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

Steve TREWICK[1], Fabrice BRESCIA[2] & Corina JORDAN[1]

[1] Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, Private Bag 11-222, Palmerston North, New Zealand.
[2] Institut Agronomique néo-Calédonien (IAC), Programme Élevage et Faune Sauvage, Nouméa, New Caledonia.
s.trewick@massey.ac.nz

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

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1996; Wadeet al.

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* ampli ed. The A2 haplotype was found in two individuals of *P. bratus* and two of *P. porphyrostomus* from the Isle of Pines but at different locations (Table 1). The C1 haplotype was found in four *P. bratus* individuals from Forêt Nord and one *P. porphyrostomus* individual from Pindaï, both sites on Grande Terre. Haplotype sharing was absent between individuals Haplotype sharing

FIG. 3. NJ tree of COI sequences from all N

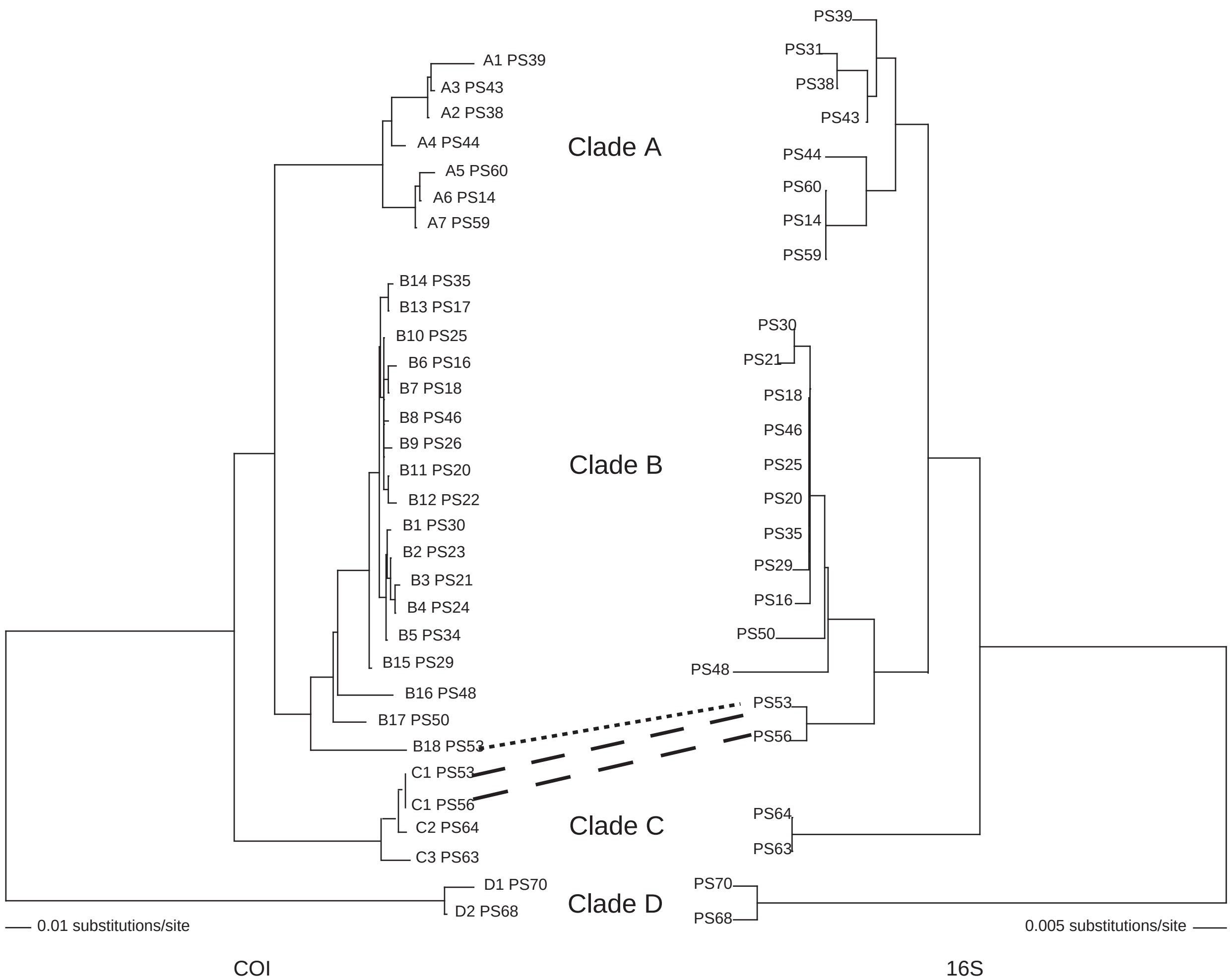

FIG. 4. NJ Phylogenies of New Caledonia *Placostylus* COI (left) and 16S (right) haplotypes. ML bootstrap values for 16S are shown at nodes. Dotted lines indicate difference in clade position of COI and 16S haplotypes from Forêt Nord *P. bratus* PS53 and PS56. Thin dotted line indicates the alternative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

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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.