RESEARCH ARTICLE

Taxonomic and conservation status of a newly discovered giant landsnail from Mount Augustus, New Zealand

Steven A. Trewick · Kath J. Walker · Corina J. Jordan

Abstract The Rhytidae (Mollusca; Gastropoda; Pulmonata) are a group of large carnivorous land snails distributed in the southern hemisphere, with a particularly rich fauna in New Zealand. The endemic genus *Powelliphanta* consists of at least 10 species and many more recognised subspecies, most of which are restricted to the western margin of South Island, New Zealand. *Powelliphanta* taxa tend to have restricted ecological and spatial ranges among the mountains of this region, with some species being limited to lowland forest and others to habitats at or above the treeline. Among recent discoveries is a population of snails occupying habitat on and around a peak called Mt Augustus, which is situated at the edge of a large and economically important coalfield. Since recognition of the potential biological significance of the Mt Augustus snails in 2004, almost all of their habitat has been destroyed by opencast mining revealing a direct conflict between economic and biodiversity prioritisation. Our analysis of mtDNA sequence data indicate *Powelliphanta* "Augustus" is a distinctive evolutionary lineage, more closely related to a nearby lowland species *Powelliphanta lignaria* than the spatial and ecological neighbour *Powelliphanta patrickensis*. *Powelliphanta* "Augustus" appears to be a specialised local endemic species. Despite a growing international awareness of the importance of biodiversity conservation, the demand for foreign earnings continues to take priority over the protection of our biota.

Keywords mtDNA · Coal mining · Extinction · Mollusc · Biodiversity · Gastropod

S. A. Trewick (✉) · C. J. Jordan
Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, Private Bag 11-222, Palmerston North, New Zealand
e-mail: s.trewick@massey.ac.nz

C. J. Jordan
e-mail: C.J.Jordan@massey.ac.nz

K. J. Walker
Research, Development and Improvement Division, Department of Conservation, Private Bag 5, Nelson 7042, New Zealand
e-mail: kwalker@doc.govt.nz

Introduction

The Rhytidae (Mollusca; Gastropoda; Pulmonata) are a group of large carnivorous land snails distributed in the southern hemisphere (South Africa, New Guinea, Australia, New Zealand and islands in the south west Pacific), with a particularly rich fauna in New Zealand (Spencer et al. 2004). *Powelliphanta* O'Connor 1945 is an endemic genus that is almost entirely restricted to the region west of the major mountain chain that dissects South Island, New Zealand. Tectonic activity has yielded a mountainous landscape in the west, with a high rainfall due to orographic lift of moist air arriving from the Tasman Sea. The genus includes the largest New Zealand rhytidid, *Powelliphanta superba prouseorum* (shell diameter up to 90 mm), and the glossy, intricately patterned and coloured shells of all *Powelliphanta* are attractive. The larger species living in relatively accessible native forests came to the attention of Western science soon after European settlement of the country in the 1860s (e.g. Pfeiffer 1862), and many were described by the 1930s and 1940s, culminating in a taxonomy consisting of 10 species and 27 subspecies (Powell 1979). However, smaller *Powelliphanta* living in remote alpine areas were more easily overlooked and a few new species are still being discovered today.

The conservation status of many species of *Powelliphanta* and other rhytidids in New Zealand is of concern as a result of habitat loss and predation by introduced predators (Walker 2003). Habitat loss and modification previously had its most pronounced impact on the species occupying relatively low altitude forest habitats, but now taxa at higher altitudes are equally threatened; the most recently discovered alpine *Powelliphanta* became critically endangered as a result of opencast coal mining almost as soon as it was found. In this paper we describe the recognition of *Powelliphanta* "Augustus' in 2004 and the destruction of almost all its remaining habitat by 2007 to provide direct material benefit to humans.

In 1996 a handful of *Powelliphanta* shells were found on Mount Augustus on the Stockton Plateau north of Westport South Island, New Zealand. Initially, these were thought to be *Powelliphanta patrickensis*, whose type locality is also the Stockton Plateau. However, in 2003, during a detailed survey of the range of *Powelliphanta patrickensis*, the Mt Augustus shells were critically examined and found to differ substantially from these in terms of shell morphology. Shell shape, colour and size indicated that the taxon was either a highly distinctive new subspecies of *Powelliphanta lignaria*, or a separate new species.

Stockton Plateau is the site of a large opencast coal mine (~2,300 ha) and by 2004 the original collection site for the *Powelliphanta* from Mt Augustus had been destroyed. A small population remained on about 5 ha of the Mt Augustus ridgeline, though this too was in the process of being mined. Exhaustive searches elsewhere failed to find any further colonies of the Mt Augustus snail, and thus the snail's taxonomic distinctiveness became a prime importance in decisions as to whether mining should continue.

The Stockton Plateau, a high (600–1,100 m asl) uplifted plain that slopes gently to the east from a precipitously steep western scarp, has been the site of coal extraction since the 1870s. The Eocene coal deposits here are generally in the form of a single seam (Mangatini) 4–15 m in depth (Nathan 1996; Barry et al. 1994). Extraction was originally by underground shafts, but later by opencast mining by the state-owned coal company, Solid Energy New Zealand Ltd. (SENZ). This involves stripping of the overburden, which at Mt Augustus includes snail habitat, to access the coal. The overburden (Fig. 1) consists primarily of quartz sandstone, and soils developed on this tend to be shallow and infertile supporting subalpine heaths rather than forests (Wardle 1991). Soil pH is low, and there is a very high rainfall (>6,000 mm/annum), which leaches what little nutrients the thin soils contain. The Mangatini coal measure extends beneath the entire western escarpment, including Mt Augustus, so mining this seam involves permanently lowering the ridgeline by 30–50 m. High quality coking coal in the Stockton mine is nearly exhausted, so the coal under the ridgeline is the last available, but unfortunately also particularly valuable. As the snails are restricted to one of the highest and wettest peaks along the ridgeline, coal extraction will result in the destruction of almost all of their available habitat.

Following the discovery of the snails, about 60% of the individuals were salvaged and taken into captivity and mining of the Augustus ridgeline continued. The vegetation, soils and substrate of almost all of the snail habitat has now been removed, and the cliffs are being "deconstructed"; this lowering of altitude may in turn impact on orographic rainfall and drainage in the sliver of snail habitat that remains on the western escarpment below the coal seam. The long-term survival of the snail population

Fig. 1 Cross-section schematic of coal deposits at Mt Augustus on the Stockton Plateau, South Island, New Zealand (based on "Stockton Opencast Mine Ridgeline Mining: Environmental Impact Assessment" by Solid Energy New Zealand, July 2005). Overburden (dark grey) indicates rock strata overlying coal measure (black) that are being removed to gain access to coal. The east–west extent of snail distribution on Mt Augustus is indicated by pale grey line, although snail density was highest at the higher elevations
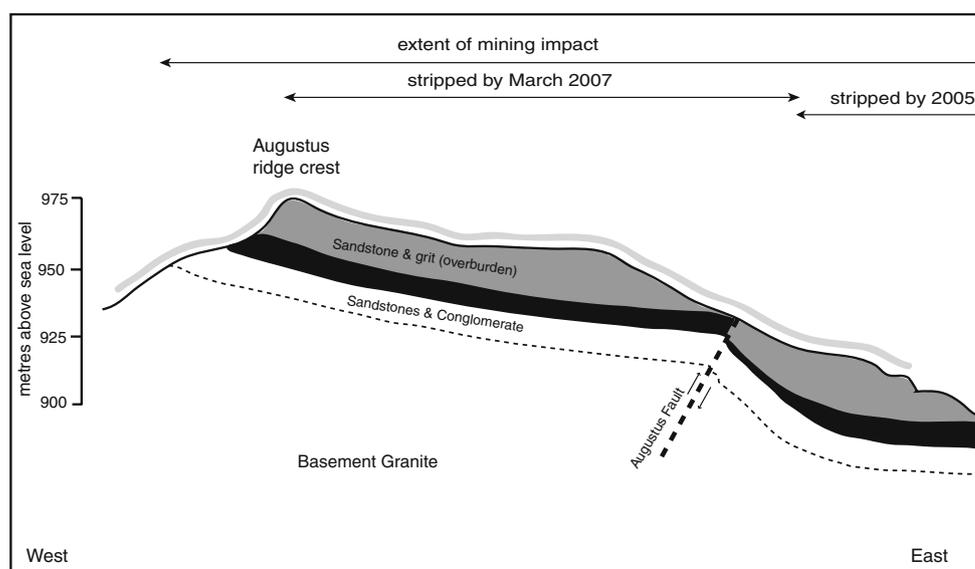
salvaged before mining is uncertain, thus it remains important to determine whether the Mt Augustus snail is a distinct evolutionary lineage and therefore a unique part of New Zealand's endemic biodiversity, or a genetically undifferentiated but isolated population of a more widespread taxon.

This study used mitochondrial DNA sequence data to place the Mt Augustus snails in a phylogenetic context. As no previous phylogenetic analysis of *Powelliphanta* has been undertaken with DNA sequence data, we used a broad sampling of the wider taxonomic and spatial range of the genus, in addition to extensive sampling in the vicinity of Mt Augustus (Fig. 2). This provided the best opportunity to assess the relative diversity within the group as a whole and identify the closest genealogical relations of the Mt Augustus snails. In particular, it allowed us to test whether Mt Augustus snails are phylogenetically closest to the alpine species *Powelliphanta patrickensis*, as initially supposed on the basis of their close proximity on the coal measures of the Stockton Plateau, or nearer the lowland taxon *P. lignaria* to which they are morphologically more similar.

Methods

Biological material

Powelliphanta snails were collected in the field, and common species were killed by freezing. Soft parts were removed and held at −80°C for genetic analysis, and shells stored dry at ambient temperatures. Most material was collected in 1987–1990 for use in an allozyme analysis of the genus (Walker 2003). Rare species were not collected until 2004, when tissue biopsies were taken before the live snails were returned to the wild. Snails from the Mt Augustus population were initially sampled in 2004 and 2005 as two whole animals found freshly dead. Soft parts were stored in ethanol and shells dried. In order to obtain a more extensive sample without further impact on an

Fig. 2 South Island, New Zealand indicating approximate location of sampling sites of *Powelliphanta* snails analysed. The species *P. traversi*, is restricted to North Island, outside the boundary of this map
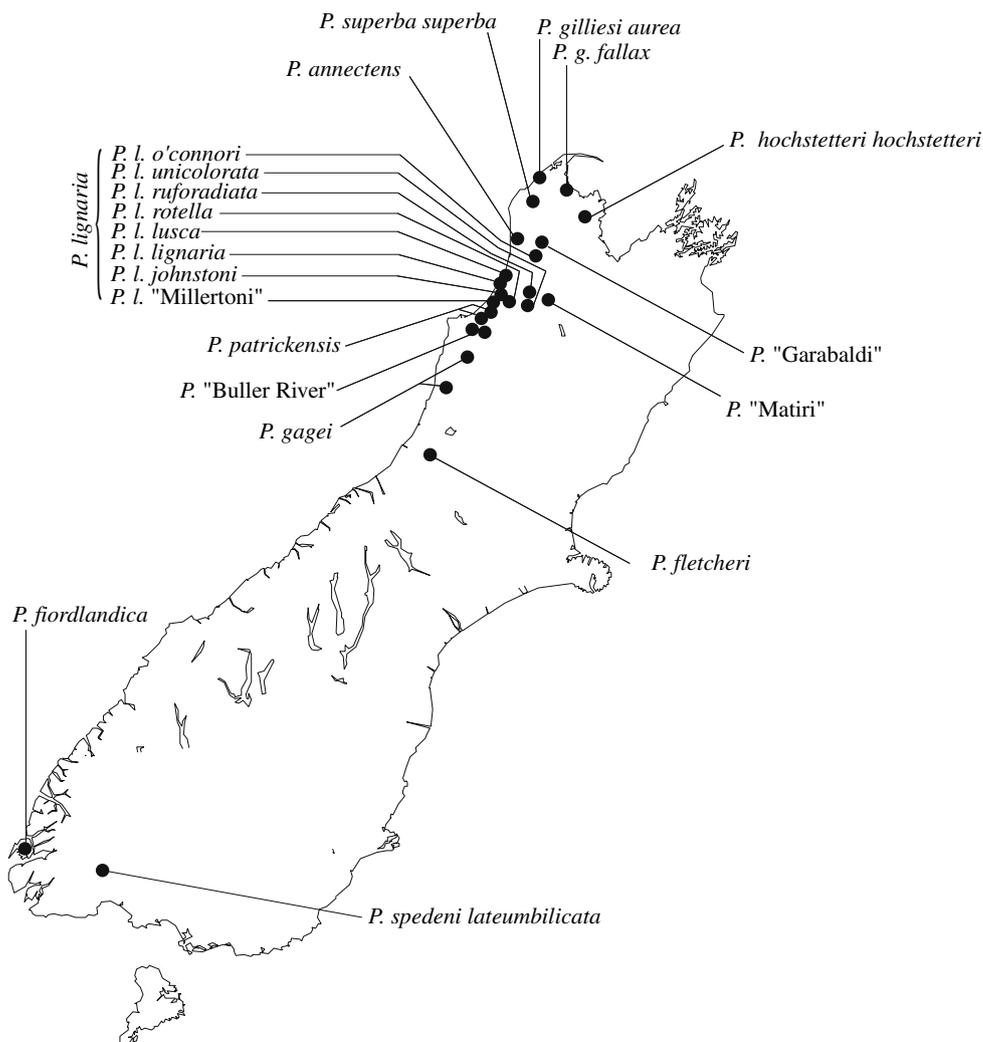
already limited population, biopsies of $\sim 1 \times 3$ mm were taken from live snails. To do this, snails were collected in the field and transported to the laboratory. Here, placed on a glass plate at room temperature, the snail emerged, allowing a biopsy to be taken from the side of the foot with a sterile scalpel. Biopsied tissue was placed immediately in 95% ethanol, and the animal returned to its container and rehydrated and cooled for recovery. The first nine snails sampled in this way were held for 3 months to confirm full recovery from this procedure, before being returned to the wild. Following legal action and recognition of the conservation issues relating to the Mt Augustus snails, individuals were collected by SENZ and maintained by the New Zealand Department of Conservation, with a view to subsequent relocation. Individuals in captivity thus became a ready source of additional biopsy tissue samples.

The taxonomy and systematics of several recently discovered *Powelliphanta* populations is currently being addressed. Based on the combined evidence of spatial distribution, morphological variation, allozyme data and ecological niche a relatively advanced hypothesis for classification of this fauna has already been developed (Walker 2003). Thus, although a number of the populations examined in our present analysis are identified by tag-names following Walker (2003), these taxa (and in many instances specific samples) have already been the subject of allozyme, spatial and morphological interpretation. Included in our sample are representatives of described species and tag-named populations from the wider Buller area, some of which are associated with elevated habitats, sometimes on coal measures (Table 1). The closest populations are *P. lignaria* "Millertoni" at 300 m asl about 4 km north of Mt Augustus, and *P. patrickensis* which occurs between 550–1,040 m asl in low coal measure heath and low forest over much of the Stockton and adjacent Denniston plateaux. On Stockton Plateau, *P. patrickensis* comes to within 1.5 km south of the Mt Augustus snail colony, along the same ridgeline (Fig. 3).

Extraction, amplification and sequencing of DNA

Genomic 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, 1.4M NaCl, 20mM EDTA), followed by a combined phenol/chloroform/isoamyl alcohol (25:24:1) cleanup based on previously described methods (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 electrophoresis on 1%

agarose gels. We targeted a portion of the mitochondrial gene, cytochrome oxidase subunit I (COI) using universal primers LCO1490, HCO2198 (Folmer et al. 1994) and H7005 (Hafner et al. 1994). The COI primers used have been effectively applied to intra- and interspecies studies of molluscs (Holland and Hadfield 2002; Ponder et al. 2003; Rundell et al. 2004; Simison and Lindberg 1999; Spencer et al. 2006; Trewick et al. 2008). PCR reactions were performed in 10 μl volumes with Red Hot ® Taq polymerase using the cycling conditions: initial denaturation at 94°C for 2 min, followed by 35 cycles of, 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 45 s, with a final extension at 72°C for 3 min. PCR products were purified using SAP/EXO (Shrimp Alkaline Phosphatase/exonuclease) enzymatic digest (USB corporation). Cycle sequencing used Perkin Elmer Bigdye ® chemistry following the manufacturer's protocols, and was read on an ABI prism DNA capillary sequencer (Applied Biosystems, Inc., Foster City, California).

Sequences were checked against the ABI trace file using Sequencher v4.6 (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 and frame shifts (Blanchard and Lynch 2000; Bennason et al. 2001). DNA sequences representing outgroup taxa (other New Zealand Rhytida) were obtained from Genbank (Spencer et al. 2006).

Phylogenetic analysis

We used PAUP* (Swofford 2002) to implement phylogenetic analyses using Neighbour Joining (NJ), Maximum Parsimony (MP), and Maximum Likelihood (ML) criteria, and MrBayes v3.1.2. (Ronquist and Huelsenbeck 2005) for Bayesian analysis. To select among models of DNA evolution for the ML and NJ analyses we used Modeltest version 3.06 (Posada and Crandall 1998). We also used NeighbourNet as implemented in Splitstree 4 (Huson and Bryant 2006) to express sequence relationships and signal conflict among the ingroup (Holland et al. 2004). Bootstrap support (Felsenstein 1985) for each node was assessed using 1,000 replicates under ML (implemented using PhyML, Guindon and Gascuel 2003), MP and NJ optimality criteria. Bayesian analyses used HKY (nst = 2) and GTR (nst = 6) models with gamma-distributed rate variation across sites (with four categories), a proportion of invariant sites and default priors. Each analysis of 10^7 generations used two independent, simultaneous runs with three heated chains and a sample frequency of 100 generations. For burnin, 25,000 samples were discarded. Node stability is indicated by credibility values.

Table 1 New Zealand *Powelliphanta* land snail samples used in the present study, including source location, sample sizes and number of haplotypes (Haps.). A total of 33 haplotypes were identified from comparison of 580 bp homologous fragments of COI sequence (i.e. the region sequenced from all individuals). Maximum shell diameter and habitat (altitude, vegetation and soil) is provided for each taxon based on our extensive collections and field observations

Taxon	Location	N	Haps.	Size (mm)	Altitude (m asl)	Vegetation	Soil
P. gilliesi fallax	Parapara Inlet, Golden Bay	1	1	48	Sea level	Dense mixed forest	Limestone soils
P. gilliesi aurea	Mangarakau	1	1	56	300	Dense mixed forest	Limestone soils
P. hochstetteri hochstetteri	Canaan, Pikikiruna Range	1	1	75	1,000	Litter and logs in *Nothofagus* forest	Mostly on calcium-rich marble
P. spedeni lateumbilicata	Lake Monowai, Fiordland	1	1	39	910–1,000	Shield ferns in *Nothofagus* forest/alpine scrub	Schist-sandstone soils
P. traversi	Lake Papaitonga, Horowhenua Plains	1	1	54	<100	Swampy lowland forest	Deep fertile soils on old sand-dunes
P. annectens	Oparara Valley, Karamea	1	1	85	610	*Nothofagus* and podocarp forest	Granite and alluvial soils
P. superba superba	Cedar Ridge, Aorere Valley, Golden Bay	1	1	80	800	Mixed *Nothofagus* forest	Leached granitic soils
P. fiordlandica	Kakapo Range, Edwardson Sound, Fiordland	1	1	32	640	Mixed *Nothofagus* forest	Schist
P. "Matiri"	Bald Knob Ridge, Buller	1	1	41	1,200	Alpine tussock-grassland	Calcium-rich soils on limestone and sandstone
P. "Garabaldi"	Garabaldi, Kahurangi NP	1	1	38	1,400	Alpine tussock-grassland	Calcium-rich soils on limestone
P. fletcheri	Mt Tuhua, mid Westland	1	1	36	1,100–1,300	Subalpine scrub and tussock-grassland	Soils on granite and schist
P. gagei	Southern Paparoa Ranges	2	2	42.5	900–1,200	Alpine scrub and tussock grassland	Infertile acidic soils & schists
P. "Buller River"	Lower Buller Gorge	8	1	40	50–100	Mixed *Nothofagus* forest	Alluvial soils with limestone talus
P. lignaria oconnori	Wilkinson Track, Kahurangi NP	1	1	52	~500	*Nothofagus* and podocarp forest	Calcium-rich limestone and mudstones
P. l. lusca	Glasseye Ck, Karamea Highway	1	1	58	<30	Mixed *Nothofagus* & podocarp forest	Alkaline soils
P. l. lignaria	Gentle Annie Point, Mokihinui River mouth	1	1	65	<100	Mixed *Nothofagus* & podocarp forest	Alkaline soils
P. l. rotella	St Andrew Stm, Mokihinui Forest	1	1	56	<100	Podocarp forest	Leached acidic poorly drained soils
P. l. johnstoni	Mumms Mill, Charming Ck	1	1	56	300	Podocarp & *Nothofagus* forest	Leached acidic poorly drained soils
P. l. ruforadiata	Maori Gully, Mokihinui River	1	1	58	150–400	*Nothofagus* forest	Calcium-rich limestone and mudstones
P. l. unicolorata	True Left, Mokihinui Rv Forks	1	1	50	80–500	Mixed *Nothofagus* forest	Alkaline soils
P. l. "Millertoni"	Millerton	1	1	53	300	Podocarp-hardwood forest	Podzolized infertile soils
P. patrickensis	Denniston/Stockton plateau	12	9	37	700–1,000	Tanglefern & manuka	Infertile Brunner coal measure soils
P. "Augustus"	Mount Augustus	47	2	43	950–1,011	Tanglefern & manuka	Infertile Brunner coal measure soils
Total		88	33				
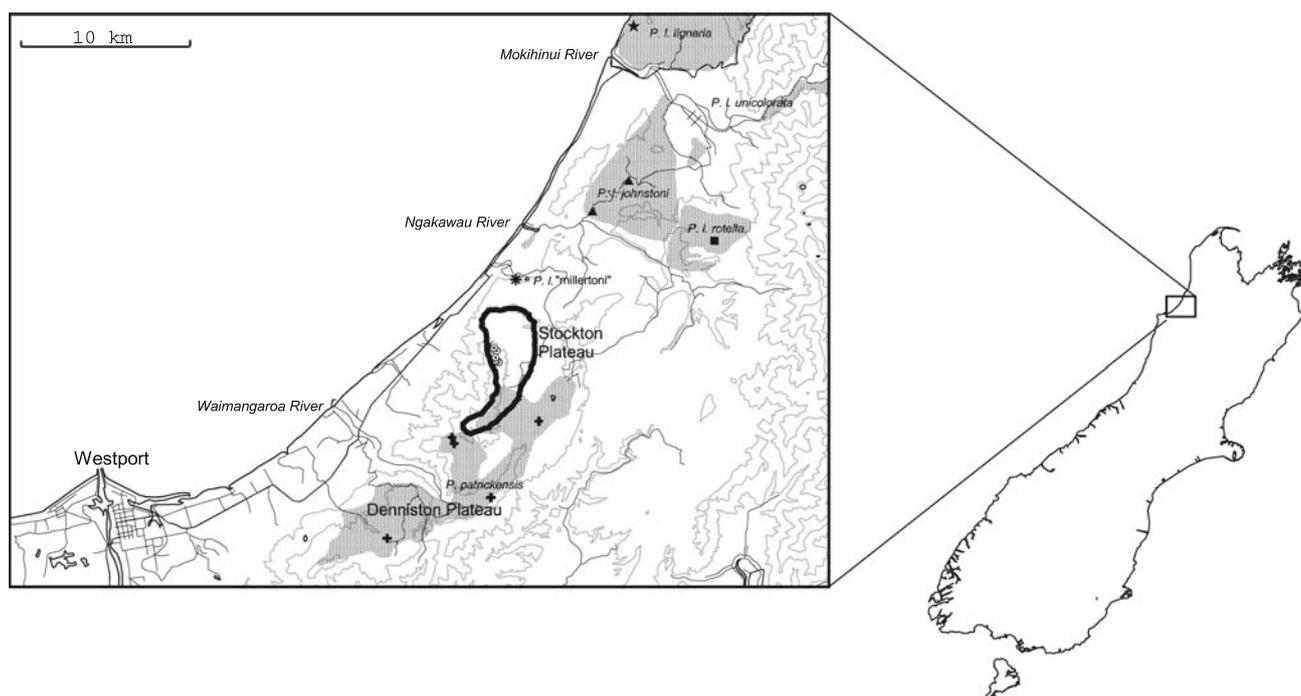
Fig. 3 Buller region, South Island, New Zealand with taxon ranges (shaded) and collecting locations (symbols) indicated: *Powelliphanta patrickensis* on Stockton and Denniston Plateaux (plus symbol), *P.* "Augustus" (circle with dot), *P. lignaria* "Millertoni" (asterisk), *P. l. rotella* (square), *P. l. johnstoni* (triangle), *P. l. lignaria* (star). *P. l. unicolorata* sampled outside boundary of this map. Thick black line indicates the current extent of opencast coal mining in the vicinity of Mt Augustus

Results

Genetic diversity

We analysed and aligned 87 sequences of 580–800 bp from the COI gene (Table 1). 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. Representative DNA sequences have been deposited on GenBank (EU265739–EU265774).

Pairwise genetic distances were estimated using the HKY+G+I model selected by ModelTest v3.06. Haplotypes were determined on the basis of the 580 bp homologous sequences obtained from all 87 samples (Table 1). Among the 47 *Powelliphanta* "Augustus" snails sampled we found two haplotypes that differed by 0.5%. These two haplotypes were distributed spatially at Mt Augustus with all 24 individuals from the ridgeline north of the Mt Augustus having one haplotype, and 23 from the ridgeline just south of Mt Augustus having the other. The spatially closest samples with different haplotypes came from sites about 700 m apart.

Eight *P. lignaria* samples representing the seven subspecies and the newly discovered taxon *P. l.* "Millertoni" each had different haplotypes. Genetic distances (HKY+ G+I) between *P. lignaria* mtDNA sequences and the *P.* "Augustus" haplotypes averaged 3.6%, compared to a mean pairwise distance of 1.1% within *P. lignaria* (maximum 1.8%). The mean genetic distance between *P.* "Augustus" and *P. patrickensis* samples was much higher, 7.7%. Higher genetic distances were found in comparisons of the *P.* "Augustus" haplotypes with all other *Powelliphanta* taxa surveyed (Table 2).

Phylogenetic relationships

Initial analysis (not shown) undertaken using outgroup representatives of the Rhytidae (*Rhytida greenwoodi* DQ298508, *Wainuia urnula* DQ298517, *Amborhytida dunniae* DQ298465, *Schizoglossa novoseelandica* DQ298516, *Paryphanta busbyi* DQ298504) revealed that *Powelliphanta fiordlandica* is sister to all other *Powelliphanta* in the present study. This confirms indications from morphology (Climo 1971), allozymes (Walker 2003) and previous molecular analysis (Spencer et al. 2006). Thus, we used *P. fiordlandica* in subsequent analyses as a preferred, closer outgroup to address the phylogenetic position of snails from Mt Augustus. Phylogenetic analyses used 36 COI sequences from *Powelliphanta* representing the DNA sequence diversity found among the sample set (Table 1). All key taxa (*P. patrickensis*, *P. lignaria* and *P.* "Augustus", plus other taxa were represented by at least one full 800 bp sequence.

Table 2 Pairwise genetic distances among samples based on mtDNA COI sequences (expressed as a proportion). ML distances were calculated using the bestfit model (HKY+G+I) identified using AIC implemented by ModelTest v3.1. Boxed values indicate pairwise genetic distances among samples of key taxa (*P. patrickensis*, *P. lignaria* and *P. "Augustus"*)

Code	Taxon	KJW373	KWJ110	KJW142	KJW129	KJW136	KJW230	KJW26	KJW29	KJW63	KJW242	KJW418	KJW246	KJW64	KJW65	KJW66	KJW72	KJW75	KJW76
KJW373	*P. s. superba*																		
KWJ110	*P. traversi*	0.117																	
KJW142	*P. annectens*	0.017	0.128																
KJW129	*P. hochstetteri hochstetteri*	0.092	0.019	0.098															
KJW136	*P. gilliesiaurea*	0.108	0.073	0.106	0.059														
KJW230	*P. gilliesifallax*	0.119	0.075	0.117	0.061	0.003													
KJW26	*P. fiordlandica*	0.367	0.363	0.346	0.316	0.255	0.274												
KJW29	*P. spedeni lateumbilicata*	0.065	0.104	0.057	0.075	0.074	0.081	0.282											
KJW63	*P. fletcheri*	0.133	0.149	0.109	0.120	0.099	0.101	0.336	0.078										
KJW242	*P. gagei*	0.105	0.132	0.092	0.106	0.094	0.096	0.355	0.073	0.026									
KJW418	*P. gagei*	0.104	0.136	0.080	0.108	0.103	0.105	0.327	0.073	0.031	0.005								
KJW246	*P. "Garabaldi"*	0.135	0.151	0.125	0.123	0.106	0.108	0.298	0.081	0.085	0.089	0.085							
KJW64	*P. patrickensis*	0.117	0.123	0.111	0.098	0.096	0.095	0.323	0.082	0.078	0.059	0.060	0.036						
KJW65	*P. patrickensis*	0.124	0.127	0.115	0.102	0.096	0.095	0.322	0.073	0.069	0.062	0.066	0.029	0.003					
KJW66	*P. patrickensis*	0.113	0.123	0.103	0.098	0.087	0.086	0.316	0.071	0.061	0.059	0.065	0.024	0.005	0.004				
KJW72	*P. patrickensis*	0.117	0.119	0.107	0.095	0.090	0.089	0.310	0.068	0.066	0.059	0.063	0.026	0.002	0.001	0.003			
KJW75	*P. patrickensis*	0.113	0.115	0.103	0.091	0.089	0.088	0.294	0.067	0.063	0.058	0.062	0.025	0.003	0.003	0.001	0.001		
KJW76	*P. patrickensis*	0.113	0.123	0.103	0.098	0.087	0.086	0.316	0.071	0.061	0.059	0.065	0.024	0.005	0.004	0.000	0.003	0.001	
KJW360	*P. patrickensis*	0.105	0.119	0.100	0.095	0.094	0.095	0.336	0.086	0.072	0.056	0.057	0.033	0.005	0.012	0.007	0.010	0.009	0.007
KJW361	*P. patrickensis*	0.119	0.123	0.103	0.098	0.098	0.097	0.350	0.087	0.073	0.059	0.067	0.035	0.007	0.014	0.005	0.012	0.010	0.005
KJW362	*P. patrickensis*	0.105	0.119	0.100	0.095	0.094	0.095	0.336	0.086	0.072	0.056	0.057	0.033	0.005	0.012	0.007	0.010	0.009	0.007
KJW396	*P. patrickensis*	0.109	0.119	0.100	0.095	0.085	0.086	0.310	0.068	0.059	0.056	0.060	0.022	0.007	0.005	0.001	0.004	0.003	0.001
KJW398	*P. patrickensis*	0.109	0.119	0.100	0.095	0.085	0.086	0.310	0.068	0.059	0.056	0.060	0.022	0.007	0.005	0.001	0.004	0.003	0.001
KJW417	*P. patrickensis*	0.105	0.119	0.100	0.095	0.086	0.088	0.318	0.079	0.068	0.056	0.057	0.028	0.005	0.009	0.003	0.007	0.005	0.003
KJW70	*P. "Buller River"*	0.109	0.127	0.107	0.102	0.093	0.095	0.316	0.071	0.061	0.062	0.066	0.027	0.014	0.012	0.008	0.011	0.010	0.008
KJW251	*P. "Matiri"*	0.117	0.140	0.107	0.106	0.093	0.095	0.291	0.066	0.061	0.059	0.063	0.024	0.029	0.024	0.021	0.023	0.024	0.021
KJW375	*P. " Augustus"*	0.112	0.141	0.097	0.113	0.091	0.101	0.269	0.073	0.100	0.076	0.074	0.095	0.089	0.096	0.088	0.089	0.084	0.088
KJW404	*P. " Augustus"*	0.100	0.127	0.093	0.100	0.101	0.109	0.283	0.068	0.077	0.073	0.071	0.070	0.079	0.071	0.063	0.067	0.063	0.063
KJW82	*P. lignaria rotella*	0.125	0.131	0.107	0.103	0.086	0.087	0.278	0.070	0.077	0.064	0.068	0.075	0.086	0.078	0.070	0.072	0.072	0.070
KJW115	*P. lignaria unicolorata*	0.113	0.139	0.099	0.103	0.107	0.109	0.281	0.072	0.092	0.064	0.063	0.103	0.087	0.099	0.091	0.092	0.087	0.091
KJW95	*P. lignarialignaria*	0.116	0.152	0.110	0.122	0.118	0.120	0.334	0.082	0.095	0.073	0.071	0.113	0.096	0.109	0.101	0.102	0.096	0.101
KJW13	*P. lignariaruforadiata*	0.121	0.139	0.107	0.111	0.098	0.101	0.294	0.079	0.092	0.070	0.069	0.095	0.080	0.092	0.084	0.085	0.080	0.084
KJW290	*P. lignariajohnstoni*	0.125	0.131	0.107	0.103	0.088	0.090	0.283	0.073	0.079	0.064	0.066	0.078	0.083	0.078	0.072	0.073	0.073	0.072
KJW348	*P. lignariamillertoni*	0.116	0.138	0.107	0.110	0.097	0.099	0.299	0.070	0.077	0.070	0.074	0.085	0.093	0.083	0.075	0.077	0.077	0.075
KJW119	*P. lignarialusca*	0.118	0.127	0.100	0.100	0.093	0.095	0.277	0.069	0.076	0.069	0.072	0.080	0.088	0.087	0.073	0.073	0.074	0.073
KJW258	*P. lignariaoconnori*	0.112	0.138	0.099	0.110	0.092	0.094	0.300	0.067	0.080	0.063	0.064	0.104	0.089	0.097	0.086	0.089	0.084	0.086

Boxed region (KJW64–KJW417) labelled: *P. patrickensis*

Table 2 continued

		KJW360	KJW361	KJW362	KJW396	KJW398	KJW417	KJW70	KJW251	KJW375	KJW404	KJW82	KJW115	KJW95	KJW13	KJW290	KJW348	KJW119	KJW258
KJW373	*P. s. superba*																		
KWJ110	*P. traversi*																		
KJW142	*P. annectens*																		
KJW129	*P. hochstetteri hochstetteri*																		
KJW136	*P. gilliesi aurea*																		
KJW230	*P. gilliesi fallax*																		
KJW26	*P. fiordlandica*																		
KJW29	*P. spedeni lateumbilicata*																		
KJW63	*P. fletcheri*																		
KJW242	*P. gagei*																		
KJW418	*P. gagei*																		
KJW246	*P. "Garabaldi"*																		
KJW64	*P. patrickensis*																		
KJW65	*P. patrickensis*																		
KJW66	*P. patrickensis*																		
KJW72	*P. patrickensis*																		
KJW75	*P. patrickensis*																		
KJW76	*P. patrickensis*																		
KJW360	*P. patrickensis*																		
KJW361	*P. patrickensis*	0.005																	
KJW362	*P. patrickensis*	0.000	0.005																
KJW396	*P. patrickensis*	0.005	0.010	0.005															
KJW398	*P. patrickensis*	0.005	0.010	0.005	0.000														
KJW417	*P. patrickensis*	0.000	0.005	0.000	0.002	0.002													
KJW70	*P. "BullerRiver"*	0.012	0.018	0.012	0.006	0.006	0.009												
KJW251	*P. "Matiri"*	0.027	0.034	0.027	0.018	0.018	0.022	0.023											
KJW375	*P. "Augustus"*	0.079	0.090	0.079	0.082	0.082	0.079	0.089	0.093										
KJW404	*P. "Augustus"*	0.073	0.082	0.073	0.061	0.062	0.069	0.064	0.073	0.005									
KJW82	*P. lignaria rotella*	0.080	0.085	0.080	0.067	0.067	0.076	0.069	0.067	0.031	0.030								
KJW115	*P. lignaria unicolorata*	0.076	0.088	0.076	0.085	0.085	0.077	0.092	0.092	0.030	0.033	0.011							
KJW95	*P. lignarialignaria*	0.086	0.098	0.086	0.094	0.094	0.086	0.101	0.095	0.043	0.046	0.017	0.016						
KJW13	*P. lignariarufioradiata*	0.070	0.081	0.070	0.078	0.078	0.071	0.085	0.078	0.033	0.036	0.007	0.010	0.012					
KJW290	*P. lignariajohnstoni*	0.077	0.091	0.077	0.068	0.068	0.074	0.070	0.068	0.030	0.031	0.001	0.010	0.016	0.007				
KJW348	*P. lignariamillertoni*	0.087	0.092	0.087	0.072	0.072	0.083	0.074	0.072	0.039	0.036	0.005	0.015	0.009	0.011	0.007			
KJW119	*P. lignarialusca*	0.082	0.096	0.082	0.076	0.074	0.079	0.079	0.076	0.038	0.043	0.008	0.010	0.024	0.018	0.010	0.011		
KJW258	*P. lignariaoconnori*	0.079	0.082	0.079	0.082	0.082	0.079	0.089	0.083	0.036	0.036	0.009	0.011	0.009	0.007	0.009	0.005	0.016	

P. "Augustus" (KJW375, KJW404)

P. lignaria (KJW82–KJW258)

Although the deeper phylogenetic structure among the *Powelliphanta* taxa in our present study is only partially resolved we found a consistent pattern of relationships with a number of main clades. Phylogenetic trees with the same or similar groupings of taxa were returned by MP, NJ, ML and Bayesian nst = 2 and nst = 6 analyses. We found that analysis of a reduced character data set of 580 bp of homologous sequence produced the same arrangement of clades as analyses using the 800 bp character set (that included some missing information). Most of the taxa in our analysis are allopatric or parapatric although close sympatry is observed in some instances. For example, three species (*P. superba*, *Powelliphanta hochstetteri* and *Powelliphanta gilliesi*) are sympatric on Parapara Peak, Golden Bay, with a forth parapatric taxon (*P.* "Anatoki Range") at higher elevation. mtDNA sequences from these sympatric taxa differ by 5.9% or more (*P. hochstetteri* and *P. gilliesi* Table 2) although many morphologically distinct allopatric *Powelliphanta* species have lower genetic divergences than this. For example the large and distinctive South Island *P. hochstetteri* and the North Island species *Powelliphanta traversi* have COI sequences that differ by just 1.9%.

All analyses revealed a sister relationship of *P. lignaria* and the Mt Augustus snails, supporting inferences from morphology (Fig. 4). We also found that, where DNA sequences from multiple individuals were analysed they formed monophyletic clades, and this included the representatives of the *P. lignaria* group. It was immediately evident that the Mt Augustus snails were not closely related to *P. patrickensis* despite the close proximity of these taxa, their occupation of related geology, ecology and landforms, and superficial similarity in the size and weight of their shells. Whilst *P.* "Augustus" is evidently not closely related to *P. patrickensis*, a number of other taxa analysed are, including *Powelliphanta* "Buller River", *Powelliphanta* "Garabaldi" and *Powelliphanta* "Matiri". However, mtDNA evidence corroborates indications from morphology and allozymes (Walker 2003) that *P.* "Buller River", *P.* "Garabaldi" and *P.* "Matiri" constitute distinct taxa in their own right (Fig. 5), and will receive further attention. We note that even within *P. patrickensis*, there is evidence for phylogeographic structure among populations on the Stockton and Denniston coal plateaux.

Discussion

Our analyses indicate two levels of phylgenetic structure; deeper relationships among taxa that are either sympatric (e.g. *P. hochstetteri* and *P. gilliesi*) or spatially widely disjunct (e.g. *P. spedeni* and *P. superba*), and shallower relationships among close allopatric or parapatric taxa (e.g. *P. patrickensis* group). This indicates that there have been
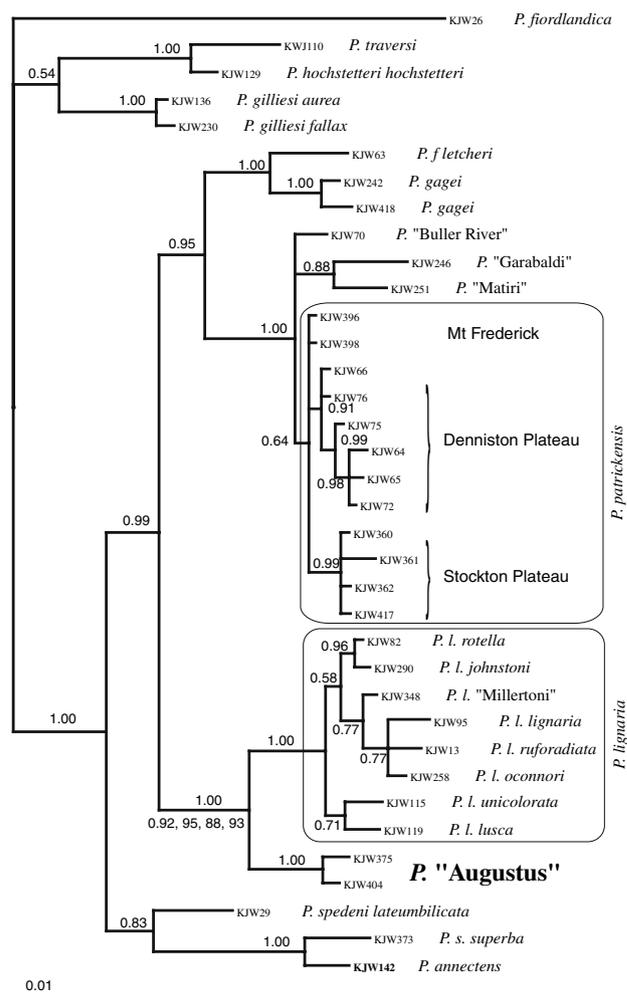
Fig. 4 Evolutionary hypothesis from a Bayesian analyses of 36 mtDNA COI sequences up to 800 bp long from *Powelliphanta* snails. Values at nodes indicate Bayesian credibility values from nst = 2 analysis. Support for the *P. lignaria/P.* "Augustus" clade from Bayesian nst = 6, ML, MP and NJ bootrap support respectively are shown below the relevant branch

episodes of speciation over a protracted timeframe. A similar scale of genetic diversity has been reported for a related group of New Zealand snails (Paryphantinae—Spencer et al. 2006).

On the basis of the present sampling it is clear that *P.* "Augustus" is distinct from other species and subspecies of *Powelliphanta*. It is closest to the low altitude *P. lignaria* but is apparently the phylogenetic sister to this entire species group rather than being a subspecies of *P. lignaria*. This finding is consistent with their spatial proximity and morphological characteristics of *P.* "Augustus" which also indicate an affinity to *P. lignaria* but not a close relationship to any particular subspecies in this group. Although distinguished by low mtDNA sequence diversity, the described subspecies of *P. lignaria* are well supported by morphology, and have discrete geographic ranges. On the basis of
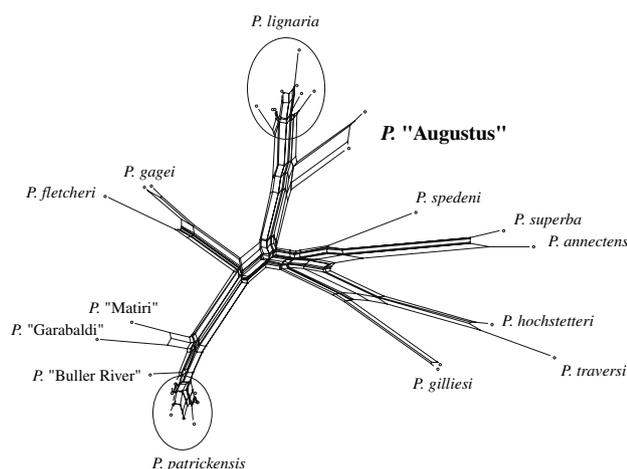
Fig. 5 Neighbournet (SplitsTree 4, Huson and Bryant 2006) analysis of mtDNA COI sequences from ingroup *Powelliphanta* snails. Box-like branches indicate relative support for alternative relationships among taxa

the current sampling, all the recognized *P. lignaria* sub-species sampled, including *P. l.* "Millertoni", are much closer to each other than they are to *P.* "Augustus".

Although recent advocates argue that mtDNA sequence divergence is (in many cases) sufficient alone to determine species status (e.g. Hebert et al. 2003), it is overly simplistic to rely on this single measure in the absence of other information (Trewick 2007). Indeed, the most striking and seemingly convincing examples of such an approach in fact draw upon extensive ecological, morphological and/or behavioural evidence to corroborate inferences from mtDNA sequence difference (e.g. Hebert et al. 2004). Low mtDNA sequence divergence is in itself not sufficient reason to constrain interpretation of specific status where other evidence is contradictory (Trewick 2001), and many studies of snail diversity reveal low genetic distances among some species (e.g. *Partulina semicarinata* and *P. variabilis* 2.5%—Holland and Hadfield 2002; *Succinea manuana* and *S. modesta* 0.2%—Rundell et al. 2004; *Paryphanta busbyi* and *P. watti* 0.25%—Spencer et al. 2006). It is however widely observed that sequence differences (for COI and other mtDNA genes) between species in most animal groups tend to be greater than 2% (Hebert et al. 2003) and a conservative threshold of 3% for sequence diversity within species has been applied to many taxa.

Thus, even on these simplistic metric terms, *P.* "Augustus" (which differs from its sister species *P. lignaria* by 3.6%) could arguably be considered a distinct species, but we prefer a holistic approach that draws on the broadest available evidence. Such an approach is in accordance with the criteria set out by Moritz (1994) and others (e.g. Mallet 1995). The most potent evidence for

species distinction when dealing with allopatric taxa comes from a combination and contrast of phylogenetic placement, genetic distance, morphological evidence and ecological specialisation. The fact that *P.* "Augustus" is not sister to the geographically and ecologically neighbouring species *P. patrickensis*, indicates that these snail lineages have independently adapted to the harsh conditions of the montane coal plateaux. *Powelliphanta* need calcium for shell and egg-case construction. Their main prey, earthworms, flourish in alkaline, moist but not saturated soils. However, the Buller coal plateaux provide only super-saturated acidic soils with very low pH, low available calcium, and low densities of earthworms. Presumably in response to the unfavourable conditions, both *P.* "Augustus" and *P. patrickensis* have a reduced adult size and, particularly in the latter, fragile shells lacking much conchin. This gives them superficial similarities in shell morphology, but the molecular data reveal this to be a case of convergence in response to local conditions.

The sister relationship of *P.* "Augustus" to *P. lignaria* provides a contrast that supports this inference. In shell-shape, colour, banding and sculpturing, there are echoes of *P. lignaria* in *P.* "Augustus". However, in contrast to the small, thin-shelled, subalpine scrub and heath-dwelling *P.* "Augustus", all subspecies of *P. lignaria*, are heavy-shelled snails, even those which only attain relatively small adult size (i.e. *P. l. unicolorata*) or live on relatively acidic substrates (i.e. *P. l. rotella* and *P. l. johnstoni*). All subspecies of *P. lignaria* are lowland forest-dwellers, with the distribution of most subspecies closely aligned to the presence of limestone.

Powelliphanta patrickensis, which occurs in both forest and heathland from below the bush line (600 m asl) to the summit of the highest peaks on the plateaux (Mt's Frederick and Rochfort at 1,100 m asl) has a much larger spatial range and apparently wider ecological tolerance than *P.* "Augustus". Although widespread burning associated with underground coal mining on the Denniston Plateau has reduced the land's carrying capacity (Walker 2003), the wider geographic range of *P. patrickensis* has protected it so far from the rapid and extreme population decline of the more localized *P.* "Augustus". As opencast coal mining on the Stockton Plateau is now beginning to permanently destroy *P. patrickensis* habitat too, this contrast is starting to be obscured, but for now it is interesting to speculate on why their ecological tolerances should be so different.

A sharp altitudinal boundary delineates the natural range of *P.* "Augustus" on the unmodified steep western slopes of Mt Augustus, with snails present in abundance above 900 m asl, but entirely absent just 100 m below. Whilst change in vegetation type may have been a contributing cause of the sharp cut-off (below 900 m short sub-alpine

vegetation rapidly gives way to taller forest communities) there is more evidence that the key factor shaping distribution is increasing moisture and decreasing temperature at the higher altitudes where *P.* "Augustus" occurred. Due simply to the steep orographic gradient, rainfall is about 1,000 mm higher per annum at 1,010 m at the top of the snail colony than at the bottom of the colony where there are very few snails. The former prominence of the peaks of Mt Augustus meant there was nearly always a wreath of mist and cloud over the snail colony, but this moist, cooling cloud is absent below 900 m. Temperature steadily drops with increasing altitude, so that average temperature is at least a degree cooler in the core of the snail colony than just below it.

An alternative explanation is that differential predation by introduced mammals, which are theoretically limited above but abundant below 900 m asl, created the striking altitudinal limit to the snails distribution, rather than *P.* "Augustus" being a habitat specialist. However, with other equally palatable snails (i.e. *P. patrickensis)* only 1.5 km away, subject to the same potential predators but still present both above and well below 900 m, and with no evidence of shells, predator-damaged or otherwise below the Mt Augustus snail colony, this theory lacks support. A similarly erroneous proposal has in the past been made to explain high altitude limitation in New Zealand's alpine scree weta (Orthoptera; Anostostomatidae) (discussed by Trewick et al. 2000).

Why should *P.* "Augustus" be so restricted to very cool and extremely wet sites, when *P. patrickensis* is not? One possibility is that *P.* "Augustus", derived from ancestral lowland *P. lignaria* stock, still retains behaviours that evolved in heavier-shelled snails in shaded forest environments where water-loss was not so likely. Paradoxically, when it is not raining or misty, the exposed coal plateaux, covered only in short grasses and heaths, can be something of a desert, so such behaviours in snails which are no longer protected by a heavy shell could be lethal. These characteristics reveal that *P.* "Augustus" is of considerable evolutionary interest and should be given appropriate conservation consideration. The species status of *P.* "Augustus" will be formally established elsewhere.

Almost no significant natural habitat now remains for the Mt Augustus snail. A third of the salvaged snails have been relocated to land just below the original colony that they were unable to occupy naturally; a third have been relocated to Mt Rochfort on the Denniston Plateau where they will be in competition with *P. patrickensis*; and a third remain in captivity awaiting identification of other possible translocation sites. These translocations are high risk as the remaining sliver of natural habitat and the two translocation sites appear to be at best sub-optimal *P.* "Augustus" habitat; being very small, modified and fragmented, and

separated from each other. Less than a decade after this species was first discovered, it is on the brink of extinction in the wild.

It is not known how many other organisms have or had distinctive lineages or species endemic to Mt Augustus. Prior to the discovery of snails, an extension of an adjacent protected area to incorporate the summit of Mt Augustus was proposed in 1998 because it was the type locality for two insect species (Click beetle—*Elatichrosis*, plant hopper—*Paracephalus*), and was identified as being botanically valuable with specialized habitat of high aesthetic value (Overmars et al. 1998). Whether the Mt Augustus snail was part of a distinct biogeographic element will never be known, so determining the true impact on national biodiversity of mining Mt Augustus will also remain unknown. The extent of coal mining in New Zealand is far from the scale of environmental devastation being wrought, for instance, in the Appalachian mountains of the United States (Orr 2007), but the philosophy underlying the practice is the same (Johns 2007). In fact the biodiversity impact may well be disproportionately high as New Zealand has a relatively small land area with a distinctive and highly endemic biota (Myers et al. 2000; Orme et al. 2005), and some areas support extreme levels of diversity (e.g. land snails—Solem et al. 1981; Barker and Mayhill 1998).

As pressure mounts to exploit dwindling fossil fuel reserves, the impacts on global biodiversity are inevitable (Johns 2007). Invertebrates are usually the last to be considered in any competition for resources, and land snails seem particularly vulnerable. Molluscs have the dubious honour of having the highest number of documented animal extinctions of any major taxonomic group (Lydeard et al. 2004). They suffer the dual disadvantages of extreme sensitivity to habitat modification and pollution, and under-recognition of taxonomic diversity. The extent of convergence of shell type (a key taxonomic feature) in distinct lineages and thus the source of under-recognition is being revealed by the application of genetic methods (e.g. Haase et al. 2003; Weaver et al. 2007). A combination of morphological, ecological and genetic information is essential if we are to disentangle the evolutionary histories of land snails and provide a sound appraisal of their biodiversity (Chiba 2003; Lydeard et al. 2004). In particular the application of genetic evidence provides a robust framework for the recognition of evolutionarily significant units (ESU) in terrestrial molluscs (Backeljau et al. 2001). For the Mt Augustus snail, the combined weight of evidence indicates that it should be treated as a distinct species, yet by the time this is formally done, the land where it lived will have ceased to exist. The sorry tale of the late discovery and quick demise of *P.* "Augustus" is the unfortunate detail behind the global decline (Lydeard et al. 2004) of non-marine molluscs.

References

Backeljau T, Baur A, Baur B (2001) Population and conservation genetics. In: Barker GM (ed) The biology of terrestrial molluscs. CAB International, UK, pp 383–412

Barker GM, Mayhill P (1998) Patterns of diversity and habitat relationships in terrestrial mollusc communities of the Pukea-maru Ecological District, northeastern New Zealand. J Biogeog 25:215–238

Barry JM, Duff SW, Macfarlane DAB (1994) Coal resources of New Zealand. Resource information report 16. Energy and Resources Division, Ministry of Commerce, New Zealand

Bensasson D, Zhang D-X, Hartl DL, Hewitt GM (2001) Mitochondrial pseudogenes: evolution's misplaced witnesses. Trends Ecol Evol 16:314–321

Blanchard JL, Lynch M (2000) Organellar genes: why do they end up in the nucleus? Trends Genet 16:315–32

Chiba S (2003) Species diversity and conservation of Mandarina, an endemic land snail of the Ogasawar Islands. Global Env Res 7:29–37

Climo FM (1971) A new species of Paryphanta (Mollusca: Paryphantidae) from south-west Fiordland, New Zealand. Rec Dom Mus 7:145–150

Felsentein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Folmer O, Black M, Hoeh W, Lutz R, Vrijnhoek R (1994) DNA primers for amplification of mitochondrial cytrochrome c oxidase subunit 1 from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Guindon S, Gascuel O (2003) PhyML—A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Sys Biol 52:696–704

Haase M, Misof B, Wirth T, Baminger H, Baur B (2003) Mitochondrial differentiation in a polymorphic land snail: evidence for Pleistocene survival within the boundaries of permafrost. J Biol Evol 16:415–428

Hafner MS, Sudman PD, Villablanca FX, Spradling TA, Demastes JW, Nadler SA (1994) Disparate rates of molecular evolution in cospeciating hosts and parasites. Science 265:1087–1090

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proc R Soc Lond, [Biol] 70:313–321

Hebert PDN, Penton EH, Burns J, Janzen DJ, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly, Astraptes fulgerator. Proc Natl Acad Sci USA 101:14812–14817

Holland BS, Hadfield MG (2002) Islands within an island: phylogeography and conservation genetics of the endangered Hawaiian tree snail Achatinella mustelina. Mol Ecol 11:365–375

Holland BR, Huber KT, Moulton V, Lockhart PJ (2004) Using consensus networks to visualise contradictory evidence for species phylogeny. Mol Biol Evol 21:1459–1461

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 23:254–267

Johns D (2007) Like it or not, politics is the solution. Conserv Biol 21:287–288

Lydeard C, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Clark SA, Cummings KS, Frest TJ, Gargominy O, Herbert DG, Hershler R, Perez KE, Roth B, Seddon M, Strong EE, Thompson FG (2004) The global decline of nonmarine Mollusks. BioScience 54:321–330

Mallet J (1995) A species definition for the modern synthesis. Trends Ecol Evol 10:294–299

Moritz C (1994) Defining 'Evolutionary significant units' for conservation. Trends Ecol Evol 9: 373–375

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–856

Nathan S (1996) Geology of the Buller Coalfield, scale 1:50,000. Institute of Geological & Nuclear Sciences geological map 23. GNS Science, Lower Hutt, New Zealand

Orr DW (2007) The politics of carbon. Conserv Biol 21:295–296

Orme et al. (2005) Global hotspots of species richness are not congruent with endemism or threat. Nature 436:1016–1019

Overmars FB, Kilvington MJ, Gibson RS (1998) Ngakawau Ecological District: survey report for the Protected Natural Areas Programme. New Zealand Protected Natural Areas Programme Series No. 11. Department of Conservation, Hokitika, New Zealand

Ponder WF, Colgan DJ, Gleeson DM, Sherley GH (2003) Relationships of Placostylus from Lord Howe Island: an investigation using the mitochondrial cytochrome c oxidase 1 gene. Molluscan Res 23:159–178

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Powell AWB (1979) New Zealand Mollusca. Collins, Auckland, New Zealand

Rambaut A (1996) Se-Al: Sequence Alignment Editor. Available (2/11/07) at http://www.tree.bio.ed.ac.uk/software/seal/

Ronquist F, Huelsenbeck JP (2005) Bayesian analysis of molecular evolution using MrBayes. In: Nielsen (ed) Statistical methods in molecular evolution. Springer, New York

Rundell RJ, Holland BS, Cowie RH (2004) Molecular phylogeny and biogeography of the endemic Hawaiian Succineidae (Gastropoda: Pulmonate). Mol Phylogenet Evol 31:246–255

Simison BW, Lindberg DR (1999) Morphological and molecular resolution of a putative cryptic species complex: a case study of Notoacmea fascicularis (Menke, 1851) (Gastropoda: patellogastropoda). J Mollusc Stud 65:99–109

Solem A, Climo FM, Roscoe DJ (1981) Sympatric species diversity of New Zealand land snails. NZ J Zool 8:453–485

Spencer HG, Marshall BA, Maxwell PA, Grant-Mackie JA, Stilwell JD, Willan RC, Campbell HJ, Crampton JS (2004) Phylum Mollusca—Chitons, clams, tusk shells, snails, squids and kin. In: Gordon DP (ed) The New Zealand inventory of biodiversity. Volume 1. Kingdom Animalia: Radiata, Lophotrochozoa and Deuterostomia. Canterbury University Press, Christchurch

Spencer HG, Brook FJ, Kennedy M (2006) Phylogeography of kauri snails and their allies from Northland, New Zealand (Mollusca: Gastropoda: Rhytidae: Paraphantinae). Mol Phylogenet Evol 38:835–842

Stine OC (1989) Cepaea nemoralis from Lexington, Virginia: the isolation and characterisation of their mitochondrial DNA, the implications for their origin and climatic selection. Malacologia 30:305–315

Swofford DL (2002) PAUP* Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts

Terrett JA (1992) The mitochondrial genome of Cepaea nemoralis: PhD. thesis, University of Nottingham

Thomaz D, Guiller A, Clarke B (1996) Extreme divergence of mitochondrial DNA within species of pulmonate land snails. Proc R Soc Lond [Biol] 263:363–368

Trewick SA, Morgan-Richards M, Wallis GP (2000) Phylogeographic pattern correlates with Pliocene mountain building in the alpine scree weta (Orthoptera: Anostostomatidae). Mol Ecol 9:657–666

Trewick SA (2001) Identity of an endangered grasshopper (Acrididae: *Brachaspis*): taxonomy, molecules and conservation. Conserv Genet 2:233–243

Trewick SA (2007) DNA Barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae). Cladistics 23:1–15

Trewick SA, Brescia F, Jordan C (2008) Diversity and phylogeny of New Caledonian *Placostylus* land snails; evidence from mitochondrial DNA. In: Grandcolas P (ed) Zoologia Neocaledonica 6. Biodiversity studies in New Caledonia. Mémoires du Muséum national d'Histoire naturelle, 196, in press

Walker KJ (2003) Recovery plans for *Powelliphanta* land snails. Threatened Species Recovery Plan 49. Department of Conservation, New Zealand, Wellington

Wardle P (1991) Vegetation of New Zealand. Cambridge Press, Cambridge

Weaver KF, Perez-Losada M, Guralnick RP, Nelson A, Blatt S, Crandall KA (2007) Assessing the conservation status of the land snail *Oreohelix perpherica wasatchensis* (Family Oreohelicidae). Conserv Genet. doi:10.1007/s10592-007-9415-y