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Correlation between shell phenotype and local environment suggests a role for natural selection in the evolution of *Placostylus* snails

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Abstract

The giant edible *Placostylus* snails of New Caledonia occur across a wide range of environmental conditions, from the dry southwest to the wetter central and northeastern regions. In large, slow-moving animals such as *Placostylus*, speciation could be assumed to be largely driven by allopatry and genetic drift as opposed to natural selection. We examined variation in shell morphology using geometric morphometrics and genetic structure within two species of *Placostylus* (*P. fibratus*, *P. porphyrostomus*), to determine the drivers of diversity in this group. Despite the current patchy distribution of snails on New Caledonia, both mtDNA and nuclear SNP data sets (>3000 loci) showed weak admixing between populations and species. Shell morphology was concordant with the genetic clusters we identified and had a strong relationship with local environment. The genetic data, in contrast to the morphological data, did not show concordance with climatic conditions, suggesting the snails are not limited in their ability to adapt to different environments. In sympatry, *P. fibratus* and *P. porphyrostomus* maintained genetic and morphological differences, suggesting a genetic basis of phenotypic variation. Convergence of shell shape was observed in two adjacent populations that are genetically isolated but experience similar habitat and climatic conditions. Conversely, some populations in contrasting environments were morphologically distinct although genetically indistinguishable. We infer that morphological divergence in the *Placostylus* snails of New Caledonia is mediated by adaptation to the local environment.

Keywords: adaptation, climate response, convergence, ecotypes

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Introduction

Adaptation of populations to their local environment may result in the formation of ecotypes, which could be the first steps towards ecological speciation (Kawecki & Ebert 2004; Rundle & Nosil 2005; Mallet 2008; Räsänen & Hendry 2008; Nosil & Feder 2012; Stankowski 2013). Local adaptation is frequently inferred for a range of morphological and physiological traits of relevance to abiotic and biotic interactions, but determining the role of natural selection in shaping geographically partitioned variation is not simple (Merilä & Hendry 2014). An observed difference in traits between regional populations does not directly demonstrate local adaptation as patterns of variation may also be shaped by genetic drift. Understanding the role of divergent ecological selection requires quantification of genetic, phenotypic and environmental variation.

Understanding how the environment confers selection pressure that maintains phenotypic variation is complicated by the fact that phenotypic variation could represent either adaptation, phenotypic plasticity or both. Variation due to phenotypic plasticity does not

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necessarily impede adaptation, and in some cases, it has been suggested to promote genetic divergence (Thibert-Plante & Hendry 2011; Fitzpatrick 2012). Phenotypic plasticity could allow individuals to successfully colonize marginal environments, exposing them to selection that subsequently leads to genetic differentiation (Fitzpatrick 2012). Local adaptation sets predictable outcomes regardless of whether a phenotype is the result of plastic or genetic adaptation. Local phenotypic adaptation would result in correspondence between phenotype and environmental variation, but no correlation between neutral genetic variation and either phenotype or environment is expected. Habitat-specific convergent evolution also provides a powerful means of testing adaptive hypotheses (Minards *et al.* 2014). Separate lineages exposed to similar environmental conditions that display similar responses (convergence) support the hypothesis that traits are adaptations resulting from local selection (Butlin *et al.* 2014).

Studies of molluscs and land snails in particular have made important contributions to several aspects of evolutionary biology including some of the best examples of ecological speciation (Woodruff & Gould 1987; Goodfriend & Gould 1996; Davison 2002; Teshima *et al.* 2003; Quesada *et al.* 2007; Greve *et al.* 2012; Stankowski 2013; Wada & Chiba 2013; Butlin *et al.* 2014). Snails (Gastropoda) provide useful models for studying local adaptation because their shells are highly responsive to environmental conditions (Giokas *et al.* 2014). Shell morphology in some species of gastropods has been shown to be plastic with respect to the environment and predators (Appleton & Palmer 1988; Trussell 2000; Doyle *et al.* 2010; Hollander & Butlin 2010; Butlin *et al.* 2014). However, shell characteristics can also reflect fixed genetic attributes (Goodfriend 1986; Johannesson 1996; Stankowski 2013). Selective advantage from phenotypic plasticity and trait-specific adaptations enable lineages to specialize to distinct environmental conditions (e.g. ecotypes of the coastal marine snail *Littorina saxatilis* (Hollander & Butlin 2010; Saura *et al.* 2012; Butlin *et al.* 2014).

The large *Placostylus* land snails belonging to Bulimidae (Neubert *et al.* 2009) occur naturally in the western Pacific: New Zealand, Vanuatu, Fiji, Papua New Guinea, Solomon Islands, Lord Howe Island and New Caledonia. New Caledonia itself is composed of a main island, Grande Terre, and several offshore islands including Ile des Pins (Isle of Pines) and Ouvéa (part of the Loyalty Islands), which are of particular interest here (Fig. 1). Positioned about 20° south of the equator in the western Pacific Ocean, New Caledonia is a biodiversity hot spot known for its plant and animal endemism and micro-endemism (Grandcolas *et al.* 2008). The New Caledonian biota is now understood to have originated by long-distance dispersal and establishment after land emerged in the Oligocene (37 MYA) (Bartish *et al.* 2005; Trewick *et al.* 2007; Grandcolas *et al.* 2008; Espeland & Murienne 2011; Nattier *et al.* 2011). The islands, which are orientated in a northwest–southeast direction, have diverse terrestrial environments from southwestern dry forest (before extensive forest clearance) to northeastern and central rain forest (Fig. 1). A broad altitudinal range amplifies local variation in conditions and is implicated in the high levels of biodiversity seen today (Grandcolas *et al.* 2008). In landscapes with variable environmental conditions such as this, selection and adaptation of local populations, resulting in the formation of ecotypes and species, is a likely component of diversification. Indeed, high levels of regional endemism in New Caledonia support this (Murienne *et al.* 2009; Nattier *et al.* 2011).

New Caledonian *Placostylus* are prominent, ecologically important, endemic invertebrates and are economically and culturally significant as human food. Six species are currently recognized on the basis of soft tissue morphology, in particular the genitalia (Neubert *et al.* 2009). Until recently, a much larger number of species were inferred from shell morphology, but taxonomic revision has largely ignored shell shape variation of New Caledonian *Placostylus*, due to concern about phenotypic variation in relation to the environment. Genetic research using mitochondrial sequence data has suggested that the current taxa might comprise additional lineages concealed by an overly conservative treatment of shell morphology (Trewick *et al.* 2008; Neubert *et al.* 2009). Four of the currently recognized species have restricted ranges. Two (*P. fibratus* and *P. porphyrostomus*) are relatively abundant and widespread although patchily distributed. These are the only legally harvested species. *Placostylus porphyrostomus* is usually the smaller of the two species with the notable exception of dwarf *P. fibratus* on Ouvéa Island. *Placostylus porphyrostomus* populations occur in the western sclerophyll forest of Grande Terre, while *P. fibratus* is found in the wetter central and eastern forests (Fig. 1) (Brescia 2011). This predominantly allopatric distribution changes on Ile des Pins where *P. fibratus* and *P. porphyrostomus* are sympatric (Fig. 1). On Ile des Pins, the two species are distinguished by size and genitalia (after dissection), but elsewhere shell size and shape variation are extensive within both species (Fig 1). The presence of two distinct morphotypes on Ile des Pins suggests that shell shape is not purely a flexible trait responding to local environment but is genetically constrained. Focusing on these two *Placostylus* taxa, and with additional data from *Placostylus caledonicus*, we tested whether shell shape variation is best explained

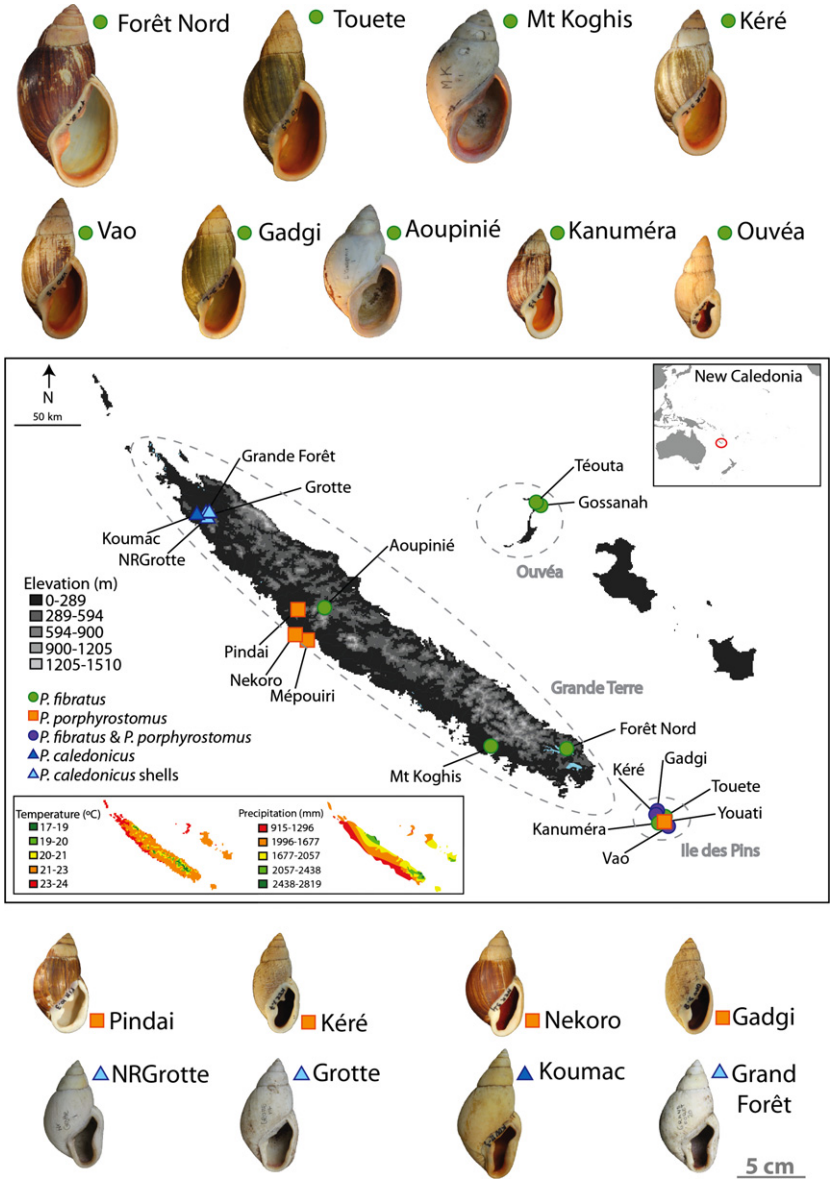

Fig. 1 Sampling locations of *Placostylus* snails in New Caledonia used to study genetic and phenotypic variation. Population samples are coded according to current taxonomy; *P. porphyrostomus* (orange squares), *P. fibratus* (green circles) and *P. caledonicus* (blue triangles). Two environmental gradients across New Caledonia, generated from Bioclim data sets, are shown on the bottom left: annual average temperature and annual precipitation. *Placostylus fibratus* is considered a wet forest specialist and is characterized by a bulging body whorl and open aperture, while *P. porphyrostomus* is considered a dry forest specialist characterized by a narrow aperture, smaller body whorl and smaller size overall. Samples from Ouvéa Island (Téouta and Gossanah) represent a dwarf form of *P. fibratus*. Representative shell forms from all sampled populations are shown to the same scale.

by genetic structuring or environmental gradients on Grande Terre, Ouvéa and Ile des Pins.

Geometric morphometric techniques, not previously applied to these snails, were used to analyse variation in shell shape and size. Genetic diversity was examined from this previously little-studied animal using mtDNA haplotype data and an extensive SNP marker data set derived from RAD sequence (Senn *et al.* 2013; Wagner *et al.* 2013). We used these tools to examine the drivers of diversity within New Caledonian *Placostylus*. If morphological differentiation has accumulated through drift alone, then we would expect morphological and neutral genetic differences among lineages to correlate and have no link with patterns of environmental variation. In contrast, selection is expected to result in correlation between shell morphology and environmental gradients but not correlate with neutral genetic structure. Convergence of morphology in independent lineages collected from adjacent sites would be strong support for local adaptation of morphological traits, the type of selection expected to lead to the formation of ecotypes in contrasting environments.

Methods

Sampling strategy

We focused on the two most common and widespread species of *Placostylus* that are patchily distributed through central and southern New Caledonia. Sampling

was carried out to encompass a range of geographic, taxonomic and environmental contrasts (Fig. 1).

Placostylus fibratus was collected from three locations on Grande Terre (Forêt Nord, Mt Koghis and Aoupinié) where it is allopatric with respect to *P. porphyrostomus*. The three locations differ in altitude, temperature and precipitation. Populations of *Placostylus fibratus* on two islands were also sampled; these represent a dwarf form on one of the Loyalty Islands (Ouvéa), and several locations on Ile des Pins where the snails are sympatric with *P. porphyrostomus*. Similarly, *Placostylus porphyrostomus* was collected from three locations in western Grande Terre where the snails are allopatric, two from coastal areas (Nekoro and Mépouiri) and one inland site (Pindai). Populations were also sampled from several locations on Ile des Pins. *Placostylus caledonicus* was sampled from four locations in the north of Grande Terre, but three of these sites did not contain live snails and provide only morphometric shell data. Thus, three northern populations (Grande Forêt, Grotte and NRGrotte) did not contribute genetic data.

Whole snails were collected by hand between 2005 and 2012 as part of a research programme directed at understanding the population structure and demography of the snails.

Geometric morphometric analysis

To characterize shell morphology, we used a geometric approach with a set of landmarks around the aperture and shell outline in ventral orientation (Fig. 1, Fig S1, Supporting information). Studies on land and marine molluscs have shown the benefit of this technique in capturing shape variation among ecotypes and species (Crampton & Gale 2005; Haase & Misof 2009; Stankowski 2011; Hills *et al.* 2012). Six permanent landmarks and 22 semi-landmarks were used in the analysis, and all imaging and digitization were undertaken by E. Dowle.

Digital images of the ventral surface of each shell were obtained using a Canon EOS 600d with EF100 mm f2.8 USM macro lens after careful positioning in a bed of sand of contrasting colour. Photographic equipment was mounted on a high-precision Kaiser stand to allow reproducible positioning and orientation. Two digital 'combs' were positioned over images of each shell using ADOBE PHOTOSHOP CS6. Combs were placed as shown in Fig S1, Supporting information. Digitizing was undertaken in TPSDIG2 2.17 (Rohlf 2013) on a Wacom Cintiq 22HD digitizing tablet. Digitized semi-landmarks were slid using SEMILAND, part of the IMP714 package (Sheets 2012; Zelditch *et al.* 2004), implementing the Procrustes distance method. Landmark X/Y coordinates were then imported into MORPHOJ 1.05F (Klingenberg 2011) where nonparametric tests were applied. The error associated with photographing and digitizing the images was examined by rephotographing and digitizing one shell. The error was found to be small (<5%) in comparison with the potentially biologically meaningful variation among populations.

Shell shape and size variation were examined with MORPHOJ. Shape was assessed using principal component analysis (PCA) across all individuals and all landmarks. Discriminant analysis used cross-validation and 1000 permutations among groups based on the current taxonomic treatment (*P. fibratus*, *P. porphyrostomus* and *P. caledonicus*) with the assumption that the shells collected from Grande Forêt, Grotte and NRGrotte represented recently deceased *P. caledonicus*. A second discriminant analysis used the groupings identified from the genetic analysis (SNP and mtDNA) with the dwarf form of *P. fibratus* and samples lacking genetic data kept separate: *P. fibratus* (Ile des Pins, Forêt Nord and Mt Koghis), *P. caledonicus*, *P. porphyrostomus* (Ile des Pins), *P. por*-nekoro (*P. porphyrostomus* from Nekoro), *P. por*-pindai (*P. porphyrostomus* from Pindai) and *P. fib*-aoupinié (*P. fibratus* from Aoupinié). A third discriminant analysis of just the two species, *P. fibratus* and *P. porphyrostomus*, from Ile des Pins was also conducted. Canonical variate analysis (CVA) of these ten putative populations was used to describe differences among them. We tested the influence of removing some semi-landmarks from the analysis using a discriminant analysis with cross-validation and 1000 permutations. Seven different combinations of semi-landmarks were tried: removing semi-landmarks 4–10, 12–21, 23–27, 4–10 and 12–21, 4–10 and 23–27, 12–21 and 23–27, and finally removing all semi-landmarks and using just the 6 permanent landmarks (Figs S1 and S2, Supporting information). The relationship between size and shape was analysed by a regression of PC1 scores (all landmarks) against centroid size (size variation) in MORPHOJ. Procrustes coordinates and centroid size were then averaged across all populations and a separate PCA applied to this data set. The PC1 and PC2 scores and centroid sizes from populations on Grande Terre and Ouvéa were used in the subsequent modelling.

MtDNA sequence

To improve genealogical resolution in relation to taxonomy and geography, we sequenced a mtDNA gene not previously surveyed in *Placostylus* that was expected to be less constrained than the more commonly used cytochrome oxidase subunit I (COI) gene (Hills *et al.* 2011). To achieve this, a genomic sample of a *Placostylus porphyrostomus* from Mépouiri was sequenced on an Illumina Hi-Seq 2000 (BGI) with an expected scale of 1 GB

of data. DNA was extracted using incubation at 55 °C 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 two rounds of clean-up using combined phenol/chloroform/isoamyl alcohol (25:24:1) to minimize polysaccharide content and elution in water. The resulting sequence data were de novo assembled using VELVET 1.1 (Zerbino & Birney 2008) with mapping in BOWTIE2 (Langmead & Salzberg 2012) and visualization in TABLET 1.12.09.03 (Milne *et al.* 2010). A large contig (>3000 bp) representing part of the mtDNA genome provided information used to design primers surrounding the ND2 gene (primers: Placo_ND2F: AAC GCA AAG GGT ATG AAC CCG TAA ATA G, Placo_ND2R: GAG CAA TCG CCG GAG GAA CGG AAA T). This gene was sequenced across all available New Caledonian *Placostylus* samples. Extractions were diluted as necessary for PCRs. PCR used the protocol: 94 °C for 3 min; 94 °C for 45 s, 56 °C for 45 s and 72 °C for 75 s repeated 36 times; followed by a 2-min annealing step. Cycle sequencing used PerkinElmer BIGDYE 3.1 chemistry following the manufacturer's protocols and analysed on an ABI Prism 377 DNA sequencer (Applied Biosystems, Inc., Foster City, CA, USA). Sequences were checked and aligned in GENEIOUS 6.0.3 (Geneious). A bootstrapped maximum-likelihood (ML) tree was inferred using RAXML via the CIPRES portal (Stamatakis 2006; Miller *et al.* 2010) with a GTR + Γ model, allowing RAXML to halt the bootstrapping when statistically appropriate.

RAD-Seq

Anonymous single nucleotide polymorphic nuclear markers were generated using high-throughput sequencing, with DNA fragments tagged, so we could assign genotypes to individual snails. The double-digest RAD-Seq protocol was used with minor modifications (Peterson *et al.* 2012). We used the restriction endonucleases PstI and BamHI to digest whole-genomic DNA from 149 individual snails. The DNA fragments were ligated (Invitrogen T4 ligase) with short DNA barcode sequences to enable identification postsequencing, before the DNA was pooled and indexed. Data generated on an Illumina Hi-Seq (New Zealand Genomics Ltd) were sorted using the STACKS 1.01 pipeline (Catchen *et al.* 2011, 2013).

Selection of nuclear markers was undertaken, so analyses would be performed on loci likely to be single copy and for which the maximum number of individuals could be genotyped. In STACKS, a range of parameter settings relating to read coverage, individual number and population coverage were implemented. Recommended read coverage settings vary in the literature (Peterson *et al.* 2012; Buerkle & Gompert 2013), so we experimented with coverage cut-offs between 7 and 30 reads with our data. Low coverage might reduce identification of heterozygotes, but we found little influence on results. Here, we report analysis using 8 reads per individual as providing a reliable set of markers for downstream analysis and excluding all stacks with a lower coverage. Potentially spurious highly repetitive stacks were removed. Within an individual, we allowed a maximum of two mismatches (SNPs) between alleles and four mismatches between primary and secondary reads within USTACKS. In CSTACKS, three mismatches were allowed between alleles in different individuals when generating the SNP set (parameter settings were as follows: -m 8 –N 2 –M 4 –n 3 -t). Analysis was restricted to a single SNP site per putative locus (always the first) avoiding potential problems of data nonindependence. We tested a range of values relating to the number of populations a SNP marker was required to be present in before being recorded. The largest data set required that each putative locus included was genotyped in individuals from two or more populations and genotyped in ≥90% of individuals within those populations. This data set was used to estimate pairwise F_{ST} values as it maximized the data available for each pairwise comparison and each comparison was independent of others. Two smaller, more stringent data sets were also examined. One required that a locus be detected in all populations for inclusion, regardless of the proportion of individuals genotyped. The other required that ≥90% of individuals be genotyped at each SNP locus in at least three populations before being recorded. However, given the agreement we found between analyses of each data set, we report only results from the largest set of SNP loci here.

Data file conversion for other programs was performed using PGDSPIDER 2.0.4.0 (Fischer *et al.* 2011). For all three data sets, BAYESCAN 2.01 was used to examine individual markers for evidence of selection using the default settings, which stipulates that prior odds of a neutral model are 10 times higher than the model with selection at a locus (Foll & Gaggiotti 2008; Foll *et al.* 2010; Fischer *et al.* 2011). Alpha was used to indicate the direction of selection with a positive value suggesting diversifying selection and a negative value suggesting balancing selection. Results were viewed in R (R Core Team 2013). Any non-neutral markers were then separated. To examine differences in population clustering, a component analysis of the neutral and non-neutral markers sets was performed using the R package ADEGENET (Jombart 2008). Pairwise F_{ST} (Weir and Cockerham) values were calculated in GENODIVE 2.0B24 (Meirmans & Van Tienderen 2004) using an AMOVA with 1000 permutations. Population genetic structure based

on nuclear genotypes of 97 snails was inferred using STRUCTURE 2.3.4 (Pritchard *et al.* 2000). Initially, all three sets of SNP loci were examined with 10 replications of an admixture model with correlated allele frequencies using a burn-in of 100 000 followed by 200 000 generations, with the number of groups (K) set from 1 to 15. Once concordance across the runs was confirmed, a longer run on the full data set was implemented using an admixture model with correlated allele frequencies, a burn-in of 100 000 followed by 500 000 generations (10 replications), for numbers of groups (K) ranging from 1 to 12. Results from STRUCTURE were examined using STRUCTURE HARVESTER (Earl & von Holdt 2012), before being averaged across the 10 replications using CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007). Output genotype structure graphs were then regenerated in DISTRUCT 1.1 (Rosenberg 2004).

To examine the genotypes in the area of sympatry, STACKS and BAYESCAN were rerun (settings as above) using all the individuals from Ile des Pins from both species (*P. fibratus* and *P. porphyrostomus*). For this, SNP loci were included if ≥50% of individuals from each species (two groups) could be genotyped. Loci where variation was restricted to a rare allele in a single individual were removed for the BAYESCAN and STRUCTURE analyses. Evidence of selection was examined using BAYESCAN 2.01 as above. Population structure was analysed using STRUCTURE with the genotype data from the 16 *P. fibratus* and 32 *P. porphyrostomus* sampled from Ile des Pines and redrawn using CLUMPP 1.1.2 and DISTRUCT 1.1. Gene flow between the two species in sympatry was estimated from the sympatric SNP loci using MIGRATE-N 3.5.1 (Beerli 2006, 2009). The *P. porphyrostomus* data were randomly resampled to generate a subsample of 16 individuals to match the number of *P. fibratus*. To assess the levels of exchange, we used the Bayesian inference strategy implemented by MIGRATE-N, which estimates effective population sizes N_e and past migration rates *M* assuming a constant mutation rate (Beerli 2006, 2009). The starting values for θ (=$4N_e\mu$, where N_e is effective population size and μ is mutation rate) and M were generated from F_{ST} estimates in the first run and ensuing runs used the θ and *M* from the prior run. Convergence was examined after each run. In total, four analyses were undertaken. The uniform prior distributions were used for both θ and *M* parameters with metropolis sampling; one long chain was run recording 6000 steps every 100 steps after a burn-in of 50 000. The final run used a static heating scheme with four chains.

Environmental modelling

We used mixed linear modelling in R 3.1.2 (R Core Team 2013) to assess whether *Placostylus* shell shape variation across New Caledonia was influenced by local adaptation to the environment or neutral drift. Modelling used morphometric distance based on principal component scores (PC1 and PC2) to summarize relative shape similarities, neutral genetic diversity, geographic distances, and environmental attributes for the *Placostylus* population sample sites.

Environmental data were gathered using DIVA-GIS (Hijmans *et al.* 2013) to access the WORLDCLIM 1.4 (Hijmans *et al.* 2005) database and download the 19 available Bioclim variables (http://www.worldclim.org/bioclim). These global climate layers have a spatial resolution of about 1 km^2. Geographic distance between each pair of sites was determined using the function earth.dist of the FOSSIL package (Vavrek 2011) in R. We modelled the relationships between pairwise genetic distance (F_{ST} values obtained from the full SNP loci set with samples from Téouta and Gossanah combined), geographic distance, morphometric distance (PC1 and PC2 scores across populations, separately) and environmental distance (Bioclim) for the Grande Terre and Ouvéa population samples with complete data sets (Ouvéa, Forêt Nord, Pindai, Aoupinié, Nekoro).

Due to the nonindependence of the pairwise genetic distance and pairwise geographic distance data, mixed linear modelling was implemented when appropriate using the R package LME4 (Zuur *et al.* 2009; Bates 2010). ANOVA scores indicated that the most suitable model for the genetic, environment and morphological data was a mixed linear model with random intercepts over a mixed linear model with random intercepts and slopes (AIC −50.355, AIC −43.533). Thus, mixed modelling was used to examine the relationship between pairwise genetic distance and three variables; environmental distance, morphometric distance and geographic distance. A correlation between pairwise genetic and geographic distances was also studied using a Mantel test with 10 000 permutations implemented in the ADE4 package in R (Chessel *et al.* 2013).

To examine the relationship between morphology (shell shape), shell size (centroid size) and environmental conditions, standard linear models were fitted as the data were independent (i.e. not pairwise). General linear models (GLMs) were then used to determine how the environmental variables that were significantly associated with morphology correlated with one another, and to examine the relationship between shell size and shape. Finally, a mixed linear model with random intercepts was fitted using geographic distance and morphometric distance to evaluate whether there was a relationship between spatial separation and morphology.

Results

Geometric morphometric analysis

Sixteen population samples from Grande Terre, Ile des Pins and Ouvéa provided geometric data for 152 *Placostylus* snails (Fig. 1). Population samples ranged from three individuals (*P. fib*-aoupinié and *P. fib*-Mt Koghis) to 39 (*P. por*-nekoro). Principal component analysis (PCA) of all individuals and all landmarks showed that 75% of observed morphological variance was explained by the first three principal components. Individuals were found to group with others of the same species based on the current taxonomic classification and could be further subdivided using genetic evidence (Figs 2 and 3). Discriminant function analysis of geometric data supported the inference that individual snails could be correctly placed into their current species delimitation

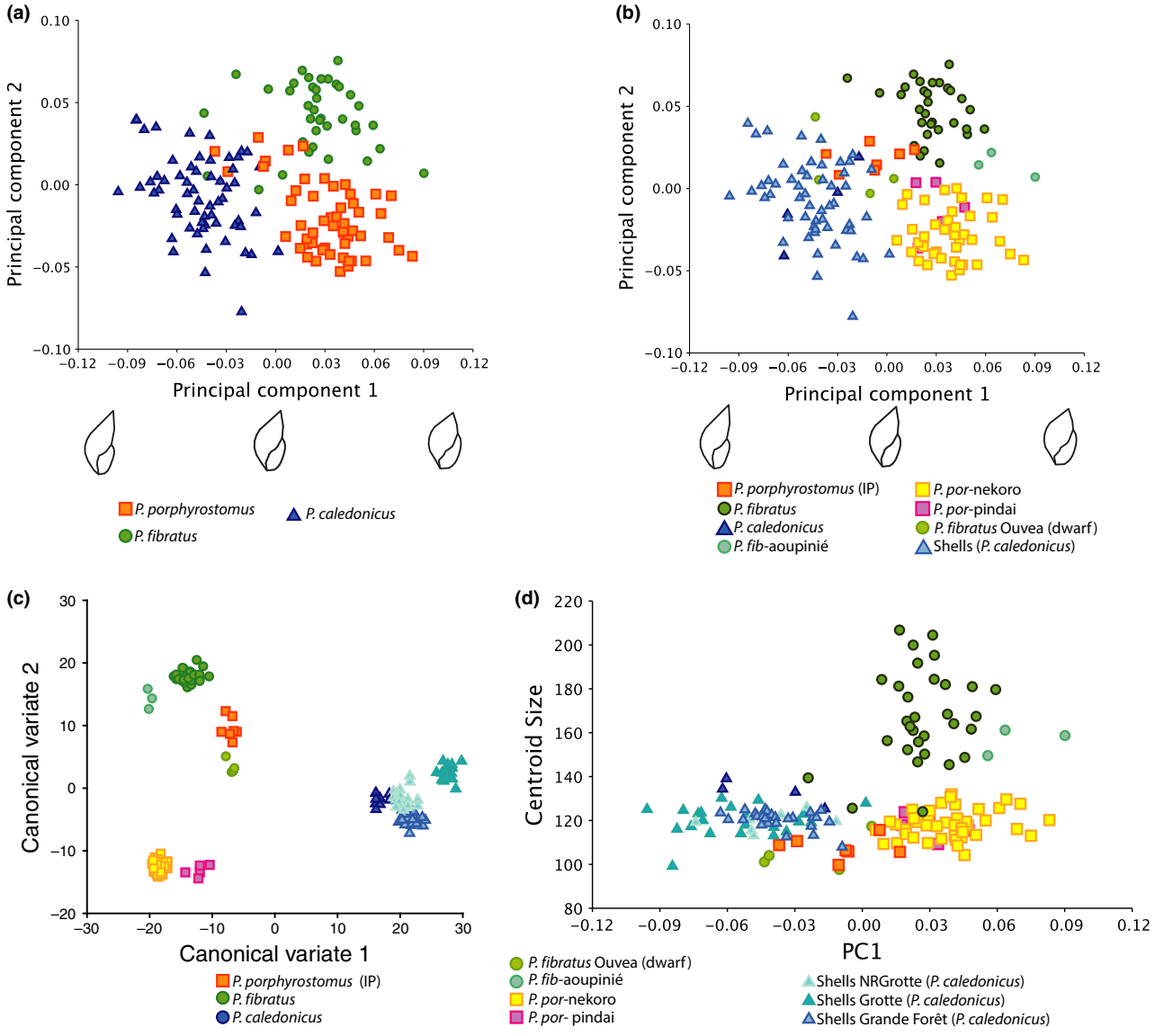

Fig. 2 Shell shape variation among three species of New Caledonian *Placostylus*, based on geometric morphometric analyses of six permanent and 22 semi-landmarks. (a) Snail shell shape as a function of the first two principal components (PCA) with individuals coloured by current taxonomy; *P. porphyrostomus* (orange squares), *P. fibratus* (green circles) and *P. caledonicus* (blue triangles). The shape deformation associated with PC1 is illustrated under the *x*-axis. (b) Snail shell shape as a function of the first two principal components with individuals coloured by genetic assignment (see Fig. 3) with the addition of a separate colour for samples of dwarf *P. fibratus* from Ouvéa (IP = Ile des Pins). (c) Canonical Variate analysis of the geometric data using genetic and location groupings (as in b). Samples that are genetically similar form morphological clusters that only partly conform to current taxonomy. (d) Linear regression of the relationship between shell shape (PC1) and shell size, samples coloured by genetic groups and location.

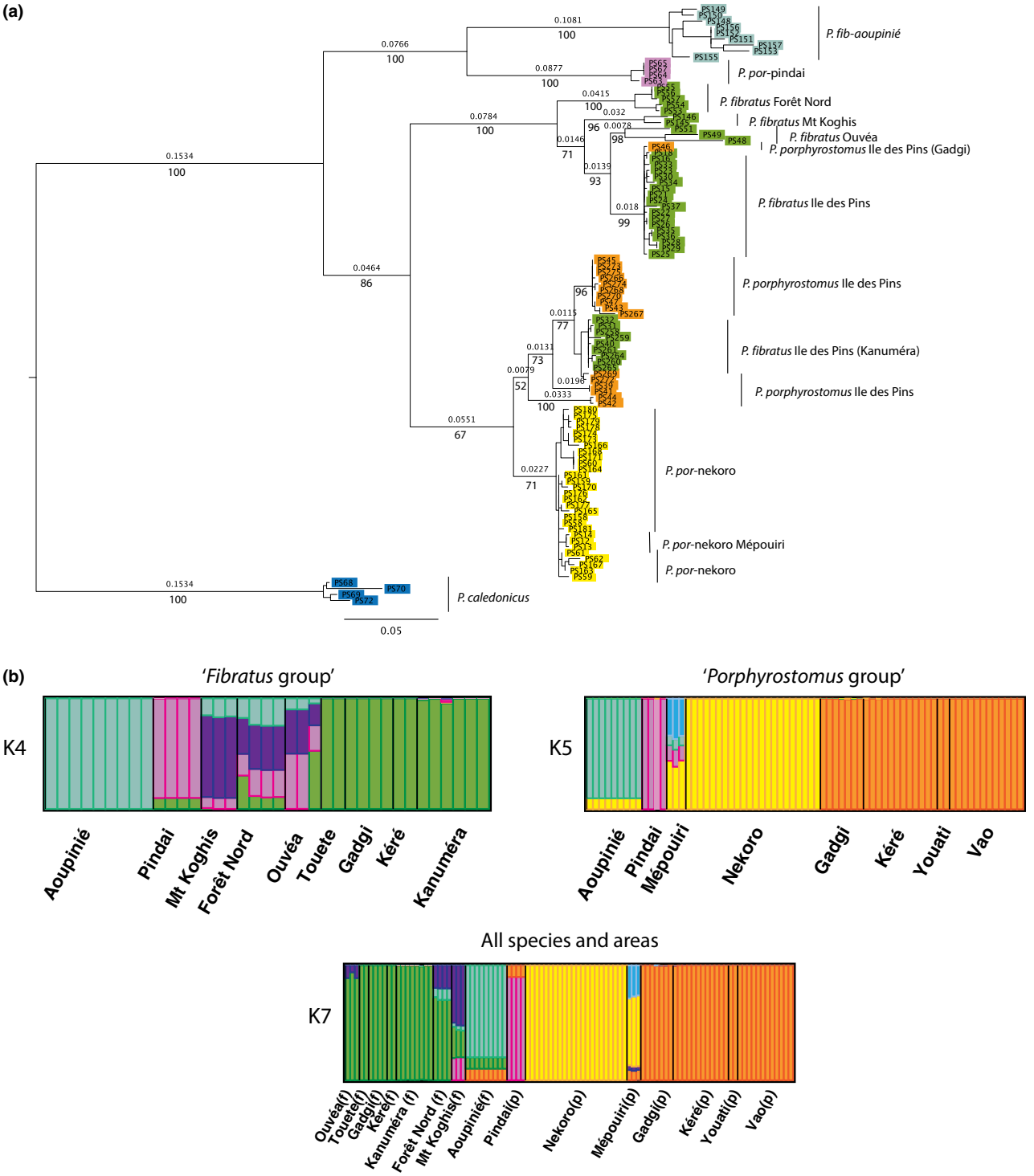

Fig. 3 Genetic variation among the New Caledonian *Placostylus*. (a) Phylogenetic relationships of New Caledonian *Placostylus* snails inferred using maximum-likelihood analysis of 830 bp of mtDNA ND2 sequence. Six genetic groupings can be identified: *P. caledonicus*, *P. fibratus* (Grande Terre and Ile des Pins), *P. por*-pindai, *P. porphyrostomus* (Ile des Pins), *P. por*-nekoro and *P. fib*-aoupinié. Colours represent the genetic assignments from the SNP data sets. (b) Bayesian assignment analysis using genotypes from 3764 nuclear SNP loci (STRUCTURE). The '*Fibratus* group' comprises nine population samples including Pindai due to uncertainty about its taxonomic placement ($K = 4$). The '*Porphyrostomus* group' analysis comprised 8 population samples, including Aoupinié due to uncertainty about its taxonomic placement. ($K = 5$). Analysis with all population samples of *P. fibratus* and *P. porphyrostomus* ($K = 7$) revealed similar clusters of individuals and concordance with the five groups identified by mtDNA sequences.

Table 1 Discriminant analysis of geometric shell shape data based on current species taxonomy, including all permanent and semi-landmarks. All samples from the three described species, *P. fibratus* (*P. fib*), *P. porphyrostomus* (*P. por*) and *P. caledonicus* (*P. cal*), were allocated to the correct group

Test	*P. fib–P. cal*	*Pfib–Pcal*	*Pfib–Ppor*	*Pfib–Ppor*	*P. cal–P. por*	*P. cal–P. por*
True Group	*P. fib*	*P. cal*	*P. fib*	*P. por*	*P. cal*	*P. por*
Allocated Group						
P. fib	38	0	38	0	—	—
P. por	—	—	0	54	0	54
P. cal	0	60	—	—	60	0

based on shell shape (Table 1). Concordance between genetic groupings and shell shape was also found with discriminant function analyses and canonical variate analysis (CVA) (Fig S2, Supporting information, Fig. 2). Only the placement of populations that lacked genetic data (putatively *P. caledonicus* based on location and shell shape) was not well resolved. The concordance of morphometric and genetic data was robust even with some (but not all) semi-landmarks removed (Fig S2, Supporting information).

Mitochondrial sequencing

In total, 100 individuals representing all sampled populations with living snails were sequenced for 830 bp of the mitochondrial gene ND2 (Fig. 3). The resulting phylogenetic tree (rooted with *P. caledonicus*) supports previous work using 16S and COI recognizing four lineages in two major clades within *P. fibratus* and *P. porphyrostomus* (Fig. 3) (Trewick *et al.* 2008). These clades did not correspond with current taxonomic classification. One clade contained two distinct lineages represented by the *P. fib*-aoupinié and *P. por*-pindai population samples. The other contained two lineages each with high levels of diversity within them: *P. fibratus* from eastern Grande Terre (Mt Koghis and Forêt Nord), Ile des Pins and Ouvéa, and *P. porphyrostomus* from Ile des Pins and *P. por*-nekoro. *Placostylus fibratus* and *P. porphyrostomus* were not represented by monophyletic clades.

Nuclear single nucleotide polymorphism data

We analysed RAD-Seq data from one read direction per fragment as this provided sufficient independent loci. After removing the second read and running process_radtags in STACKS, >40 million reads were retained with individuals averaging >3 000 000 reads each. A total of 150 532 loci were identified in the data set. In total, 149 New Caledonian *Placostylus* were sequenced for SNP analysis, but due to fragmented DNA, not all yielded adequate coverage. The individuals with poorest coverage were removed along with all *P. caledonicus* samples leaving 97 individuals for subsequent analysis. The more distantly related *P. caledonicus* shared a much lower proportion of polymorphic loci than was shared among *P. fibratus* and *P. porphyrostomus*. Removing poorer quality samples and *P. caledonicus* increased the number of loci retained per population when using a population proportional representation requirement for locus selection.

Evidence for selection was found in 65 SNP loci (Fig S3, Supporting information) using BAYESCAN, with diversifying selection inferred at 60 SNP loci. Non-neutral loci were separated from the neutral SNP loci, resulting in a total of 3764 neutral loci used in downstream analyses (Fig S3, Supporting information, Table 2). PCA of non-neutral SNP loci found similar population clusters as returned by analysis of mtDNA and neutral SNP loci (Figs 3 and 4). The populations *P. fib*-aoupinié and *P. por*-pindai clustered together in both neutral and non-neutral SNP sets (Fig. 4). Evidence for at least five genetically distinct groups (Fig. 3) was inferred from the Bayesian assignments of individual genotypes from STRUCTURE. Groupings inferred from the nuclear SNP data were similar to those inferred from their mtDNA haplotype data (Table 3). Two genetic clusters were evident within putative *P. fibratus*, the specimens from Aoupinié (*P. fib*-aoupinié) being genetically distinct from the others. Three genetic clusters were evident within putative *P. porphyrostomus*. Although Nekoro and Mépouiri samples (*P. por*-nekoro) were not genetically identical on SNP data, they did cluster together and apart from other populations. A second cluster (*P. por*-pindai) comprised the snails sampled from Pindai in Grande Terre, and third comprised all *P. porphyrostomus* sampled from Ile des Pins. There was some evidence of admixture between the Grande Terre *P. fibratus* (Mt Koghis and Forêt Nord) and *P. por*-pindai and *P. fib*-aoupinié samples. The F_{ST} results largely corroborated the assignment of genotype clusters inferred using STRUCTURE (Table 4).

Environmental modelling

We expected that morphological variation in *Placostylus* resulting from local adaptation would strongly correlate with environmental variation but that neutral genetic variation would be partitioned independently. To examine this, we compared the neutral genetic data with the environmental data and then morphological data. No environmental variables were found to have a relationship with neutral genetic variation (*t*-value: <1.395, *P*-value: >0.1). A model with variable intercepts comparing pairwise genetic distance (F_{ST}) to morphometric distance (PC1 and PC2) was also nonsignificant (*t*-value: 1.258, *P*-value: 0.224; *t*-value: −0.567, *P*-value: 0.578). Thus, we inferred that neutral genetic variation among these *Placostylus* snail populations was independent of both environment and shell morphology.

Table 2 Genotypes of New Caledonian *Placostylus* snails were generated using nuclear SNP loci, with two main data sets used in analyses. 'Raw SNP' indicates the output from STACKS and 'SNP neutral' indicates the total neutral SNP loci available. 'Total SNP no singles' indicates the final SNP set after removing loci that had a nucleotide substitution in just a single individual

SNP set	Sample size	Raw SNP	Neutral SNP	Total SNP no singles
All	97	5872	5807	3764
Sympatry	48	843	842	661

Next, we examined the relationship between shell morphology and the environment, which we would expect to be correlated under a local adaptation model. We found that six environmental variables were correlated significantly with morphology using PC1 (Fig. 5). All significant variables were associated with temperature: annual mean temperature, isothermality, minimum temperature of coldest month, mean temperature of wettest quarter, mean temperature of warmest quarter and mean temperature of coldest quarter. Linear modelling of these temperature-related environmental variables found them to be highly correlated with each other (*P*-value: <0.01). In contrast, the independent PC2 scores did not correlate significantly with any environmental variables (Fig S4, Supporting information) (*t*-values: <1.782, *P*-values: >0.11).

Importantly, we found that relevant variation in shell morphology reflected shell shape not size, as there was a nonsignificant relationship between shell size and morphometric distance for PC1 and PC2 (R^2: 0.4366, *P*-value: 0.063; R^2: −0.08012, *P*-value: 0.490). We note too that there was no relationship between morphological similarity (PC1 and PC2) and geographic distance (*t*-value: 0.000, *P*-value: 1; *t*-value: 0.000, *P*-value: 1). Our mixed linear model comparing F_{ST} to geographic distance provided no support for a correlation between the two (*t*-value: 0.625, *P*-value: 0.539). This lack of a positive relationship was confirmed using a Mantel test between pairwise genetic and geographic distance (*P*-value: 0.2139). Thus, the distribution of nuclear SNP

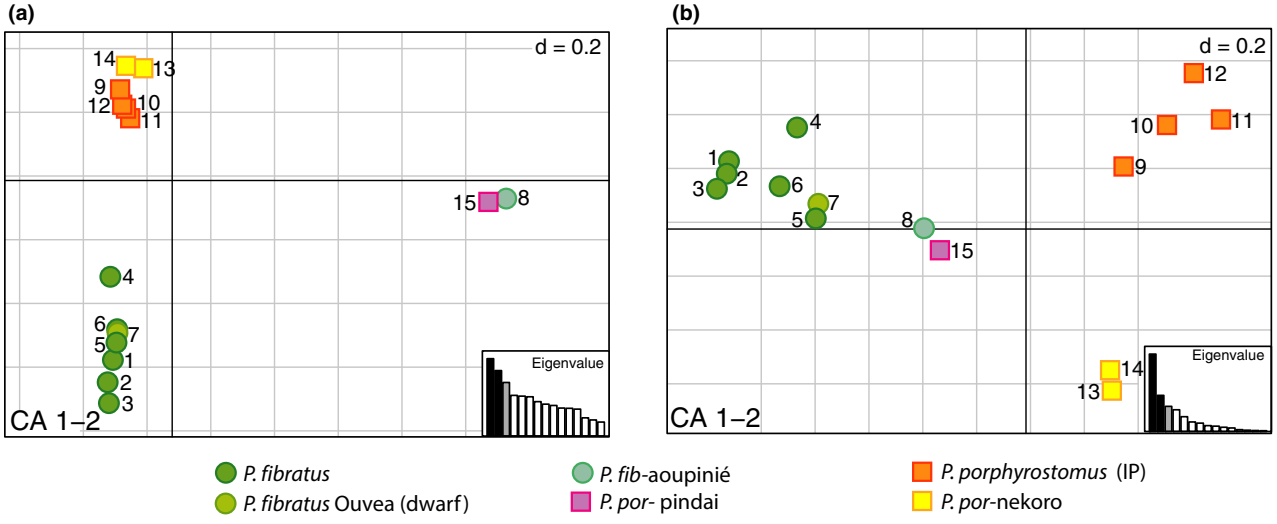

Fig. 4 Principle component analysis of 3764 neutral (a) and 65 non-neutral (b) loci identified in two *Placostylus* snail species from New Caledonia. Populations sampled: 1, *P. fib*-Touete; 2, *P. fib*-Gadgi; 3, *P. fib*-Kéré; 4, *P. fib*-Kanuméra; 5, *P. fib*-Forêt Nord; 6, *P. fib*-Mt Koghis; 7, *P. fib*-Ouvéa; 8, *P. fib*-aoupinié; 9, P. por–Gadgi; 10, *P. por*-Kéré; 11, *P. por*-Youati; 12, *P. por*-Vao; 13, *P. por*-nekoro; 14, *P. por*-Mépouiri; and 15, *P. por*-pindai. Eigenvalues show much of the variation is not displayed by the first two components particularly in the neutral loci.

Table 3 Estimates of genetic differentiation among population samples of *Placostylus* snails from New Caledonia. F_{ST} results based on the full SNP data set of 3764 loci using GENODIVE. Here, Ouvéa contains the data from samples collected at both Téouta and Gossanah. Populations from the Ile des Pins are identified as IP. The remaining populations are from Grande Terre except for the Ouvéa sample. Current species taxonomy is indicated in brackets, *P. fibratus* (f) and *P. porphyrostomus* (p)

	IP:Touete (f)	IP:Gadgi (f)	Forêt Nord (f)	IP:Kéré (f)	IP:Kanuméra (f)	Nekoro (p)	Mt Koghis (f)	Aoupinié (f)	Pindai (p)	IP:Gadgi (p)	IP:Kéré (p)	Mépouiri (p)	IP:Youati (p)	IP:Vao (p)	Ouvéa (f)
IP:Touete (f)	0	0.093	0.4	0.08	0.182	0.651	0.458	0.584	0.643	0.678	0.545	0.604	0.657	0.551	0.509
IP:Gadgi (f)	0.093	0	0.411	0.075	0.263	0.67	0.5	0.67	0.704	0.7	0.603	0.652	0.709	0.585	0.484
Forêt Nord (f)	0.4	0.411	0	0.424	0.588	0.6	0.385	0.603	0.666	0.68	0.63	0.633	0.672	0.601	0.563
IP:Kéré (f)	0.08	0.075	0.424	0	0.208	0.688	0.487	0.636	0.692	0.689	0.596	0.612	0.694	0.576	0.539
IP:Kanuméra (f)	0.182	0.263	0.588	0.208	0	0.697	0.533	0.735	0.875	0.369	0.607	0.654	0.628	0.55	0.202
Nekoro (p)	0.651	0.67	0.6	0.688	0.697	0	0.612	0.622	0.657	0.385	0.476	0.231	0.448	0.513	0.614
Mt Koghis (f)	0.458	0.5	0.385	0.487	0.533	0.612	0	0.582	0.596	0.634	0.561	0.561	0.6	0.522	0.514
Aoupinié (f)	0.584	0.67	0.603	0.636	0.735	0.622	0.582	0	0.49	0.684	0.631	0.575	0.72	0.68	0.614
Pindai (p)	0.643	0.704	0.666	0.692	0.875	0.657	0.596	0.49	0	0.74	0.689	0.585	0.685	0.646	0.715
IP:Gadgi (p)	0.678	0.7	0.68	0.689	0.369	0.385	0.634	0.684	0.74	0	0.058	0.494	0.005	0.031	0.689
IP:Kéré (p)	0.545	0.603	0.63	0.596	0.607	0.476	0.561	0.631	0.689	0.058	0	0.48	0.083	0.119	0.602
Mépouiri (p)	0.604	0.652	0.633	0.612	0.654	0.231	0.561	0.575	0.585	0.494	0.48	0	0.348	0.44	0.474
IP:Youati (p)	0.657	0.709	0.672	0.694	0.628	0.448	0.6	0.72	0.685	0.005	0.083	0.348	0	0.058	0.672
IP:Vao (p)	0.551	0.585	0.601	0.576	0.55	0.513	0.522	0.68	0.646	0.031	0.119	0.44	0.058	0	0.598
Ouvéa (f)	0.509	0.484	0.563	0.539	0.202	0.614	0.514	0.614	0.715	0.689	0.602	0.474	0.672	0.598	0

Table 4 Estimates of gene flow between two New Caledonian *Placostylus* snail species on Ile des Pins using a Bayesian approach implemented in MIGRATE-N with 661 SNP loci. There is evidence for very low levels of gene flow between *P. fibratus* and *P. porphyrostomus* on Ile des Pins. Showing $\theta = 4\ N_e\mu$, where N_e is effective population size and μ is mutation rate. Numbers of immigrants per generation (Nm) were calculated from mutation scaled population size and migration rate estimates from MIGRATE $\theta_1 M_{2->1} = 4Nm_1$

All Loci	Mean	0.025	0.975
θ1 *P. fibratus*	0.0209	0.0120	0.0297
θ2 *P. porphyrostomus*	0.0213	0.0123	0.0277
Nm *P. fibratus*	0.1134	0.0141	0.2819
Nm *P. porphyrostomus*	0.1156	0.0145	0.2819

not explained by geographic distance (IBD) in New Caledonian *Placostylus*.

Sympatric snails

Sympatric *P. fibratus* and *P. porphyrostomus* on the Ile des Pins belong to separate mtDNA clades (Fig. 6), and principal component analysis of geometric data showed clear morphological differences between them. There was no overlap in shape using the first two principal components (Fig. 6).

Analysis of SNP data told a similar tale. Of 843 SNP loci that met the required criteria, single nucleotide substitutions that were observed in only one individual (rare alleles) accounted for 182 loci leaving a data set of 661 SNP loci for BAYESCAN and STRUCTURE analyses. Of these, a single SNP locus was identified as non-neutral by BAYESCAN. Clear population distinction between the two morphotypes/species on Ile des Pins was resolved with STRUCTURE (Fig. 6). Low levels of inferred gene flow between the two species were estimated as ~0.1 total migrants per generation (Table 4), with similar estimates in each direction.

Discussion

The patterns of phenotypic variation in nature and the processes that produce distinct forms and varieties are central to evolutionary biology. By seeking to understand the processes that give rise to morphological diversity within a lineage and the formation of local forms, we are closer to understanding the origin of new species. Ecological speciation is thought to begin with the formation of ecotypes, which arise through local adaptation to local conditions (Rundle & Nosil 2005; Hendry *et al.* 2007; Räsänen & Hendry 2008). The local adaptation model generates predictable patterns, including convergence of phenotype in reproductively

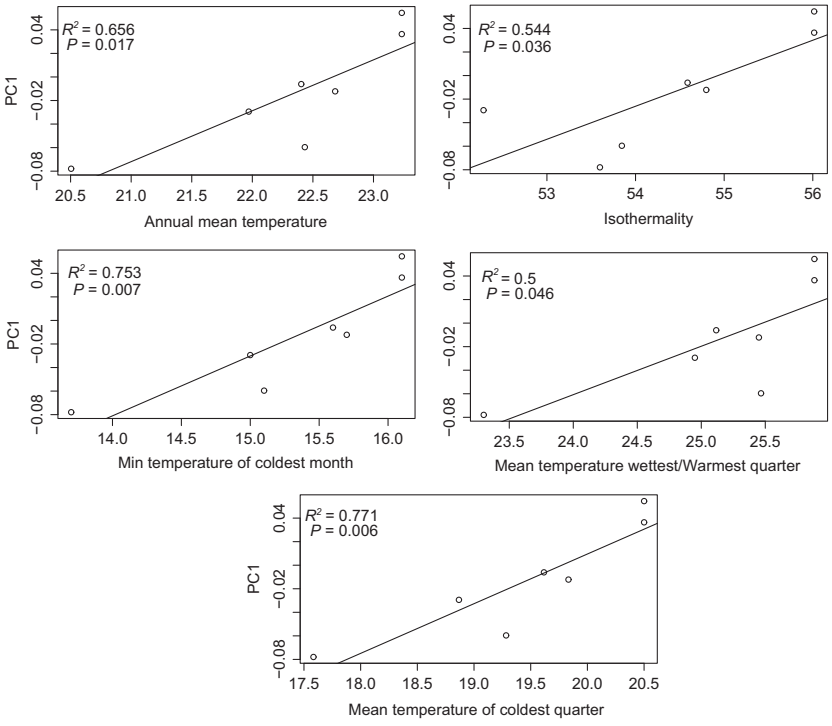

Fig. 5 Environmental conditions are associated with New Caledonian *Placostylus* snail shell shape as revealed by the relationship between temperature and morphology (PC1) across Grande Terre and Ouvéa.

isolated lineages via the same selective forces, and a lack of correlation between phenotype and neutral markers or geographic distance.

Within the New Caledonian *Placostylus* snails studied here, shell shape and genetic data are largely concordant, supporting five distinct lineages where two species had previously been recognized based on soft tissue traits (Neubert *et al*. 2009). Ile des Pins is the only currently known region within New Caledonia where two *Placostylus* species exist in sympatry. MtDNA and nuclear SNP data reveal strong genetic signal for separation between the two described species here, *P. fibratus* and *P. porphyrostomus*. In line with this, geometric morphometric analysis of Ile des Pins snails also revealed distinct phenotypic clusters concordant with current taxonomy and genotypic clusters. The successful discrimination of individuals into species based on just shell morphometrics is strong evidence that neither shell plasticity nor hybridization is blurring the phenotypic distinction of these two taxa. Ecological differences between the two species have yet to be formally described, but fine-scale habitat partitioning as has been described in other sympatric snail species (e.g. *Partula*) is possible (Johnson *et al.* 1993; Murray *et al.* 1993). On Ile des Pins, *Placostylus porphyrostomus* might be more common in dryer coastal areas, while *P. fibratus* is predominantly found in denser inland forest (Brescia 2011) although our sampling included individuals of both species from the same sites (e.g. Gadgi). Despite the clear genetic and morphological clustering revealed, we also detected evidence for low levels of gene flow between the two species on Ile des Pins in the mtDNA introgression and genotype assignment probabilities for a small number of individuals. Symmetrical gene flow was estimated to involve fewer than 0.12 (0.01–0.3) migrants per generation. That this has resulted in neither extensive introgression nor loss of distinctive shell shape suggests divergent ecological selection is maintaining these separate lineages (Mallet 2008; Nosil 2008; Nosil & Schluter 2011).

Morphological variation may result from genetic differences or phenotypic plasticity. The presence of two species with distinct forms in sympathy on Ile des Pins strongly suggests a genetic component to shell shape. The environmental variables used to examine the relationship between environment and shell shape in New Caledonia were chosen to reflect broad scale changes in environment between distinct populations. Geometric morphometrics allowed us to study shell shape variation in greater detail than previously possible and without the confounding influence of size. We determined that shell shape variation among *Placostylus* populations was greater than that within populations. We found a strong relationship between some morphological variation and local temperatures, as expected if selection by the local environment was driving variation in shell shape across New Caledonia. In contrast, there was no relationship between neutral genetic divergence and the

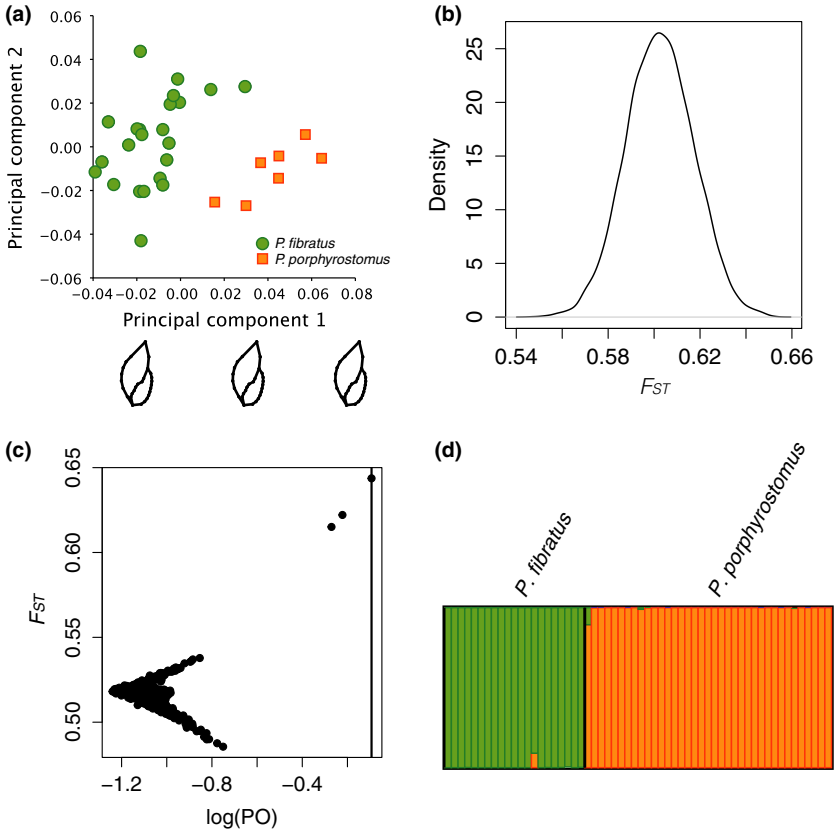

Fig. 6 Two species of *Placostylus* in sympatry on Ile des Pins, New Caledonia, are clearly distinguished by shell shape. (a) Snail shell shape as a function of the first two principal components shows no overlap between samples of *P. fibratus* and *P. porphyrostomus*. The deformation (i.e. translation of landmarks) inferred from the shape changes associated with PC1 are indicated along the *x*-axis. (b) Frequency distribution of F_{ST} estimates between *P. fibratus* and *P. porphyrostomus* from each of 661 nuclear SNP loci. (c) Almost all 661 SNP loci were neutral as indicated by BAYESCAN plot, but a single locus showed evidence of selection. Posterior odds (log(PO)) of significance is plotted against F_{ST} estimates; the vertical line is the 5% threshold of false discovery. (d) Inference of population assignment of snails from Ile des Pins using genotypes from 661 SNP loci and a Bayesian approach implemented in STRUCTURE, $K = 2$. Genotype clusters separate the two sympatric species, *P. porphyrostomus* and *P. fibratus*. The two individuals with the highest amounts of genetic admixture were from Gadgi and Kanuméra, the same two population samples that revealed mtDNA sharing in the haplotype tree (Fig. 3).

environment variables, suggesting the various shell forms across New Caledonia are the result of independent local adaptation to the environment.

Land snails are sensitive to environmental conditions and desiccation is a major environmental factor for *Placostylus* as in other land snails (Giokas *et al.* 2014). This is likely reflected in the shell shape differentiation among populations. On Grande Terre, slimmer shell apertures and compact shell shapes are associated with warmer and drier western forest (Pindai and Nekoro), while wider apertures with elongated shell shapes are found in the cooler and wetter central/eastern areas (Aoupinié, Mt Koghis and Forêt Nord). Morphological adaptation of this type can be rapid in snails (e.g. Chiba 1996; Stankowski 2013; Wada & Chiba 2013). In New Caledonia, a dwarf form of *P. fibratus* exists on the young island of Ouvéa, which emerged from the seabed with the other Loyalty Islands within the last 2 million years (Pelletier 2006; Neall & Trewick 2008). The snails in the Ouvéa population differ from *P. fibratus* on Grande Terre primarily in their much smaller size, although shape also contributes to the difference. However, the Grande Terre and Ouvéa population samples did not differ significantly based on neutral markers. Genetic similarity and geological youth of the landscape indicate a comparatively rapid rate of morphological change, although a plastic phenotypic response may contribute. Overall reduction in shell size (rather than shape) is a likely outcome of growth in an environment with some resource limitation (e.g. aridity) (Machin 1967; Perrott *et al.* 2007; Proćków *et al.* 2012). Phenotypic plasticity might be of greater importance in regard to microhabitat variation within populations, which were not considered in this study. Laboratory studies

(Schilthuizen & Kellermann 2014) or transplantation experiments, which have successfully shown adaptive phenotypic plasticity in *Littorina saxatilis* snails (Hollander & Butlin 2010), would be needed to determine the extent of the *Placostylus* species plasticity.

The relationship between morphological adaptation and the environment is particularly compelling when examining instances of convergent evolution. We found that convergence of shell shape between the populations at Pindai and Nekoro has resulted in them being classified as the same species, *P. porphyrostomus*, because of their similar shell shapes (Figs 1 and 2). The populations occur in similar local environments in close proximity to one another, yet genetically, *P. por*-pindai and *P. por*-nekoro samples form two distinct genetic clusters and are associated with two separate lineages. Together these data suggest a common selective force, probably linked to environmental temperature extremes, has driven independent local adaptation and convergence of shell morphology. This strongly supports the idea that morphological variation is the result of adaptation not drift.

Evidence of phenotypic divergence between geographically and genetically neighbouring populations of New Caledonian *Placostylus* snails was detected. In central Grande Terre, the phylogenetic sister populations, *P. por*-pindai and *P. fib*-aoupinié, are spatially adjacent but occur in contrasting environments (Fig. 1). The two species cluster together in both the neutral and non-neutral SNP loci examined. Nevertheless, these populations are morphologically and genetically distinct, suggesting divergent ecological selection could be driving morphological evolution. On Grande Terre, population clustering based on the neutral-nuclear SNP data suggests that populations of *P. fibratus* at Mt Koghis and Forêt Nord were formerly subject to admixing from the *P. fib*-aoupinié and *P. por*-pindai populations. Although *Placostylus* populations on Grande Terre are now allopatric, the sharing of some nuclear alleles suggests gene flow between populations was probably more common in the recent past. With near-continuous forest across the island prior to European colonization, snail populations on Grande Terre would have been more contiguous. In such circumstances, diverging ecological selection can drive adaptation of populations to local environmental conditions, despite some gene flow, and result in the formation of ecotypes (Rundle & Nosil 2005; Mallet *et al*. 2009).

New Caledonia has a diverse environment with extremely high levels of biodiversity. The high level of plant and animal micro-endemism is thought to be predominantly the product of allopatric speciation (Grandcolas *et al*. 2008), and it might therefore have been predicted that speciation in slow-moving giant land snails would be dominated by spatial geography. Our results are contrary to this. Detecting shell shape variation that is specialized to local environmental conditions provides evidence of natural selection at work rather than random genetic drift (Giokas *et al*. 2014). Convergence in separate lineages further supports an instrumental role of adaptation (Davison & Chiba 2006), and the ecotypes that result could be the first steps in the origin of new species (Stankowski 2013). Although shell shape variation in *Placostylus* has been considered to be arbitrary and uninformative, the hidden diversity we found within two New Caledonian *Placostylus* taxa appears to have been driven by independent adaptation to local conditions.

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References

Appleton RD, Palmer AR (1988) Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. *Proceedings of the National Academy of Sciences*, **85**, 4387–4391.

Bartish IV, Swenson U, Munzinger JRM, Anderberg AA (2005) Phylogenetic relationships among New Caledonian Sapotaceae (Ericales): molecular evidence for generic polyphyly and repeated dispersal. *American Journal of Botany*, **92**, 667–673.

Bates DM (2010) lme4: Mixed-effects modeling with R. URL http://lme4. r-forge. r-project. org/book.

Beerli P (2006) Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. *Bioinformatics*, **22**, 341–345.

Beerli P (2009) *How to Use MIGRATE or Why are Marko Chain Monte Carlo Programs Difficult to Use?* Cambridge University Press, New York.

Brescia F (2011) *Ecology and population trends in New Caledonian Placostylus snails (Mollusca: Gastropoda: Bulimulidae): a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Zoology at Massey University, Palmerston North, New Zealand*.

Buerkle A, Gompert Z (2013) Population genomics based on low coverage sequencing: how low should we go? *Molecular Ecology*, **22**, 3028–3035.

Butlin RK, Saura M, Charrier G *et al.* (2014) Parallel evolution of local adaptation and reproductive isolation in the face of gene flow. *Evolution*, **68**, 935–949.

Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH (2011) Stacks: building and genotyping loci *de novo* from

short-read sequences. *G3: Genes, Genomes, Genetics*, **1**, 171–182.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. *Molecular Ecology*, **22**, 3124–3140.

Chessel D, Dufour A, Thioulouse J (2013) The ade4 package. *R NEWS*.

Chiba S (1996) A 40,000-year record of discontinuous evolution of Island snails. *Paleobiology*, **22**, 177–188.

Crampton JS, Gale AS (2005) A plastic boomerang: speciation and intraspecific evolution in the Cretaceous bivalve Actinoceramus. *Paleobiology*, **31**, 559–577.

Davison A (2002) Land snails as a model to understand the role of history and selection in the origins of biodiversity. *Population Ecology*, **44**, 0129–0136.

Davison A, Chiba S (2006) The recent history and population structure of five Mandarina snail species from subtropical Ogasawara (Bonin Islands, Japan). *Molecular Ecology*, **15**, 2905–2919.

Doyle S, MacDonald B, Rochette RM (2010) Is water temperature responsible for geographic variation in shell mass of Littorina obtusata (L.) snails in the Gulf of Maine? *Journal of Experimental Marine Biology and Ecology*, **394**, 98–104.

Earl D, von Holdt B (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

Espeland M, Murienne J (2011) Diversity dynamics in New Caledonia: towards the end of the museum model? *BMC Evolutionary Biology*, **11**, 254.

Fischer MC, Foll M, Excoffier L, Heckel G (2011) Enhanced AFLP genome scans detect local adaptation in high-altitude populations of a small rodent (Microtus arvalis). *Molecular Ecology*, **20**, 1450–1462.

Fitzpatrick BM (2012) Underappreciated consequences of phenotypic plasticity for ecological speciation. *International Journal of Ecology*, **2012**, 256017.

Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a bayesian perspective. *Genetics*, **180**, 977–993.

Foll M, Fischer MC, Heckel G, Excoffier L (2010) Estimating population structure from AFLP amplification intensity. *Molecular Ecology*, **19**, 4638–4647.

Geneious version 6.1 created by Biomatters. Available from http://www.geneious.com/.

Giokas S, Påll-Gergely B, Mettouris O (2014) Nonrandom variation of morphological traits across environmental gradients in a land snail. *Evolutionary Ecology*, **28**, 323–340.

Goodfriend GA (1986) Variation in Land-snail Shell form and Size and its Causes: a Review. *Systematic Biology*, **35**, 204–223.

Goodfriend GA, Gould SJ (1996) Paleontology and chronology of two evolutionary transitions by hybridization in the bahamian land snail cerion. *Science*, **274**, 1894–1897.

Grandcolas P, Murienne JRM, Robillard T *et al.* (2008) New caledonia: a very old darwinian island? *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 3309–3317.

Greve C, Gimnich F, Hutterer R, Misof B, Haase M (2012) Radiating on oceanic islands: patterns and processes of speciation in the land snail genus Theba (Risso 1826). *PLoS ONE*, **7**, e34339.

Haase M, Misof B (2009) Dynamic gastropods: stable shell polymorphism despite gene flow in the land snail Arianta arbustorum. *Journal of Zoological Systematics and Evolutionary Research*, **47**, 105–114.

Hendry AP, Nosil P, Rieseberg LH (2007) The speed of ecological speciation. *Functional Ecology*, **21**, 455–464.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hijmans RJ, Guarino L, Bussink C *et al.* (2013) DIVA-GIS. Vsn. 7.5. A geographic information system for the analysis of species distribution data. Manual available at http://www.diva-gis.org.

Hills SFK, Trewick SA, Morgan-Richards M (2011) Phylogenetic information of genes, illustrated with mitochondrial data from a genus of gastropod molluscs. *Biological Journal of the Linnean Society*, **104**, 770–785.

Hills SFK, Crampton JS, Trewick SA, Morgan-Richards M (2012) DNA and morphology unite two species and 10 million year old fossils. *PLoS ONE*, **7**, e52083.

Hollander J, Butlin R (2010) The adaptive value of phenotypic plasticity in two ecotypes of a marine gastropod. *BMC Evolutionary Biology*, **10**, 333.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801–1806.

Johannesson B (1996) Population differences in behaviour and morphology in the snail Littorina saxatilis: phenotypic plasticity or genetic differentiation? *Journal of Zoology*, **240**, 475–493.

Johnson M, Murray J, Clarke B *et al.* (1993) The ecological genetics and adaptive radiation of Partula on Moorea. *Oxford Surveys in Evolutionary Biology*, **9**, 167–238.

Jombart T (2008) Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**, 1403–1405.

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters*, **7**, 1225–1241.

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, **11**, 353–357.

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nature Methods*, **9**, 357–359.

Machin J (1967) Structural adaptation for reducing water-loss in three species of terrestrial snail. *Journal of Zoology*, **152**, 55–65.

Mallet J (2008) Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 2971–2986.

Mallet J, Meyer A, Nosil P, Feder JL (2009) Space, sympatry and speciation. *Journal of Evolutionary Biology*, **22**, 2332–2341.

Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, **4**, 792–794.

Merilä J, Hendry AP (2014) Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications*, **7**, 1–14.

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees, 1–8.

Milne I, Bayer M, Cardle L *et al.* (2010) Tablet–next generation sequence assembly visualization. *Bioinformatics*, **26**, 401–402.

Minards NA, Trewick SA, Godfrey AJR, Morgan-Richards M (2014) Convergent local adaptation in size and growth rate but not metabolic rate in a pair of parapatric Orthoptera species. *Biological Journal of the Linnean Society*, **113**, 123–135.

Murienne J, Guilbert E, Grandcolas P (2009) Species' diversity in the New Caledonian endemic genera Cephalidiosus and Nobarnus (Insecta: Heteroptera: Tingidae), an approach using phylogeny and species' distribution modelling. *Biological Journal of the Linnean Society*, **97**, 177–184.

Murray J, Clark B, Johnson MS (1993) Adaptive radiation and community structure of partula on moorea. *Proceedings: Biological Sciences*, **254**, 205–211.

Nattier R, Robillard T, Desutter-Grandcolas L, Couloux A, Grandcolas P (2011) Older than new caledonia emergence? A molecular phylogenetic study of the eneopterine crickets (Orthoptera: Grylloidea). *Journal of Biogeography*, **38**, 2195–2209.

Neall VE, Trewick SA (2008) The age and origin of the Pacific islands: a geological overview.

Neubert E, Cherel-Mora C, Bouchet P (2009) Polytypy, clines, and fragmentation: the bulimes of New Caledonia revisited (Pulmonata, Orthalicoidea, Placostylidae). *Zoologia Neocaledonica*, **7**, 37–131.

Nosil P (2008) Speciation with gene flow could be common. *Molecular Ecology*, **17**, 2103–2106.

Nosil P, Feder JL (2012) Genomic divergence during speciation: causes and consequences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **367**, 332–342.

Nosil P, Schluter D (2011) The genes underlying the process of speciation. *Trends in Ecology & Evolution*, **26**, 160–167.

Pelletier B (2006) Geology of the New Caledonia region and its implications for the study of the New Caledonian biodiversity. In: *Compendium of Marine Species from New Caledonia*, Doc. Sci. Tech. II7, seconde édition, 17–30. IRD, Nouméa.

Perrott JK, Levin II, Hyde EA (2007) Morphology, distribution and desiccation in the brown garden snail (Cantareus aspersus) in northern New Zealand. *New Zealand Journal of Ecology*, **31**, 60.

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double Digest RADseq: an inexpensive method for *de novo* SNP discovery and genotyping in model and non-model species. *PLoS ONE*, **7**, e37135.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Proćków M, Kuźnik-Kowalska E, Lewandowska M (2012) Differences in population dynamics of Bradybaena fruticum (OF Müller, 1774)(Gastropoda: Pulmonata: Bradybaenidae) in a submontane and lowland area of Poland. *Animal Biology*, **62**, 451–462.

Quesada H, Posada D, Caballero A, Morán P, Rolán-Alvarez E (2007) Phylogenetic evidence for multiple sympatric ecological diversification in a marine snail. *Evolution*, **61**, 1600–1612.

R Core Team (2013) *R: A Language and Environment for Statistical Computing*. R foundation for Statistical Computing, Vienna, Austria. Retrieved from http//http://www.R-project.org.

Räsänen K, Hendry AP (2008) Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecology Letters*, **11**, 624–636.

Rohlf FJ (2013) TpsDig2, Version 2.17. Available at: http://life.bio.sunysb.edu/morph/.

Rosenberg NA (2004) Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.

Rundle HD, Nosil P (2005) Ecological speciation. *Ecology Letters*, **8**, 336–352.

Saura M, Rivas MJ, Diz AP, Caballero A, Rolan-Alvarez E (2012) Dietary effects on shell growth and shape in an intertidal marine snail, Littorina saxatilis. *Journal of Molluscan Studies*, **78**, 213–216.

Schilthuizen M, Kellermann V (2014) Contemporary climate change and terrestrial invertebrates: evolutionary versus plastic changes. *Evolutionary Applications*, **7**, 56–67.

Senn H, Ogden R, Cezard T *et al.* (2013) Reference-free SNP discovery for the Eurasian beaver from restriction site–associated DNA paired-end data. *Molecular Ecology*, **22**, 3141–3150.

Sheets HD (2012) IMP714 – Integrated Morphometrics Package. Available from: http://www3canisius.edu/~sheets/imp714.htm.

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

Stankowski S (2011) Extreme, continuous variation in an island snail: local diversification and association of shell form with the current environment. *Biological Journal of the Linnean Society*, **104**, 756–769.

Stankowski S (2013) Ecological speciation in an island snail: evidence for the parallel evolution of a novel ecotype and maintenance by ecologically dependent postzygotic isolation. *Molecular Ecology*, **22**, 2726–2741.

Teshima H, Davison A, Kuwahara Y *et al.* (2003) The evolution of extreme shell shape variation in the land snail Ainohelix editha: a phylogeny and hybrid zone analysis. *Molecular Ecology*, **12**, 1869–1878.

Thibert-Plante X, Hendry AP (2011) The consequences of phenotypic plasticity for ecological speciation. *Journal of Evolutionary Biology*, **24**, 326–342.

Trewick SA, Paterson AM, Campbell HJ (2007) Hello New Zealand. *Journal of Biogeography*, **34**, 1–6.

Trewick S, Brescia F, Jordan C (2008) Diversity and phylogeny of New Caledonian Placostylus land snails; evidence from mitochondrial DNA. *Zoologia Neocaledonica*, **6**, 421–436.

Trussell GC (2000) Phenotypic clines, plasticity, and morphological trade-offs in an intertidal snail. *Evolution*, **54**, 151–166.

Vavrek MJ (2011) Fossil: palaeoecological and palaeogeographical analysis tools. *Palaeontologia Electronica*, **14**, 16.

Wada S, Chiba S (2013) The dual Protection of a micro land snail against a micro predatory snail. *PLoS ONE*, **8**, e54123.

Wagner CE, Keller I, Wittwer S *et al.* (2013) Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. *Molecular Ecology*, **22**, 787–798.

Woodruff DS, Gould SJ (1987) Fifty years of interspecific hybridization: genetics and morphometrics of a controlled experiment on the land snail Cerion in the Florida Keys. *Evolution*, **41**, 1022–1045.

Zelditch ML, Swiderski DL, Sheets HD, Fink WL (2004) *Geometric Morphometrics for Biologists: A Primer*. Elsevier, San Diego, California.

Zerbino D, Birney E (2008) Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Research*, **18**, 821–829.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York.

E.J.D., S.A.T., M.M.R. and F.B. designed project and undertook field sampling. E.J.D. performed laboratory research and analysis. E.J.D., M.M.R., S.A.T. and F.B. wrote and edited manuscript.

Data accessibility

Data are accessible through Dryad: doi:10.5061/dryad.nb526. This includes all raw reads, morphometric data, environmental data and SNP data sets.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Geometric morphometric analysis of New Caledonian *Placostylus* snails used six permanent landmarks (grey/green) and 22 semi-landmarks (black/red).

Fig. S2 The genetic data generated for New Caledonian *Placostylus* snails contained mostly neutral loci.

Fig. S3 No significant relationship was found between PC2 and any environmental conditions across Grande Terre and Ouvéa.

Table S1 Morphometric analysis of New Caledonian *Placostylus* snails using discriminate function analysis of the geometric data using the genetic groupings; *P. por* (*P. porpyrostomus* from Ile des Pins), *P. fib* (*P. fibratus* from Ile des Pins, Ouvéa, Forêt Nord and Mt Koghis), *P. cal* (*P. caledonicus* from Koumac), *P.* 'nek' (*P. por*-nekoro), *P.* 'pin' (*P. por*-pindai), *P.* 'aou' (*P. fib*-aoupinié), and the three locations from which no genetic data were collected and therefore have unknown genetic groupings: *P.* 'GR' (Grotte), *P.* 'NG' (NRGrotte) and *P.* 'GF' (Grande Forêt).