimens from New Guinea are major enforcement issues in Australia, the determination of distinct mitochondrial lineages from Australia, southern and northern New Guinea provides a basis for the enforcement of protective legislation. However, as sample sizes available to us from New Guinea were small, the haplotypic diversity present in each region needs further investigation before estimates of haplotype frequencies can be regarded as statistically robust for forensic applications.

Acknowledgments

We thank K. Aplin, S. Irwin, W. Mannion, J. Menzies, D. McCrae, S. Richards, D. Storch, T. Reardon, and F. Yuwono for supplying tissues, J. Armstrong for laboratory assistance and T. Bertozzi for assistance with the figures, S.N. Prijono and I. Sidik of the Bogor Museum and H. S. Basuki for assistance in Indonesia. Field work by LHR was supported by a D.R. Stranks Travelling Fellowship.

References

Ashton, K.G., de Queiroz, A., 2001. Molecular systematics of the western rattlesnake, *Crotalus viridis* (Viperidae), with comments on the utility of the D-Loop in phylogenetic studies of snakes. Mol. Phylogenet. Evol. 21, 176–189.

Axelrod, D.I., Raven, P.H., 1982. Paleobiogeography and origin of the New Guinea flora. In: Gressitt, J.L. (Ed.), Biogeography and Ecology of New Guinea. W. Junk, The Hague, pp. 919–941.

Banks, C., 1999. TAG Action Plan 1999. Directing the Management of Reptiles and Amphibians in Australasian Zoos. ARAZPA, Mosman, Sydney.

Barker, D.G., Barker, T.M., 1994. Pythons of the World: Australia. Advanced Vivarium Systems Inc, Lakeside, CA.

Burbrink, F., Lawson, R., Slowinski, J.B., 2001. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution 54, 2107–2118.

Cavalli-Sforza, L.L., Edwards, A.W.F., 1967. Phylogenetic analysis: models and estimation procedures. Evolution 32, 550–570.

Colgan, D., Flannery, T.F., Trimble, J., Aplin, K., 1993. Electrophoretic and morphological analysis of the systematics of the *Phalanger orientalis* (Marsupialia) species complex in Papua New Guinea and the Solomon Islands. Aust. J. Zool. 41, 355–378.

Creer, S., Malhotra, A., Thorpe, R.S., Chou, W.-H., 2001. Multiple causation of phylogeographical pattern as revealed by nested clade analysis of the bamboo viper (*Trimeresurus stejnegeri*) within Taiwan. Mol. Ecol. 10, 1967–1981.

de Queiroz, K., 1998. The general lineage concept of species, species criteria and the process of speciation. In: Howard, D.J., Berlocher, S.H. (Eds.), Endless Forms, Species and Speciation. Oxford University Press, Oxford, pp. 57–75.

Donnellan, S.C., Adams, M., Hutchinson, M., Baverstock, P.R., 1993. The identification of cryptic species in the Australian herpetofauna—a high research priority. In: Lunney, D., Ayers, D. (Eds.), Herpetology in Australia: A Diverse Discipline Transactions of the Royal Zoological Society of N.S.W. Special Edition. Surrey Beatty and Sons, Chipping Norton, pp. 121–126.

Donnellan, S.C., Hutchinson, M.N., Saint, K.M., 1999. Molecular evidence for the phylogeny of Australian gekkonoid lizards. Biol. J. Linn. Soc. 67, 97–118.

Dow, D.B., 1977. Geological synthesis of Papua New Guinea. Bureau Mineral Resources, Geol. Geophys. Bull. 201.

Dumbacher, J.P., Fleischer, R.C., 2001. Phylogenetic evidence for colour pattern convergence in toxic pitohuis: Müllerian mimicry in birds? Proc. R. Soc. London B 268, 1971–1976.

Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1995. Testing significance of incongruence. Cladistics 10, 315–319.

Galloway, R.W., Löffler, E., 1972. Aspects of geomorphology and soils in the Torres Strait region. In: Walker, D. (Ed.), Bridge and Barrier: the Natural and Cultural History of Torres Strait. Australian National University, Canberra, pp. 11–28.

Haig, D.W., Medd, D., 1996. Latest miocene to early pliocene bathymetric cycles related to tectonism, Puri anticline, Papuan basin, Papua New Guinea. Aust. J. Earth Sci. 43, 451–465.

Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22, 160–174.

Henderson, R.W., Hedges, S.B., 1995. Origin of west Indian populations of the geographically widespread Boa *Corallus enydris* inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 4, 88–92.

Hill, K.C., Gleadow, A.J.W., 1989. Uplift and thermal history of the Papuan Fold Belt, Papua New Guinea: Apatite fission track analysis. Aust. J. Earth Sci. 36, 515–539.

Hill, K.C., Grey, A., Foster, D., Barrett, R., 1993. An alternative model for the Oligo-Miocene evolution of northern PNG and the Sepik–Ramu basins. In: Carman, G.J., Carman, Z. (Eds.), Petroleum Exploration and Development in Papua New Guinea: Proceedings of the Second PNG Petroleum Convention, Port Moresby, pp. 241–259.

Hillis, D.M., Dixon, M.T., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Quart. Rev. Biol. 66, 411–453.

Johns, R.J., 1982. Plant zonation. In: Gressitt, J.L (Ed.), Monographiae Biologiae, vol. 42. W. Junk, The Hague, pp. 309–330.

Johns, G.C., Avise, J.C., 1998. A comparative summary of genetic distances in vertebrates from the mitochondrial cytochrome *b* gene. Mol. Biol. Evol. 15, 1481–1490.

Keogh, J.S., Barker, D., Shine, R., 2001. Heavily exploited but poorly known: systematics and biogeography of commercially harvested pythons (*Python curtus* group) in southeast Asia. Biol. J. Linn. Soc. 73, 113–129.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.

Kornet, D J, Turner, H., 1999. Coding polymorphism for phylogeny reconstruction. Syst. Biol. 48, 365–379.

Kumazawa, Y., Nishida, M., 1996. Variations in mitochondrial tRNA gene organization of reptiles as phylogenetic markers. Mol. Biol. Evol. 12, 759–772.

Kumazawa, Y., Ota, H., Nishida, M., Ozawa, T., 1996. Gene rearrangements in snake mitochondrial genomes: highly concerted evolution of control region-like sequences duplicated and inserted into a tRNA gene cluster. Mol. Biol. Evol. 13, 1242–1254.

Kumazawa, Y., Ota, H., Nishida, M., Ozawa, T., 1998. The complete nucleotide sequence of a snake (*Dinodon simicarinatus*) mitochondrial genome with two identical control regions. Genetics 150, 313–329.

McDowell, D., 1997. Wildlife Crime Policy and the Law An Australian Study. Australian Government Publishing Service, Canberra, Australia.

McDowell, S.B., 1975. A catalogue of the snakes of New Guinea and the Solomons, with special reference to those in the Bernice P. Bishop Museum. Part II. Aniliodea and Pythoninae. J. Herp. 9, 1–79.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. Simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.

Murphy, R.W., Sites, J.W., Buth, D., Haufler, C.H., 1996. Proteins I: Isozyme electrophoresis. In: Hillis, D.M., Moritz, C., Mable, B. (Eds.), Molecular Systematics. Sinauer, Sunderland, MA, pp. 51–120.

Nix, H.A., Kalma, J.D., 1972. Climate as a dominant control in the biogeography of northern Australia and New Guinea. In: Walker, D. (Ed.), Bridge and Barrier: the Natural and Cultural History of Torres Strait. Australian National University, Canberra, pp. 61–92.

O'Shea, M., 1996. A Guide to the Snakes of Papua New Guinea. Independent Publishing, Independent Group Pty Ltd., Port Moresby, PNG.

Parker, F., 1982. The Snakes of the Western Province. PNG Dept. of Lands and Environment 82/1.

Pigram, C.J., Davies, H.L., 1987. Terranes and the accretion history of the New Guinea orogen. BMR J. Aust. Geol. Geophys. 10, 193–211.

Posada, D., Crandell, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Pratt, T.K., 1982. Biogeography of birds in New Guinea. In: Gressit, J.L. (Ed.), Monographieae Biologicae, vol. 42. W. Junk, The Hague, pp. 815–836.

Read, J., Hope, G., 1996. Ecology of *Nothofagus* forests of New Guinea and New Caledonia. In: Veblen, T.T., Hill, R.S., Read, J. (Eds.), The Ecology and Biogeography of Nothofagus Forests. Yale University, pp. 200–256.

Richardson, B.J., Baverstock, P.R., Adams, M., 1986. Allozyme Electrophoresis: A Handbook for Animal Systematics and Population Structure. Academic Press, Sydney.

Rodriguez-Robles, J.A., De Jesus-Escobar, J.M., 2000. Molecular systematics of New World gopher, bull, and pinesnakes (*Pituophis*: Colubridae), a transcontinental species complex. Mol. Phylogenet. Evol. 14, 35–50.

Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49, 369–381.

Simpson, G.G., 1951. The species concept. Evolution 5, 285–298.

Strimmer, K., Vonhaeseler, A., 1996. Quartet puzzling—a quartet maximum-likelihood method for reconstructing tree topologies. Mol. Biol. Evol. 13, 964–969.

Swofford, D., 1999. PAUP*. Phylogenetic Analysis Using Parsimony. (* and Other Methods). Sinauer, Sunderland, MA.

Thomaz, D., Guiller, A., Clarke, B., 1996. Extreme divergence of mitochondrial DNA within species of pulmonate land snails. Proc. R. Soc. London Ser. B 263, 363–368.

Truswell, E.M., 1993. Vegetation changes in the Australian Tertiary in response to climatic and phytogeographic forcing factors. Aust. Syst. Bot. 6, 533–557.

Wiley, E.O., 1978. The evolutionary species concept reconsidered. Syst. Zool. 27, 17–26.

Winter, J.W., 1997. Responses of non-volant mammals to late quaternary climatic changes in the wet tropics region of north-eastern Australia. Wild Res. 24, 493–511.

Zamudio, K.R., Greene, H.W., 1997. Phylogeography of the bushmaster (*Lachesis muta*, Viperidae)—Implications for neotropical biogeography, systematics, and conservation. Biol. J. Linn. Soc. 62, 421–442.

Zhang, D.X., Hewitt, G.M., 1996. Nuclear integrations: challenges for mitochondrial DNA markers. TREE 11, 247–251.