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

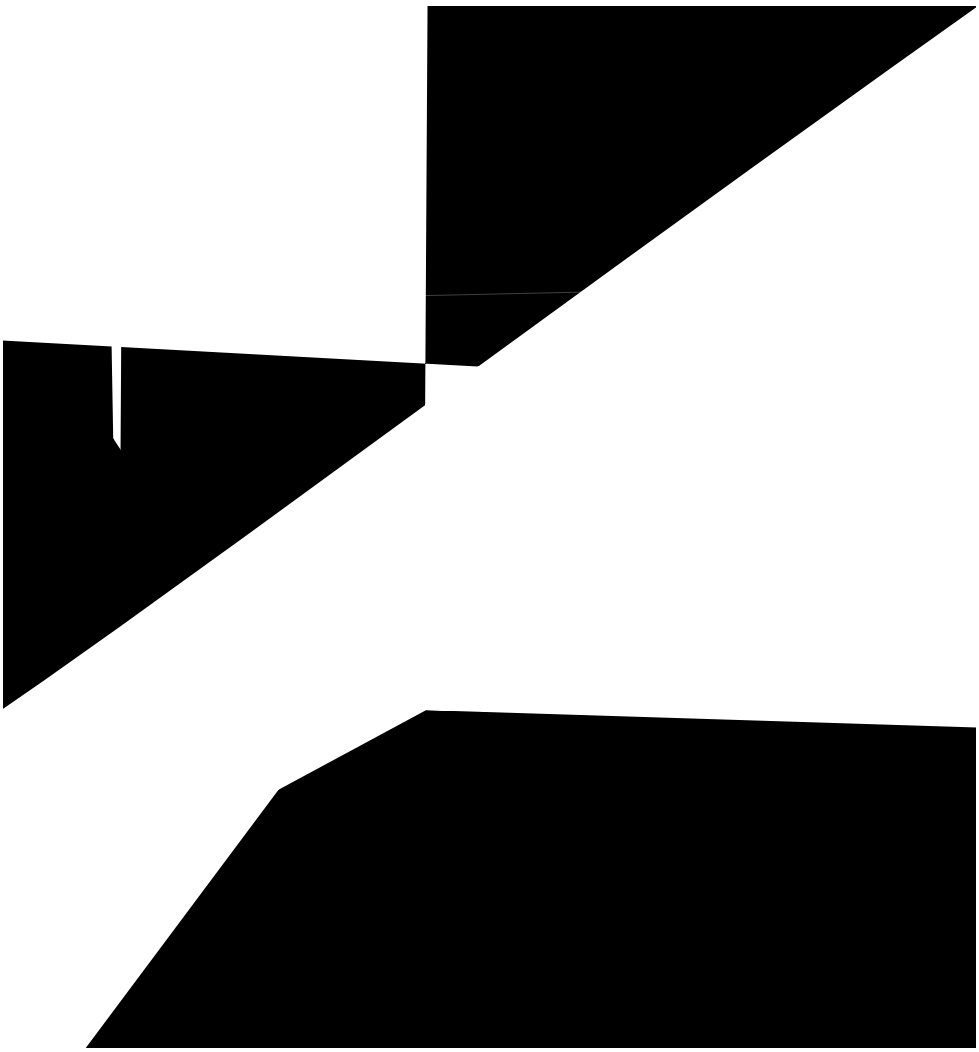
salvaged before mining is uncertain, thus it remains important to determine whether the Mt Augustus snail is a distinct evolutionary lineage and therefore a unique part of New Zealand's endemic biodiversity, or a genetically undifferentiated but isolated population of a more widespread taxon.

This study used mitochondrial DNA sequence data to place the Mt Augustus snails in a phylogenetic context. As no previous phylogenetic analysis of *P e a a* 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 *P e a a a c e* , as initially supposed on the basis of their close proximity on the coal measures of the Stockton Plateau, or nearer the lowland taxon *P. g a a* to which they are morphologically more similar.

Methods

Biological material

P e a a 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



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

Table 1 New Zealand *P e a a* 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) diameter	Habitat		
					Altitude (m asl)	Vegetation	Soil
P. g e fa a	Parapara Inlet, Golden Bay	1	1	48	Sea level	Dense mixed forest	Limestone soils
P. g e a ea	Mangarakau	1	1	56	300	Dense mixed forest	Limestone soils
P. c e e c e e	Canaan, Pikikiruna Range	1	1	75	1,000	Litter and logs in	

Results

Genetic diversity

We analysed and aligned 87 sequences of 580–800 bp from the COI gene (Table 1). Translation of COI nucleotide seque

Table 2 Pairwise genetic distances among samples based on mtDNA COI sequences (expressed

Table 2 continued

		KJW360	KJW361	KJW362	KJW396	KJW398	KJW417	KJW70	KJW251	KJW375	KJW404	KJW82	KJW115	KJW95	KJW13	KJW290	KJW348	KJW119	KJW258
KJW373	*P. . ʷ e ba*																		
KWJ110	*P. a e*																		
KJW142	*P. a ec e*																		
KJW129	*P. c e e c e e*																		
KJW136	*P. g e aʷ ea*																		
KJW230	*P. g e fa a*																		
KJW26	*P. d a d ca*																		
KJW29	*P. ede a eʷ b ca a*																		
KJW63	*P. e c e*																		
KJW242	*P. gage*																		
KJW418	*P. gage*																		
KJW246	*P.* "Garabaldi"																		
KJW64	*P. a c e*																		
KJW65	*P. a c e*																		
KJW66	*P. a c e*																		
KJW72	*P. a c e*																		
KJW75	*P. a c e*																		
KJW76	*P. a c e*																		
KJW360	*P. a c e*																		
KJW361	*P. a c e*	0.005																	
KJW362	*P. a c e*	0.000	0.005																
KJW396	*P. a c e*	0.005	0.010	0.005															
KJW398	*P. a c e*	0.005	0.010	0.005	0.000														
KJW417	*P. a c e*	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	*P.* "Augustus"									
KJW404	*P.* "Augustus"	0.073	0.082	0.073	0.061	0.062	0.069	0.064	0.073	0.005									
KJW82	*P. g a a e a*	0.080	0.085	0.080	0.067	0.067	0.076	0.069	0.067	0.031	0.030	*P. g a a*							
KJW115	*P. g a a ʷ c a a*	0.076	0.088	0.076	0.085	0.085	0.077	0.092	0.092	0.030	0.033	0.011							
KJW95	*P. g a a g a a*	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. g a a ʷf ad a a*	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. g a a*	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. g a a e*	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. g a a ʷ ca*	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. g a a c*	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	

Although the deeper phylogenetic structure among the *P e a a* 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. ʻ e ba*, *P e a a c e e* and *P - e a a g e*) 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. c e e* and *P. g e* Table 2) although many morphologically distinct allopatric *P e a a* species have lower genetic divergences than this. For example the large and distinctive South Island *P. c e e* and the North Island species *P e a a a e* have COI sequences that differ by just 1.9%.

All analyses revealed a sister relationship of *P. g a a* 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. g a a* group. It was immediately evident that the Mt Augustus snails were not closely related to *P. a c e* 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. a c e* , a number of other taxa analysed are, including *P e a a* ''Buller River'', *P e - a a* ''Garabaldi'' and *P e a a* ''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. a c e* , 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. c e e* and *P. g e*) or spatially widely disjunct (e.g. *P. ede* and *P. ʻ e ba*), and shallower relationships among close allopatric or parapatric taxa (e.g. *P. a c e* group). This indicates that there have been 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 *P e a a*. It is closest to the

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. a c e)* 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

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