Diversity and phylogeny of New Caledonian *Placostylus* land snails; evidence from mitochondrial DNA

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ABSTRACT

Placostylus is a genus of large terrestrial pulmonate snails distributed in the western Pacific, with endemic species in New Zealand, Solomon Islands, Fiji, New Caledonia, Vanuatu, Papua New Guinea and Lord Howe Island. In New Caledonia, as elsewhere, the taxonomy of *Placostylus* has been poorly resolved with more than 100 species being described in the 1900's. Recent revisions have indicated that despite considerable variance in shell morphology among populations, the New Caledonian fauna consists of far fewer species (< 10). All are threatened to a greater or lesser extent by habitat loss and predation by introduced mammals and humans. Two species in particular remain important food sources for local people, *P. fibratus* and *P. porphyrostomus*, and these are the subject of intense research focussed at conserving the taxa and maintaining a traditional food source via culturing and protection. Even within these two taxa substantial morphological variation exists among populations. We used mitochondrial DNA sequence to survey diversity and phylogeny within these two species across their geographic range, and to elucidate their relationship to other *Placostylus* in New Caledonia and beyond. We find evidence of three clades among these two species with *P. porphyrostomus* being paraphyletic with respect to *P. fibratus*. We also find evidence of recent interspecific hybridisation in the form of haplotype sharing. Movement of snails by people might explain this pattern of exchange. Comparison of sequence data from New Caledonian *Placostylus* and representatives of the genus from New Zealand and Lord Howe Island, indicate that the New Caledonian radiation may have originated by dispersal from these southern locations.

RÉSUMÉ

Diversité et phylogénie des escargots terrestres Placostylus de Nouvelle-Calédonie : apports de l'ADN mitochondrial.
Placostylus est un genre d'escargots pulmonés terrestres distribués dans le Pacifique ouest, avec des espèces endémiques de Nouvelle-Zélande, des Îles Salomon, des Fiji, de la Nouvelle-Calédonie, des Vanuatu, de la Papouasie Nouvelle-Guinée et de l'Île de Lord Howe.

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En Nouvelle-Calédonie, comme ailleurs, la taxonomie de *Placostylus* a été pauvrement traitée avec plus de 100 espèces décrites au siècle dernier. Des révisions récentes indiquent qu'en dépit d'une variation considérable dans la morphologie de la coquille entre les populations, la faune de Nouvelle-Calédonie comprend en fait beaucoup moins d'espèces (<10). Toutes sont mises en danger à des degrés variables par la destruction des habitats, la prédation par les mammifères introduits et les hommes. Deux espèces en particulier, *P. fibratus* et *P. porphyrostomus*, restent une source importante de nourriture pour les populations humaines locales et elles sont le sujet d'une recherche intense en biologie de la conservation pour maintenir leur qualité de nourriture traditionnelle via des élevages tout en assurant leur protection. Même entre ces deux taxons, une variation morphologique non négligeable est observée entre les populations. Nous avons utilisé des séquences d'ADN mitochondrial pour examiner la diversité et la phylogénie au sein de ces deux espèces à travers leur aire de répartition géographique, et leur relation aux autres *Placostylus* en Nouvelle-Calédonie et au-delà. Trois clades ont été mis en évidence au sein de ces deux espèces, avec *P. porphyrostomus* qui est paraphylétique par rapport à *P. fibratus*. La répartition des haplotypes plaide aussi pour l'existence d'une hybridation récente interspécifique. Un déplacement des escargots par les populations humaines pourrait expliquer ce résultat. La comparaison de séquences de *Placostylus* néo-calédoniens et de représentants du genre en Nouvelle-Zélande et sur l'Île de Lord Howe, indique que la radiation néo-calédonienne a pu trouver son origine par dispersion depuis ces localités du sud.

INTRODUCTION

Pulmonate land snails of the genus *Placostylus* are found only in the Western Pacific, in northern New Zealand and on islands between New Zealand and Melanesia (Solomon Islands, Fiji, New Caledonia, Vanuatu, Papua New Guinea and Lord Howe) (Gaskoin 1855; Abbott 1989; Parrish *et al.*1995; Ponder & Chapman 1999; Ponder *et al.* 2003), a distribution that is seen to be consistent with an ancient Gondwana origin (Hedley 1893; Stanisic 1981).

The taxonomy of the genus *Placostylus* is unstable due largely to variation in shell morphology exhibited by populations that has resulted in the description of a great many distinct taxa. This is very probably misrepresentative of the true diversity, with much of this variation tending to be homoplastic. Indeed, the shell morphology of some of the New Caledonian taxa is very similar to that of Lord Howe Island and the New Zealand taxa (Suter 1916; Ponder *et al.* 2003). The taxonomy of New Caledonian *Placostylus* was revised by Chérel-Mora (1983), using mainly soft tissue anatomy, who proposed a radical reorganisation resulting in a reduction of New Caledonian *Placostylus* species from the ~140 described during last century to just four species. More recently Neubert (2001) has re-appraised Chérel-Mora's work in light of more extensive sampling, and confirmed her hypothesis with respect to the fauna of the southern half of New Caledonia and the Isle of Pines, but came to a different conclusion for the North (Bouchet pers com. 2006). Collection of further northern specimens, including a variety of morphotypes, led to the recognition of 6 species and approximately 20 geographic subspecies (Neubert 2001, and in prep.). The four species recognised by Chérel-Mora (1983) are *P. fibratus* (Martyn, 1789), *P. porphyrostomus* (Pfeffer, 1851), *P. caledonicus* (Petit, 1845) and *P. eddystonensis* (Pfeffer, 1855). All are endemic and more or less endangered. These species show considerable geographic variation affecting shell and anatomic characters, with rainfall, humidity, altitude, and population density affecting population morphotypes (Chérel-Mora 1983).

These large (5-9cm in length) land snails are slow moving and slow developing, with *P. fibratus* maturing at approximately 3-5 years and living up to 20 years (Salas *et al.* 1997). They are mainly ground dwelling where they remain concealed in leaf litter, and have limited home ranges which makes them highly susceptible to predation by humans and animals, as well as habitat loss caused by deforestation and changing climates (Triggs & Sherley 1993; Salas *et al.* 1997; Sherley & Parish 1989).

The biology and ecology of the two most common New Caledonian *Placostylus* have been the subject of scientific research for the last few years. *P. fibratus,* the type species of the genus, is the most morphologically variable, and it is found throughout New Caledonia, including the Loyalty Islands where a dwarf form occurs (Chérel-Mora 1983). The species is an important food source for local people, but the only snails to be marketed for consumption are those from the Isle of Pines, a coral island at the South of the archipelago. In 1993 the harvest reached 48 tonnes (about

700,000 snails), generating an economic activity assessed at $180,000 US (Salas *et al.* 1997; Poellabauer 1995). Since 1993 a number of control measures have been imposed by local authorities, including restriction of marketing to the Isle of Pines and a ban on exportation towards Grande Terre. The number of snails harvested for consumption from the natural populations now remains constant at about 120,000 (Brescia 2005). Though harvesting is now being restricted, a field survey conducted from 1993-1999 showed a 30% reduction in *P. fibratus* numbers on the Isle of Pines during that time (Poellabauer 2002).

The second, relatively common species *Placostylus porphyrostomus* inhabits sclerophyllous forests that develop in areas with low rainfall (< 1100mm per year). The sclerophyllous forest is currently the most threatened of the New Caledonian biomes with only 1% (45 km^2) of the original area remaining. *Placostylus* snail populations within these forests are under severe threat from habitat destruction and modification by the introduced rusa deer *(Cervus timorensis russa),* and predation by rodents and pigs (*Sus scrofa)* (Brescia & Poellabauer 2004, 2005). Other species of *Placostylus*, *P. eddystonensis* and *P. caledonicus*, are rare and restricted to localised habitats in northern Grande Terre (Brescia pers. obs.).

Current research on the conservation of New Caledonia *Placostylus* is directed at producing young on mass for release into the forest to restock local populations. The development of a farming method is currently being explored with success, and attempts to preserve natural stock using captive bred snails to supplement small populations have been initiated (Brescia 2000, 2005). The focal species are *P. porphyrostomus* and *P. fibratus*. The preservation of genetic diversity is an important component of the conservation programme for a species (Frankel & Soule 1981). Successful conservation of *Placostylus* depends on understanding the ecology of the snails and this requires identification of distinct taxa (or genetically independent subgroups) so that the range of genetic variation within a species and the distribution of that variation among populations can be maintained (Triggs & Sherley 1993).

Characters such as shell colour, size, banding patters, whorl expansion rate, aperture size, reproductive organs and presence or absence of an umbilicus are commonly used to describe variation among land snails. Studies of several families of pulmonate gastropods (Bradybaenidae; Clausiliidae; Bulimulidae) indicate that variation of these kinds of morphological traits can be the result of developmental plasticity and not just population history (Chérel-Mora 1983; Engelhard & Silk 1994; Teshima *et al.* 2003; Triggs & Sherley 1993; Welter-Schultes 2000).

Where morphological features fail to give clear information about relationships, molecular techniques have proved invaluable (Wade *et al.* 2001). The development of molecular genetic techniques such as allozyme electrophoresis, PCR and genomic sequencing, has allowed important advances in the analysis of genetic variation, as differences resolved by molecular techniques almost always represent simple heritable variation.

In this paper we surveyed the extent and distribution of mitochondrial sequence variation in the two most abundant New Caledonian species, *P. porphyrostomus* and *P. fibratus* in order to address their taxonomic status and provide a measure of genetic diversity within and between populations for conservation purposes.

MATERIALS AND METHODS

BIOLOGICAL MATERIAL

Specimens of *Placostylus fibratus, P. porphyrostomus* and *P. caledonicus* were collected by hand on Grande Terre, Ouvéa (Loyalty Islands), and the Isle of Pines, New Caledonia (Fig. 1). Muscle tissue from the specimens was preserved in 95% ethanol and stored at 4°C. DNA sequences for potential outgroup taxa were obtained from Genbank (*P. bivaricosus* AY165838, *P. ambagiosus* AY148560, *P. hongii hongii* AY290744, *P. annectens watti* AY290740, *P. bollonsi arbutus* AY290741). The non-placostylid helicoidal pulmonate outgroup taxa were *Euhadra herklotsi* (Z71701) and *Albinaria caerulea* (NC_001761).

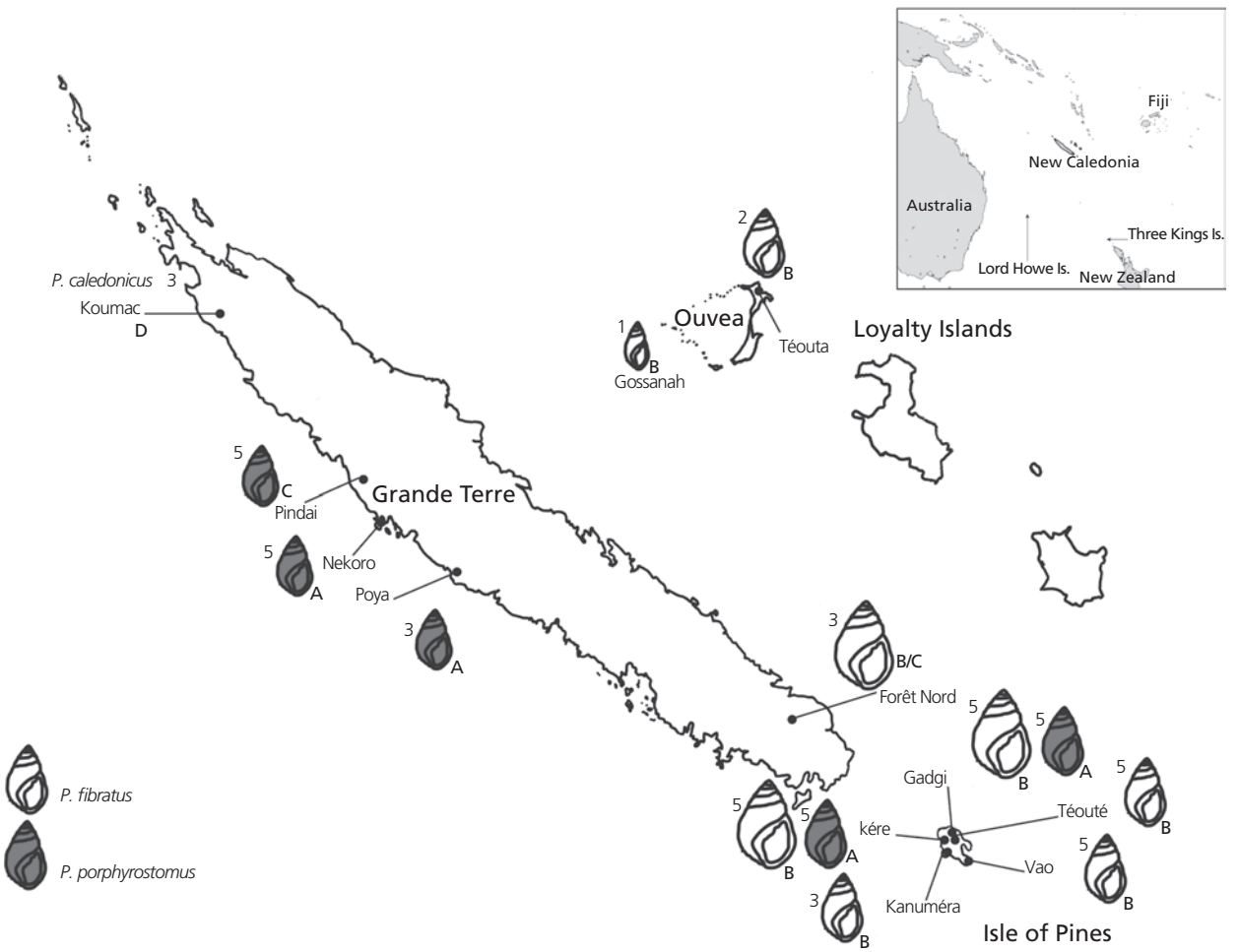

FIG. 1. Location of sampling sites on New Caledonia showing species location & sample numbers. The dominant COI clade (A-D) is indicated for each species location combination are also indicated.

EXTRACTION, AMPLIFICATION AND SEQUENCING OF DNA

Genomic DNA was extracted from sixty one specimens using incubation at 55ºC with Proteinase K and a CTAB buffer (2% Hexadecyltrimethylammonium bromide, 100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA), followed by a combined phenol-chloroform-isoamyl alcohol (25: 24: 1) cleanup as described by (Thomaz *et al.* 1996; Stine 1989; Terrett 1992). Extracted DNA was re-suspended in TE buffer (10 mM Tris, 0.1 mM EDTA) and the quality and quantity checked using a NanoDrop® ND-1000 (NanoDrop Technologies) and 1% agarose gel electrophoresis. Several primer combinations were trialled but the most reliable amplification over all samples was obtained using two pairs of primers that target parts of the Cytochrome oxidase subunit I gene (COI) and the large ribosomal subunit rRNA gene (16S). These primers were 1490-2198 for COI (Folmer *et al.* 1994), and LR-J-12887 and LR-N-13398 for 16S (Simon *et al.* 1994). The COI fragment used has been effectively applied to intra- and interspecies studies of a wide range of invertebrate taxa (Lunt *et al.* 1996; Simon *et al.* 1994), including molluscs (Holland *et al.* 2002; Ponder *et al.* 2003; Rundell *et al.* 2004; Simison & Lindberg 1999; Spencer *et al.* 2006; Trewick *et al.* 2008). Similarly, the 16S fragment has proved informative in species level studies of insects and molluscs (Simon *et al.* 1994; Thomaz *et al.*

1996; Wade *et al.* 2001). PCR reactions were performed in 10 µl volumes with Red Hot ® Taq polymerase (ABgene) using the Biometra T1 thermocycler, under the cycling conditions: initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 45 sec, with a final extension at 72°C for 3 min. PCR yielded COI and 16S fragments of approximately 650 bp and 425 bp respectively. PCR products were purified using SAP/EXO (Shrimp Alkaline Phosphatase/Exonuclease) enzymatic digest (USB corporation), and cycle sequenced with the forward primers using Perkin Elmer Bigdye ® chemistry following the manufacturer's protocols, and read on a Prism 377 DNA capillary sequencer (Applied Biosystems, Inc., Foster City, California).

SEQUENCE ANALYSIS

Sequences were checked against the ABI trace file and aligned manually using SeqEd v1.0.3 (Applied Biosystems, Inc., Foster City, California) and Sequencher v4.1 (Applied Biosystems, Inc., Foster City, California) and aligned by eye in SeAl v2.0a11 (Rambaut 1996). The nucleotide sequence for COI was translated to check for the presence of stop codons, frame shifts, or unexpected mutations that might indicate the presence of nuclear copies (Blanchard & Lynch 2000; Benasson *et al.* 2001).

PHYLOGENETIC ANALYSIS

We used PAUP* 4.0b10 (Swofford 2002) to implement phylogenetic analyses using Neighbour Joining (NJ), Maximum Parsimony (MP), and Maximum Likelihood (ML) criteria, and MrBayes v3.1.2. (Huelsenbeck & Ronquist 2001) for Bayesian analysis. To select the most appropriate model of DNA evolution for the ML and NJ analyses we used Modeltest version 3.06 (Posada & Crandall 1998). Modeltest implements a hierarchical choice test, based on log likelihood scores, in which permutations of alternative nucleotide substitution and among-site rate variation models (I- invariable sites, and **Γ**- gamma distribution) are assessed by comparing likelihood scores for a suite of 56 models.

For each gene-taxon data combination we undertook unweighted MP analysis, ML, and NJ analyses using the models, Trn + I and GTR + I + **Γ** selected using the AIC and hLRT choice tests respectively using Modeltest 3.06. However, for the 16S data and the New Caledonian COI data we observed that some substitution types (*e.g.,* T-G) did not occur among the ingroup sequences. We therefore also applied a less parameter-rich model (HKY85) in ML and NJ analyses of these data. For the ML and NJ analysis we re-estimated base composition and rate matrices for each combination of sequences, and for the NJ analysis we also used the Kimura-2 parameter model (K2P) for estimation of pairwise genetic distances. Overall we analysed four data sets: a reduced COI haplotype data set with outgroups, all New Caledonian individuals for COI, New Caledonian COI haplotypes only, and all New Caledonian 16S haplotypes. Bootstrap support (Felsenstein 1985) for each node was assessed using 500 replicates in ML and 1000 replicates in MP and NJ trees. Gaps in 16S sequence alignments were treated as missing data.

As noted above, although all classes of substitutions could be inferred in comparisons of the full taxon set for COI data (*i.e.* sequences from New Caledonian, New Zealand and Lord Howe *Placostylus*, plus outgroup taxa), comparisons of sequences from New Caledonian taxa alone indicated that not all substitution classes were represented. Therefore, modelling of GTR rates might be inappropriate for these data. Thus, in Bayesian analysis using MrBayes v3.1.2. (Huelsenbeck & Ronquist 2001) we ran analyses under two different models: a GTR model with 6 substitution types ("nst = 6") and rate variation across sites modelled using a gamma distribution with a proportion of sites being invariant ("rates = invgamma") (*i.e.* GTR + I + **Γ**), and an HKY model with two substitution types ("nst = 2"). The Markov-chain Monte-Carlo search comprised two independent, simultaneous runs with three heated chains. MCMC was run for 7,000,000 or 10,000,000 generations with trees sampled every 100 generations. Approximately the first 20% of trees were discarded as "burnin" following examination of the distributions of tree likelihood scores.

Pairwise genetic distances for COI were calculated using K2P and TrN + I models using PAUP*, and within and between clade distances were compared (Table 2; Appendix 1).

TABLE 1. Summary of New Caledonian *Placostylus* sampled at each location with its respective COI haplotype.

REGION	SITE	SPECIES	n	A1	A2	A3	A4	A5	A6	A7	B1	B2	B3	B4	B5	B6	B7	B8
Grande-Terre	Foret Nord	*P. fibratus* (giant)	3															
Grande-Terre	Pindaï	*P. porphyrostomus*	5															
Grande-Terre	Nékoro	*P. porphyrostomus*	5					1		4								
Grande-Terre	Poya	*P. porphyrostomus*	3						3									
Grande-Terre	Koumac	*P. caledonicus*	3															
Isle of Pines	Kere	*P. fibratus* (giant)	5															
Isle of Pines	Kere	*P. porphyrostomus*	5	2	2		1											
Isle of Pines	Gadgi	*P. fibratus* (giant)	5													1	2	
Isle of Pines	Gadgi	*P. porphyrostomus*	5			3	1											1
Isle of Pines	Kanumera	*P. fibratus*	3		2						1							
Isle of Pines	Touete	*P. fibratus*	5									1	1	1				
Isle of Pines	Vao	*P. fibratus*	5												1			
Ouvea	Gossanah	*P. fibratus* (dwarf)	1															
Ouvea	Teouta	*P. fibratus*	2															
	Total	*P. fibratus*	29		x						x	x	x	x	x	x	x	
	Total	*P. porphyrostomus*	23	x	x	x	x	x	x	x								x
	Total	*P. caledonicus*	3															
	TOTAL		55	2	4	3	2	1	3	4	1	1	1	1	1	1	2	1

REGION	SITE	SPECIES	n	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	C1	C2	C3	D1	D2
Grande-Terre	Foret Nord	*P. fibratus* (giant)	3										1	2				
Grande-Terre	Pindaï	*P. porphyrostomus*	5											2	2	1		
Grande-Terre	Nékoro	*P. porphyrostomus*	5															
Grande-Terre	Poya	*P. porphyrostomus*	3															
Grande-Terre	Koumac	*P. caledonicus*	3														1	2
Isle of Pines	Kere	*P. fibratus* (giant)	5	1	2					2								
Isle of Pines	Kere	*P. porphyrostomus*	5															
Isle of Pines	Gadgi	*P. fibratus* (giant)	5					1	1									
Isle of Pines	Gadgi	*P. porphyrostomus*	5															
Isle of Pines	Kanumera	*P. fibratus*	3															
Isle of Pines	Touete	*P. fibratus*	5			1	1											
Isle of Pines	Vao	*P. fibratus*	5			2			2									
Ouvea	Gossanah	*P. fibratus* (dwarf)	1								1							
Ouvea	Teouta	*P. fibratus*	2									2						
	Total	*P. fibratus*	29	x	x	x	x	x	x	x	x	x	x	x				
	Total	*P. porphyrostomus*	23											x	x	x		
	Total	*P. caledonicus*	3														x	x
	TOTAL		55	1	2	3	1	1	3	2	1	2	1	4	2	1	1	2

RESULTS

SEQUENCE DIVERSITY

We analysed aligned, unambiguous sequences of 618 bp and 420 bp respectively for COI and 16S from a total of 55 individuals of three species. Analysis of COI base composition gave nucleotide frequencies of A = 0.31, C = 0.11, G = 0.13, T = 0.44. Translation of COI nucleotide sequence to amino acid sequence revealed little variation and no frame shifts or stop codons that might indicate the presence of nuclear copies. However, among the New Caledonian sequences, a serine/alanine substitution distinguished the clade D haplotypes (*P. caledonicus*) from all others, and an isoleucine/valine substitution distinguished the clade C and D haplotypes from the rest. 16S nucleotide frequencies were A = 0.36, C = 0.16, G = 0.11, T = 0.36. Overall AT composition averaged 73% for these mitochondrial DNA sequences.

Among the 55 snails sequenced for part of the COI gene we found 30 haplotypes (A1-7, B1-18, C1-3 & D1-2). 25 snails sequenced for 16S yielded 18 haplotypes. Two COI haplotypes (D1-D2) were found only in the three individuals of *P. caledonicus* amplified. The A2 haplotype was found in two individuals of *P. fibratus* and two of *P. porphyrostomus* from the Isle of Pines but at different locations (Table 1). The C1 haplotype was found in four *P. fibratus* individuals from Forêt Nord and one *P. porphyrostomus* individual from Pindaï, both sites on Grande Terre. Haplotype sharing was absent between individuals of giant morphotype *P. fibratus* from different geographical locations (Gadgi, Kere, Forêt Nord). The remaining haplotypes, including those from the Ouvéa sample were unique, but the phylogenetic relationship among these revealed an interesting structure.

PHYLOGENETICS

Overall we found a high degree of congruence, with good support for overall tree topology, clade structure, and composition, among trees yielded by different methods of phylogenetic reconstruction, models of DNA evolution, and data sets. In all analyses the sequences from New Caledonia *Placostylus* were monophyletic with respect to the outgroup taxa employed (*Placostylus* from New Zealand and Lord Howe Island) (Fig. 2). Bayesian analysis of the COI fragment using non-placostylid helicoidal pulmonates *Euhadra herklotsi* and *Albinaria caerulea* revealed a topology in which the New Caledonian clade is nested within the New Zealand and Lord Howe *Placostylus* (Fig. 2b).

The same four New Caledonian clades (A-D) were revealed in analyses with and without outgroup taxa (Figs 2, 3). From the COI and 16S data we infer a single *P. caledonicus* clade (D) comprising unique haplotypes from Koumac which is basal to the other New Caledonian sequences obtained (Figs 1, 2). Clade A is dominated by sequences from *P. porphyrostomus* collected on the Isle of Pines plus Nékora and Poya in Grand Terre. Clade B comprises sequences predominantly from individuals of *P. fibratus* collected on the Isle of Pines and Ouvéa. The fourth clade (C) comprises haplotypes found in individuals of *P. porphyrostomus* from Pindaï on Grande Terre (but see below) (Figs 3, 4).

Clades A, B and C all include sequences found in more than one morpho-species. Clade A includes *P. porphyrostomus* from Gadgi and Kere on the Isle of Pines, and Nékora and Poya from the northern-central part of Grande Terre. Haplotype A2 is shared by two individuals of *P. porphyrostomus* from Kere and of two *P. fibratus* from Kanuméra, neighbouring locations on the Island of Pines (Fig. 3). 16S sequences from the same individuals differ by only one nucleotide. Clade B comprises *P. fibratus* from the Isle of Pines, and sister to these, *P. fibratus* from Ouvéa, plus one haplotype (B8) from a *P. porphyrostomus* morphotype from Gadgi (Fig. 3). The 16S sequence from this individual is identical to that from several *P. fibratus* in this clade. Clade C in the COI trees includes haplotypes from Pindaï (Grande Terre) *P. porphyrostomus*, and Forêt Nord (Grande Terre) *P. fibratus*, although this pattern is not evident in the 16S data.

Overall clade structure, and sharing of similar or identical haplotypes by snails of different shape and taxon, suggests recent interspecies mating (introgression) and this is supported by data from both genes. Moreover, at least one snail sampled had more than one haplotype for a single gene. Individuals PS53 and PS56 (*P. fibratus* from

Forêt Nord) are placed differently in the COI and 16S trees (Fig. 4). This anomaly was examined further using repeat DNA extraction and sequencing from these individuals. Phylogenetic analysis of 16S sequence consistently places these two individuals with other clade B *P. fibratus*, as might be expected. A similar result was obtained in analysis of COI with one sequence from PS53, but the other COI sequences obtained from PS53 and PS56 are placed withn clade C (Figs 3, 4). Indeed, PS53 and PS56 (*P. fibratus* from Forêt Nord, Grande Terre) had the same COI haplotypes as individuals of *P. porphyrostomus* from Pindaï (Grande Terre). Repeated DNA extraction, PCR and sequencing failed to reveal more than one 16S haplotype for these samples, but confirmed that the PS53 sample contained copies of two different COI haplotypes (Fig. 4). The different phylogenetic position of COI and 16S sequences from these individuals could be the product of differential PCR bias in the amplification of these genes from alternative mitochondrial genomes, which indicates that this snail at least (PS53) is heteroplasmic (Passamonti *et al.* 2003; Quesada *et al.* 2003). An alternative explanation is that one lineage is in fact a nuclear homologue, but there are no stops, frame-shifts or apparent inconsistencies in substitution pattern that might indicate this. Furthermore, the none of these sequences is of the "fossilised" form typical of nuclear copies and some haplotypes are identical to those in other taxa.

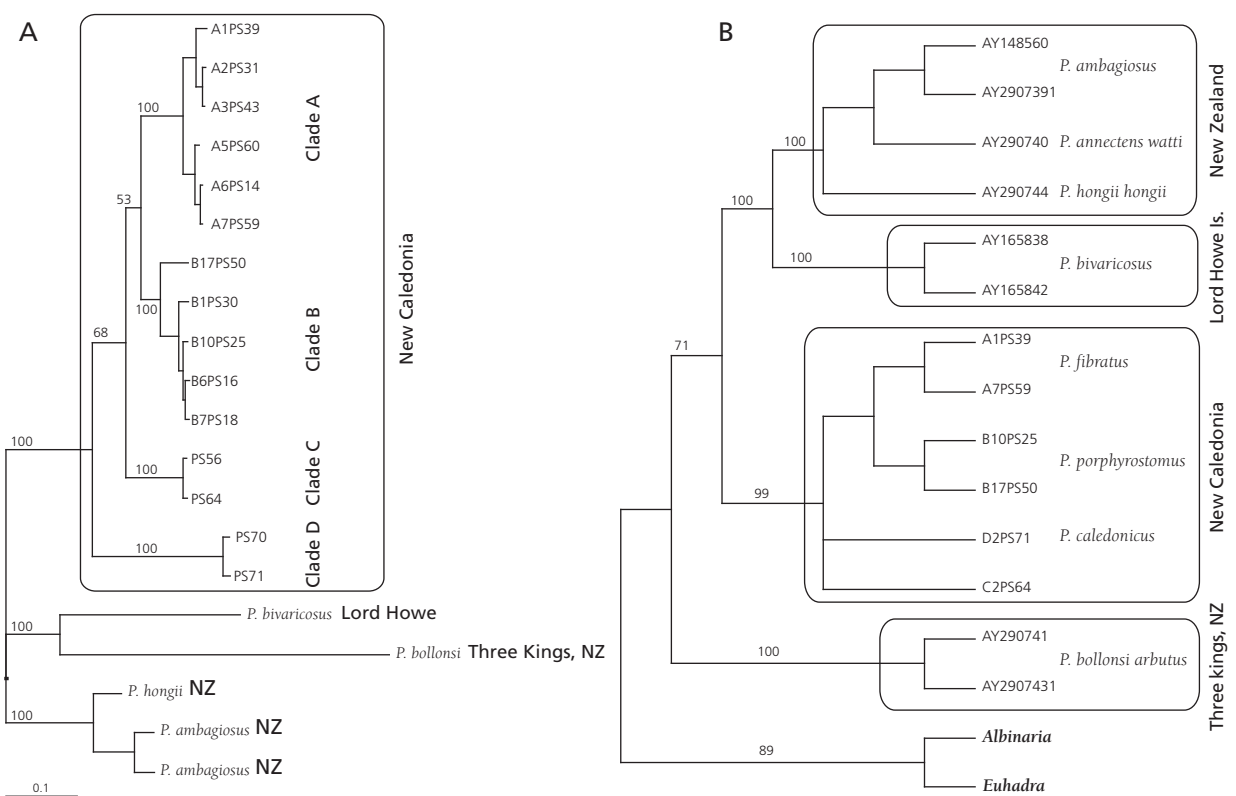

FIG. 2. Bayesian analysis of representative COI haplotypes from New Caledonian and outgroup *Placostylus*. Values at nodes are Bayesian posterior probabilities. (a), results of MCMC analysis with ten million generations with non-New Caledonian *Placostylus* outgroups; (b), results of MCMC analysis with seven million generations on reduced sample of sequences representing New Caledonian *Placostylus*, Lord Howe and New Zealand *Placostylus*, and two other helicoidean pulmonates.

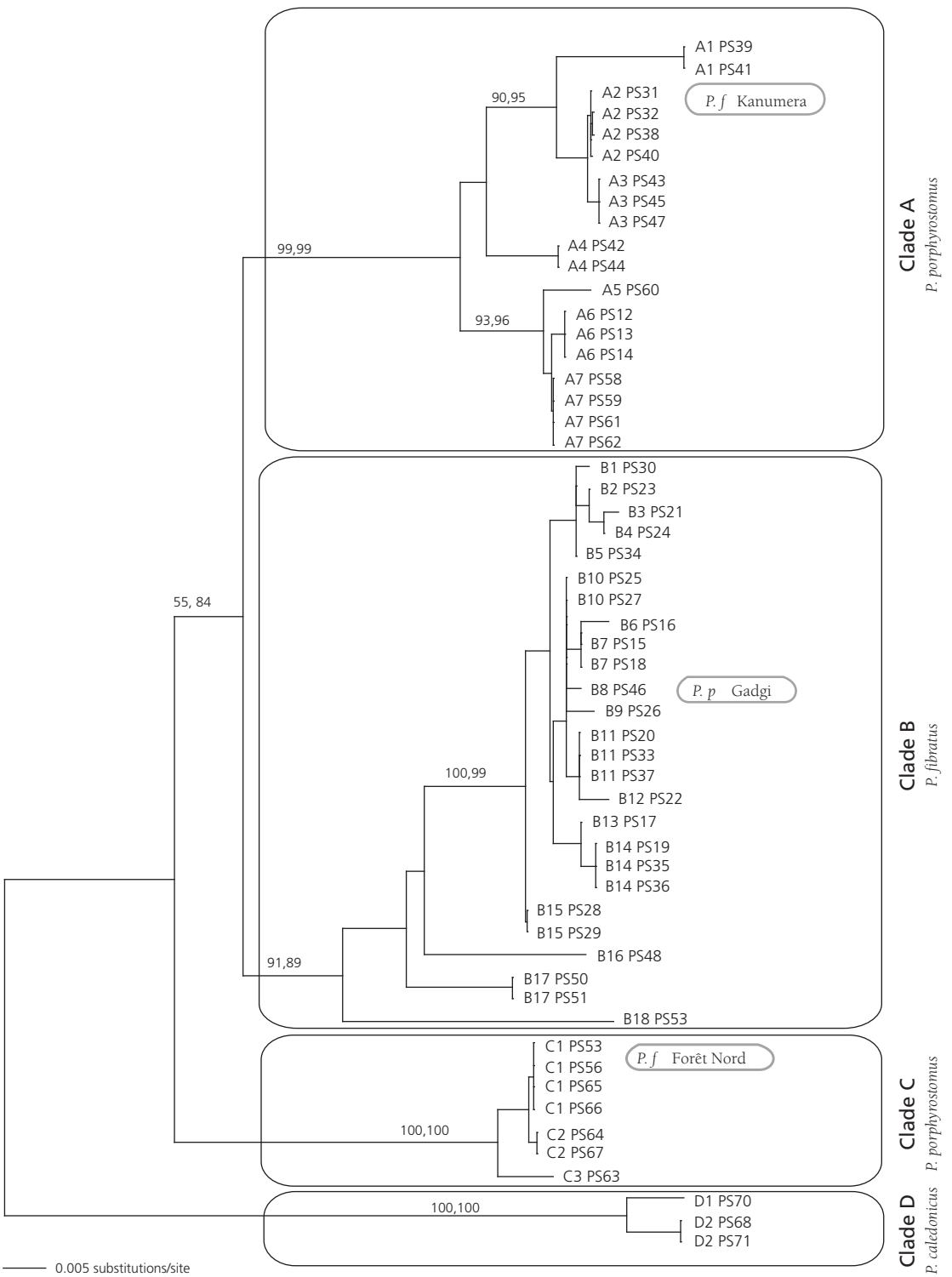

FIG. 3. NJ tree of COI sequences from all New Caledonian *Placostylus* surveyed, using K2P distances. Clades are labelled A-D with predominant species composition and examples of haplotype introgression indicated (grey elipses).

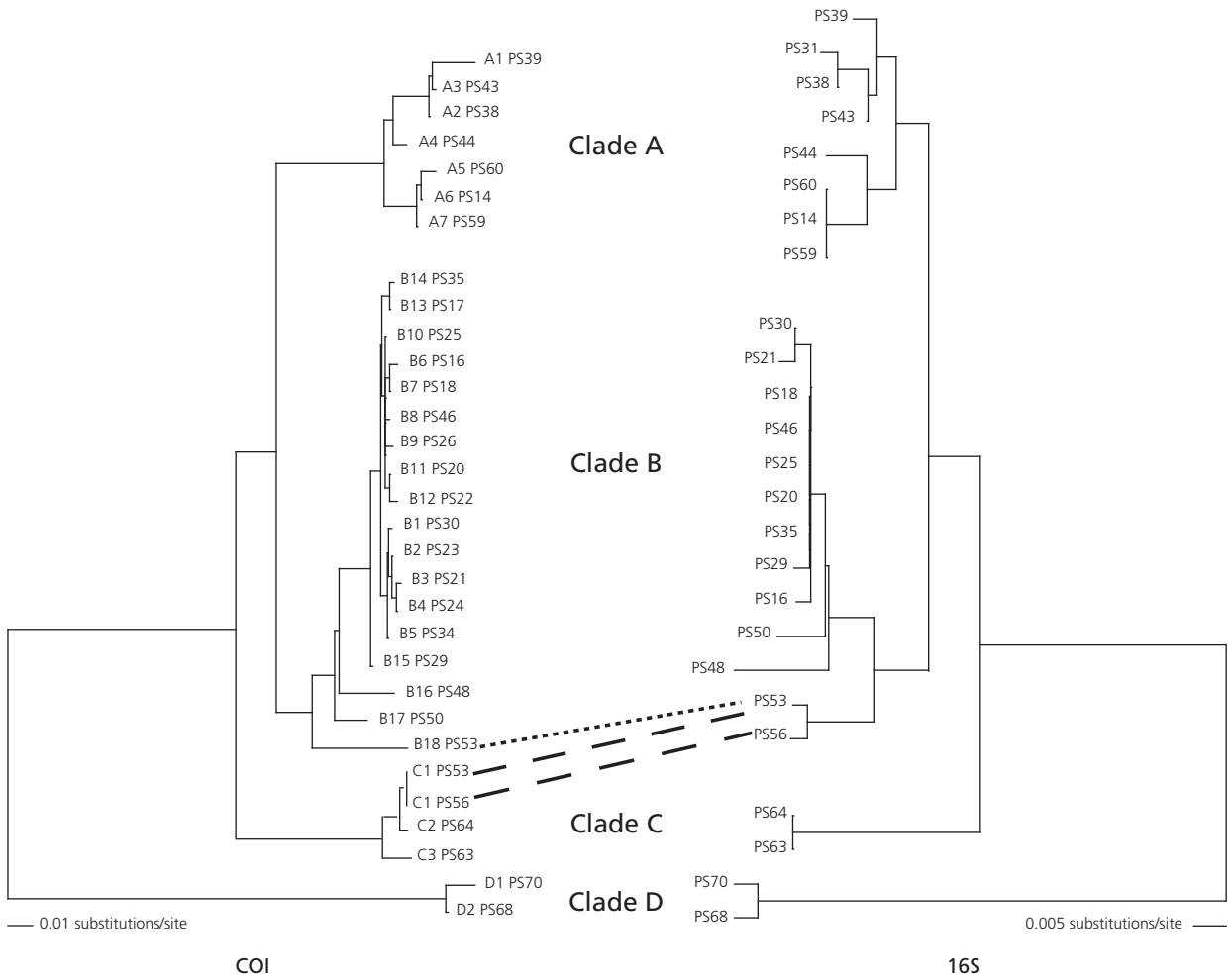

FIG. 4. NJ Phylogenies of New Caledonia *Placostylus* COI (left) and 16S (right) haplotypes. ML bootstrap values for 16S are shown at nodes. Dotted lines indicate difference in clade position of COI and 16S haplotypes from Forêt Nord *P. fibratus* PS53 and PS56. Thin dotted line indicates the alternative COI sequences obtained for PS53.

TABLE 2. Average ± SD pairwise genetic distances within and between clades using the K2P and TrN + I DNA evolutionary models. Outgroup refers to non-New Caledonian *Placostylus*.

COMPARISONS	K2P	TrN + I
Within clades	Mean ± SD	Mean ± SD
A	0.026 ± 0.013	0.024 ± 0.012
B	0.020 ± 0.020	0.018 ± 0.019
C	0.006 ± 0.005	0.005 ± 0.005
D	0.013 ± 0.00	0.012 ± 0.00
Outgroup	0.162 ± 0.079	0.643 ± 0.438

COMPARISONS	K2P	TrN + I
Between clades		
A-B	0.091 ± 0.009	0.089 ± 0.008
B-C	0.104 ± 0.007	0.102 ± 0.007
C-D	0.177 ± 0.004	0.218 ± 0.011
D-outgroup	0.252 ± 0.038	0.667 ± 0.217

GENETIC DIVERSITY

We found relatively high genetic distances within New Caledonia with the overall pattern of genetic variation being characterised by higher among population variation than within population variation (Table 2). Pairwise genetic distances (TrN + I) between *P. caledonicus* and other New Caledonian samples ranged from 0.18 to 0.23 indicating prolonged separation, while reaching only 0.012 within the *P. caledonicus* clade. Among clade variation for *P. porphyrostomus* and *P. fibratus* (clades A-C) ranged from 0.073 to 0.13, while within clade variation ranged from 0.001 to 0.063. Comparison of sequences from New Caledonian species with outgroup *Placostylus* (New Zealand, Three Kings Islands, Lord Howe) indicate genetic distances between 0.38 and 0.084 (TrN + I) (Table 2).

DISCUSSION

Our analyses support the monophyly of the New Caledonian taxa surveyed in relation to outgroup taxa from Lord Howe and New Zealand, and other helicoidean pulmonates (Fig. 2). The topology revealed by Bayesian and MP analyses with multiple New Caledonian taxon representatives is consistent with that obtained by Ponder *et al.* (2003) using a single *P. fibratus* sample. There is a clear split between the New Zealand *P. hongi*/*P. ambagiosus* and New Zealand Three Kings *P. bollonsi* radiations. Intriguingly, the New Zealand, Lord Howe and Three Kings Islands *Placostylus* are apparently basal to the New Caledonian radiation. This pattern hints at an unexpected northward directionality in dispersal of *Placostylus* among these islands, but further analysis with additional data and outgroup taxa is required to test this. Our data indicate that the Lord Howe *P. bivaricosus* is not the product of anthropogenic introduction from New Zealand or New Caledonian stock as has been speculated (Ponder *et al.* 2003).

We observed similar levels of within and among species pairwise distance estimates with K2P model in the New Caledonian and New Zealand snail radiations. Within species diversity averaged < 0.1 while between species genetic diversity was > 0.2. Estimates with more parameter rich models of DNA evolution return much higher inferred distances in comparisons of sequences from New Caledonian and outgroup *Placostylus* (> 0.6 Trn + I). Among our sample, the *P. caledonicus* specimens from Koumac formed a basal branch in all trees. COI haplotypes from *P. caledonicus* differed from *P. fibratus* and *P. porphyrostomus* haplotypes by between 0.18 and 0.23 (TrN + I).

DNA sequences from *P. fibratus* from the Isle of Pines grouped with *P. fibratus* sequences from Ouvéa in clade B, and include the dwarf morphotype from Gossanah. *Placostylus fibratus* from the Loyalty Islands (Ouvéa) and Forêt Nord (Grande Terre) are distinguished from the Isle of Pines *P. fibratus* as distinct genetic lineages. These lineages deserve further treatment and consideration as distinct units for conservation purposes. Of the sixteen clade B haplotypes sequenced, two were shared by *P. fibratus* individuals from more than one location on the Isle of Pines. One of these shared haplotypes (B14) was present in both the giant and typical morphotypes of *P. fibratus*, while the other (B11) involved *P. fibratus* individuals of the typical morph from two locations (Touete and Vao).

DNA sequences from individuals of *P. porphyrostomus* fall into two distinct groups: the small forms from the dry forests of central New Caledonia (Poya and Nékoro) group with the samples from the Isle of Pines (Kere, Kanuméra, Gadgi) in clade A. In contrast, COI and 16S DNA sequences from *P. porphyrostomus* obtained from the dry forest site at Pindaï form a separate clade (C), although some closely related COI sequences from Forêt Nord *P. fibratus* individuals group with these. The clustering with Forêt Nord *P. fibratus* is most easily explained as the result of recent hybridisation although the implied retention in these *P. fibratus* of two distinct mitochondrial lineages requires further investigation. Aside from this, *P. porphyrostomus* appears to be paraphyletic with respect to *P. fibratus*. This phylogenetic pattern is taxonomically unstable and could be resolved by splitting *P. porphyrostomus* (clade A and clade C) into two separate species. Although, this separation would not, on the current evidence, coincide with morphological evidence (Chérel-Mora 1983), it would be appropriate to recognise these lineages as discrete evolutionarily significant units (ESU, Waples 1991). Indeed the mismatch between genealogy and shell morphology is an interesting feature of these snails that probably reflects at least some degree of morphological plasticity in response to local environmental conditions such as aridity.

An intriguing observation from this study is that some *Placostylus* individuals yield evidence of more than one distinct mitochondrial lineage. Heteroplasmy has been well documented in some species of the marine bivalves of the genus *Mytilus* and the venerid Manila clam *Tapes phylipppinarun* which display separate paternal and maternal inheritance (doubly uniparental inheritance) (Quesada *et al.* 2003; Passamonti *et al.* 2003). But, so far heteroplasmy has not been documented in Pulmonate land snails, which are considered to be simultaneous hermaphrodites (combining a female and male function) and which can be facultative and obligate selfing and outcrossing (Haase & Baur 1995; Davison 2000; Dillon *et al.* 2005; Duncan 1975). Repeated, independent, temporally isolated rounds of DNA extraction, PCR and sequencing support the conclusion that these results are unlikely to be an artefact of intersample contamination. The extent of sequence difference and phylogenetic pattern among haplotypes indicates that the putative heteroplasmy observed in *Placostylus* is not of the type seen in *Mytilus*, and also indicates that nuclear copying is an unlikely source. Instead it is more similar to the sporadic instances of heteroplasmy that have been observed in a range of animals (*e.g.,* mosquito, Donnelly *et al.* 2004; thrips, Frey & Frey 2004; cricket, Harrison *et al.* 1987; bird, Kvist *et al.* 2003; brittlestar, Steel *et al.* 2000; flatworm, Vilas *et al.* 2005) that is thought to arise from the failure to reject paternal mitochondria during fertilisation (Ballard & Rand 2005). Evidence for these events generally comes in concert with evidence for introgression among divergent lineages; retention of paternal mitochondria could easily remained undetected where parents have similar or identical haplotypes. This is revealed in our data by the presence of two haplotypes of COI (in one snail) and lack of concordance of COI and 16S trees. Our data very probably underestimates the true extent of heteroplasmy, and the hybridsation that has made it evident.

We can draw tentative conclusions about the history of these snails. The observed incidence of haplotype sharing between two individuals of *P. porphyrostomus* from Kere and two individuals of *P. fibratus* from Kanuméra (clade A), and between the *P. porphyrostomus* from Pindaï and *P. fibratus* from Forêt Nord (clade C), plus the placement of one *P. porphyrostomus* from Gadgi within the *P. fibratus* clade (B) is best explained by recent introgression (*i.e.* hybridisation between individuals of the two species). The true extent of introgression will only be determined with more extensive sampling and inclusion of biparentally inherited DNA markers. Opportunities for contact between lineages (and species) were probably frequent on and between Grande Terre and Isle of Pines during the Pleistocene when climatic cycling would have led to shifting vegetation and habitat. In contrast the Loyalty Islands have probably been isolated from Grande Terre since the start of the Pleistocene despite the subsequent sea level variation (Dubois *et al.* 1974; Paris 1981; Cabioch 2003). The genetic distinctiveness of the Ouvéa samples probably indicates over-sea colonisation and subsequent isolation soon after the emergence of the Loyalty Islands 2 million years ago. The fine scale haplotype sharing (*i.e.* similar or identical haplotypes in two species) could conceivably reflect hybridisation induced by translocation of snails among locations by indigenous people. If this were the case, it may have considerable implications for current conservation efforts.

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APPENDIX 1. Pairwise genetic distances using Kimura-2-parameter (lower left) and Trn + I (upper right). Boxes indicate clades. GeneBank accession numbers are indicated for non-New Caledonian samples.

	A1 PS39	A2 PS38	A3 PS43	A4 PS44	A5 PS60	A6 PS14	A7 PS59	B14 PS35	B13 PS17
A1 PS39	CLADE A	0,018	0,017	0,029	0,036	0,038	0,039	0,100	0,103
A2 PS38	0,021		0,001	0,022	0,032	0,034	0,032	0,086	0,084
A3 PS43	0,019	0,002		0,020	0,031	0,032	0,031	0,089	0,086
A4 PS44	0,032	0,024	0,022		0,022	0,022	0,024	0,083	0,081
A5 PS60	0,039	0,035	0,033	0,024		0,006	0,007	0,090	0,087
A6 PS14	0,041	0,037	0,035	0,024	0,007		0,001	0,090	0,087
A7 PS59	0,043	0,035	0,033	0,026	0,008	0,002		0,087	0,085
B14 PS35	0,104	0,089	0,091	0,087	0,091	0,091	0,089	CLADE B	0,001
B13 PS17	0,106	0,086	0,089	0,084	0,089	0,089	0,086	0,002	
B10 PS25	0,109	0,089	0,091	0,087	0,091	0,086	0,084	0,007	0,005
B6 PS16	0,106	0,086	0,089	0,084	0,089	0,084	0,081	0,012	0,010
B7 PS18	0,112	0,091	0,094	0,089	0,094	0,089	0,086	0,008	0,007
B8 PS46	0,106	0,086	0,089	0,089	0,094	0,089	0,086	0,008	0,007
B9 PS26	0,115	0,094	0,096	0,092	0,096	0,091	0,089	0,010	0,008
B11 PS20	0,112	0,091	0,094	0,089	0,094	0,089	0,086	0,008	0,007
B12 PS22	0,111	0,091	0,093	0,089	0,093	0,088	0,086	0,012	0,010
B1 PS30	0,115	0,094	0,096	0,092	0,091	0,086	0,084	0,010	0,008
B2 PS23	0,115	0,094	0,096	0,092	0,086	0,081	0,079	0,010	0,008
B3 PS21	0,115	0,094	0,096	0,097	0,091	0,086	0,084	0,013	0,012
B4 PS24	0,112	0,091	0,094	0,094	0,089	0,084	0,081	0,012	0,010
B5 PS34	0,112	0,091	0,094	0,089	0,089	0,084	0,081	0,008	0,007
B15 PS29	0,101	0,081	0,084	0,080	0,089	0,084	0,081	0,008	0,007
B16 PS48	0,111	0,095	0,098	0,086	0,093	0,088	0,085	0,041	0,039
B17 PS50	0,087	0,082	0,084	0,071	0,079	0,075	0,077	0,037	0,035
B18 PS53 orig	0,110	0,099	0,102	0,082	0,102	0,091	0,089	0,070	0,067
C1 PS53 (1)	0,128	0,128	0,131	0,112	0,125	0,122	0,122	0,103	0,101
C1 PS56	0,128	0,128	0,131	0,112	0,125	0,122	0,122	0,103	0,101
C2 PS64	0,127	0,127	0,130	0,111	0,124	0,121	0,121	0,103	0,100
C3 PS63	0,131	0,131	0,134	0,115	0,131	0,125	0,125	0,106	0,103
D1 PS70	0,168	0,149	0,152	0,153	0,174	0,165	0,161	0,203	0,200
D2 PS68	0,174	0,155	0,158	0,166	0,181	0,178	0,174	0,196	0,192
AY148560 *P. ambagiosus*	0,176	0,170	0,167	0,176	0,172	0,169	0,166	0,169	0,166
AY165838 *P. bivaricosus*	0,220	0,208	0,204	0,212	0,216	0,207	0,204	0,208	0,204
AY290740 *P. annectens watti*	0,161	0,155	0,152	0,155	0,163	0,154	0,151	0,163	0,160
AY290741 *P. bollonsi arbutus*	0,253	0,234	0,231	0,238	0,238	0,238	0,234	0,234	0,230
AY290744 *P. hongii hongii*	0,165	0,153	0,150	0,151	0,165	0,153	0,150	0,150	0,147

	B10 PS25	B6 PS16	B7 PS18	B8 PS46	B9 PS26	B11 PS20	B12 PS22	B1 PS30	B2 PS23
A1 PS39	0,105	0,103	0,108	0,103	0,110	0,107	0,109	0,110	0,110
A2 PS38	0,086	0,084	0,089	0,084	0,091	0,089	0,090	0,091	0,091
A3 PS43	0,089	0,087	0,091	0,086	0,093	0,091	0,092	0,093	0,094
A4 PS44	0,083	0,081	0,086	0,085	0,087	0,085	0,086	0,087	0,088
A5 PS60	0,090	0,087	0,092	0,092	0,094	0,092	0,093	0,089	0,084
A6 PS14	0,085	0,083	0,088	0,087	0,090	0,087	0,088	0,084	0,080
A7 PS59	0,083	0,081	0,085	0,085	0,087	0,085	0,086	0,082	0,078
B14 PS35	0,006	0,011	0,007	0,007	0,009	0,007	0,011	0,009	0,009
B13 PS17	0,004	0,009	0,006	0,006	0,007	0,006	0,009	0,007	0,007
B10 PS25		0,004	0,001	0,001	0,003	0,001	0,004	0,006	0,006
B6 PS16	0,005		0,003	0,006	0,007	0,006	0,009	0,011	0,011
B7 PS18	0,002	0,003		0,003	0,004	0,003	0,006	0,007	0,008
B8 PS46	0,002	0,007	0,003		0,004	0,003	0,006	0,007	0,007
B9 PS26	0,003	0,008	0,005	0,005		0,004	0,007	0,009	0,009
B11 PS20	0,002	0,007	0,003	0,003	0,005		0,003	0,007	0,007
B12 PS22	0,005	0,010	0,007	0,007	0,008	0,003		0,011	0,011
B1 PS30	0,007	0,012	0,008	0,008	0,010	0,008	0,012		0,003
B2 PS23	0,007	0,012	0,008	0,008	0,010	0,008	0,012	0,003	
B3 PS21	0,010	0,015	0,012	0,012	0,013	0,012	0,015	0,007	0,003
B4 PS24	0,008	0,013	0,010	0,010	0,012	0,010	0,013	0,005	0,002
B5 PS34	0,005	0,010	0,007	0,007	0,008	0,007	0,010	0,002	0,002
B15 PS29	0,005	0,010	0,007	0,007	0,008	0,007	0,010	0,008	0,008
B16 PS48	0,037	0,043	0,039	0,039	0,041	0,039	0,043	0,041	0,041
B17 PS50	0,033	0,039	0,035	0,035	0,037	0,035	0,035	0,033	0,033
B18 PS53 orig	0,065	0,067	0,067	0,067	0,065	0,067	0,067	0,065	0,065
C1 PS53 (1)	0,103	0,106	0,106	0,106	0,103	0,101	0,100	0,103	0,098
C1 PS56	0,103	0,106	0,106	0,106	0,103	0,101	0,100	0,103	0,098
C2 PS64	0,103	0,106	0,106	0,106	0,103	0,100	0,100	0,106	0,100
C3 PS63	0,106	0,109	0,109	0,109	0,106	0,106	0,106	0,106	0,101
D1 PS70	0,189	0,189	0,192	0,192	0,196	0,185	0,185	0,189	0,196
D2 PS68	0,182	0,182	0,185	0,185	0,189	0,178	0,178	0,182	0,189
AY148560 *P. ambagiosus*	0,169	0,179	0,172	0,172	0,176	0,169	0,169	0,169	0,166
AY165838 *P. bivaricosus*	0,204	0,212	0,208	0,208	0,208	0,204	0,204	0,204	0,204
AY290740 *P. annectens watti*	0,157	0,166	0,160	0,160	0,163	0,157	0,157	0,157	0,155
AY290741 *P. bollonsi arbutus*	0,237	0,245	0,241	0,241	0,245	0,237	0,237	0,237	0,241
AY290744 *P. hongii hongii*	0,144	0,153	0,147	0,142	0,150	0,147	0,147	0,144	0,142

	B3 PS21	B4 PS24	B5 PS34	B15 PS29	B16 PS48	B17 PS50	B18 PS53 orig	C1 PS53 (1)	C1 PS56
A1 PS39	0,110	0,108	0,107	0,098	0,110	0,083	0,104	0,126	0,126
A2 PS38	0,091	0,089	0,089	0,079	0,095	0,079	0,096	0,127	0,127
A3 PS43	0,094	0,092	0,091	0,082	0,098	0,082	0,098	0,129	0,129
A4 PS44	0,093	0,090	0,085	0,076	0,084	0,068	0,078	0,109	0,109
A5 PS60	0,089	0,087	0,087	0,087	0,093	0,078	0,099	0,124	0,124
A6 PS14	0,084	0,082	0,082	0,083	0,089	0,073	0,090	0,122	0,122
A7 PS59	0,082	0,080	0,080	0,080	0,086	0,076	0,088	0,122	0,122
B14 PS35	0,012	0,011	0,007	0,007	0,038	0,035	0,063	0,102	0,102
B13 PS17	0,011	0,009	0,006	0,006	0,036	0,033	0,061	0,099	0,099
B10 PS25	0,009	0,008	0,004	0,004	0,035	0,031	0,059	0,102	0,102
B6 PS16	0,014	0,012	0,009	0,009	0,040	0,036	0,061	0,104	0,104
B7 PS18	0,011	0,009	0,006	0,006	0,036	0,033	0,061	0,105	0,105
B8 PS46	0,011	0,009	0,006	0,006	0,036	0,033	0,061	0,104	0,104
B9 PS26	0,012	0,011	0,007	0,007	0,038	0,035	0,059	0,102	0,102
B11 PS20	0,011	0,009	0,006	0,006	0,036	0,033	0,061	0,099	0,099
B12 PS22	0,014	0,012	0,009	0,009	0,040	0,033	0,061	0,101	0,101
B1 PS30	0,006	0,004	0,001	0,007	0,038	0,031	0,059	0,102	0,102
B2 PS23	0,003	0,001	0,001	0,008	0,038	0,031	0,059	0,097	0,097
B3 PS21		0,001	0,004	0,011	0,042	0,035	0,063	0,102	0,102
B4 PS24	0,002		0,003	0,009	0,040	0,033	0,061	0,099	0,099
B5 PS34	0,005	0,003		0,006	0,036	0,029	0,057	0,099	0,099
B15 PS29	0,012	0,010	0,007		0,029	0,026	0,053	0,094	0,094
B16 PS48	0,045	0,043	0,039	0,031		0,031	0,064	0,122	0,122
B17 PS50	0,037	0,035	0,031	0,028	0,033		0,054	0,091	0,091
B18 PS53 orig	0,070	0,067	0,063	0,058	0,069	0,058		0,117	0,117
C1 PS53 (1)	0,103	0,101	0,101	0,096	0,121	0,091	0,120	CLADE C	0,000
C1 PS56	0,103	0,101	0,101	0,096	0,121	0,091	0,120	0,000	
C2 PS64	0,106	0,103	0,103	0,095	0,121	0,093	0,122	0,002	0,002
C3 PS63	0,106	0,103	0,103	0,098	0,124	0,093	0,123	0,010	0,010
D1 PS70	0,196	0,192	0,192	0,185	0,175	0,175	0,183	0,178	0,178
D2 PS68	0,189	0,185	0,185	0,178	0,168	0,168	0,183	0,172	0,172
AY148560 *P. ambagiosus*	0,166	0,163	0,169	0,169	0,185	0,173	0,188	0,198	0,198
AY165838 *P. bivaricosus*	0,200	0,204	0,204	0,196	0,202	0,193	0,208	0,198	0,198
AY290740 *P. annectens watti*	0,155	0,152	0,157	0,152	0,169	0,155	0,169	0,172	0,172
AY290741 *P. bollonsi arbutus*	0,237	0,237	0,237	0,227	0,230	0,224	0,253	0,237	0,237
AY290744 *P. hongii hongii*	0,142	0,139	0,144	0,144	0,153	0,145	0,168	0,161	0,161

	C2 PS64	C3 PS63	D1 PS70	D2 PS68	AY148560	AY165838	AY290740	AY290741	AY290744
A1 PS39	0,128	0,129	0,211	0,217	0,441	0,725	0,388	0,854	0,326
A2 PS38	0,129	0,130	0,192	0,198	0,450	0,751	0,394	0,887	0,316
A3 PS43	0,131	0,132	0,196	0,202	0,451	0,758	0,392	0,894	0,311
A4 PS44	0,111	0,112	0,187	0,199	0,465	0,742	0,417	0,878	0,271
A5 PS60	0,125	0,130	0,220	0,225	0,511	0,770	0,486	0,880	0,333
A6 PS14	0,123	0,125	0,210	0,223	0,506	0,781	0,485	0,874	0,310
A7 PS59	0,123	0,125	0,207	0,219	0,509	0,788	0,488	0,881	0,305
B14 PS35	0,103	0,104	0,239	0,228	0,471	0,776	0,460	0,912	0,285
B13 PS17	0,101	0,102	0,236	0,225	0,473	0,784	0,461	0,920	0,280
B10 PS25	0,103	0,105	0,224	0,213	0,488	0,789	0,463	0,907	0,274
B6 PS16	0,106	0,107	0,225	0,214	0,489	0,778	0,467	0,896	0,295
B7 PS18	0,106	0,107	0,228	0,217	0,494	0,786	0,470	0,903	0,285
B8 PS46	0,106	0,107	0,227	0,216	0,486	0,781	0,461	0,899	0,268
B9 PS26	0,103	0,104	0,229	0,219	0,484	0,781	0,460	0,892	0,285
B11 PS20	0,101	0,105	0,221	0,210	0,488	0,789	0,463	0,907	0,280
B12 PS22	0,102	0,106	0,226	0,215	0,529	0,831	0,511	0,942	0,302
B1 PS30	0,106	0,104	0,227	0,216	0,481	0,784	0,453	0,903	0,269
B2 PS23	0,101	0,099	0,236	0,225	0,474	0,784	0,443	0,898	0,259
B3 PS21	0,105	0,104	0,236	0,225	0,474	0,793	0,443	0,906	0,259
B4 PS24	0,103	0,102	0,233	0,222	0,476	0,784	0,444	0,906	0,253
B5 PS34	0,103	0,102	0,230	0,219	0,481	0,784	0,453	0,903	0,269
B15 PS29	0,096	0,097	0,222	0,211	0,490	0,800	0,444	0,923	0,276
B16 PS48	0,124	0,125	0,207	0,196	0,487	0,710	0,459	0,922	0,291
B17 PS50	0,095	0,094	0,217	0,206	0,514	0,843	0,477	0,897	0,288
B18 PS53 orig	0,121	0,119	0,223	0,218	0,517	0,758	0,507	0,856	0,354
C1 PS53 (1)	0,001	0,009	0,218	0,207	0,549	0,680	0,538	0,909	0,356
C1 PS56	0,001	0,009	0,218	0,207	0,549	0,680	0,538	0,909	0,356
C2 PS64		0,011	0,228	0,216	0,581	0,721	0,579	0,944	0,390
C3 PS63	0,012		0,240	0,227	0,496	0,618	0,471	0,848	0,366
D1 PS70	0,181	0,184	CLADE D	0,012	0,568	0,725	0,518	1,042	0,495
D2 PS68	0,174	0,177	0,013		0,564	0,724	0,505	1,049	0,483
AY148560 *P. ambagiosus*	0,197	0,202	0,245	0,234		0,689	0,026	1,101	0,082
AY165838 *P. bivaricosus*	0,197	0,195	0,240	0,233	0,182		0,683	1,051	0,673
AY290740 *P. annectens watti*	0,171	0,176	0,227	0,224	0,028	0,179		1,107	0,063
AY290741 *P. bollonsi arbutus*	0,237	0,238	0,328	0,319	0,236	0,249	0,229		0,954
AY290744 *P. hongii hongii*	0,161	0,164	0,236	0,232	0,078	0,165	0,061	0,216	