

## Relationships of *Placostylus* from Lord Howe Island: an investigation using the mitochondrial cytochrome *c* oxidase 1 gene

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### Abstract

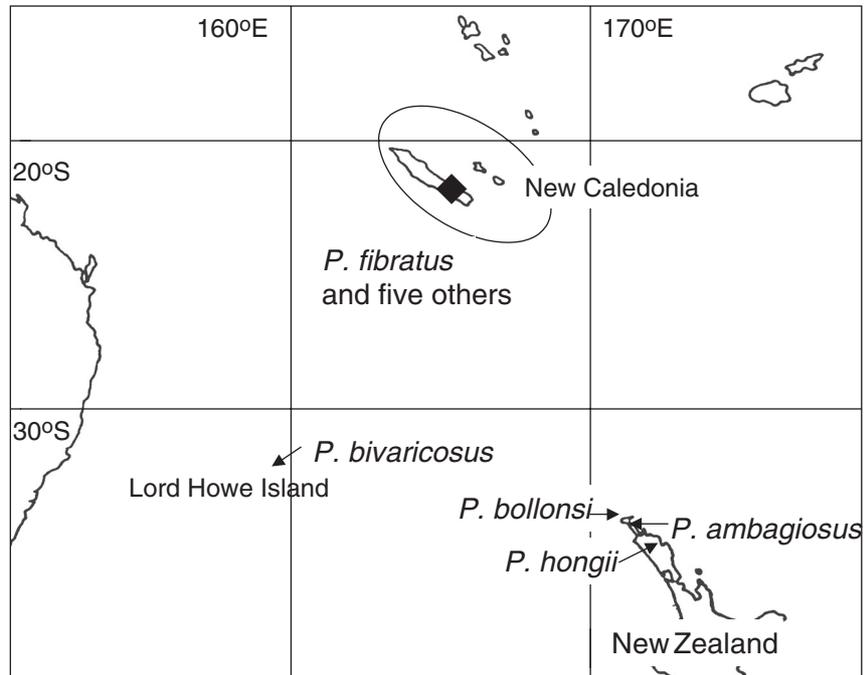
Large (5–9 cm in length) land snails of the genus *Placostylus* are found in New Caledonia and the Loyalty Islands, northern New Zealand, the Three Kings Islands just north of New Zealand and on Lord Howe Island. Their presence on Lord Howe, an oceanic island less than 6 million years old, has been an intriguing biogeographical question. Maximum parsimony and maximum likelihood analyses using cytochrome *c* oxidase subunit I sequence data suggest that the Lord Howe Island and mainland New Zealand taxa are sisters, but that the Three Kings taxon is independently derived, possibly from New Caledonian stock. *Placostylus* colonies throughout the area of the present study are under considerable threat, with many intraspecific forms and some species threatened and some listed as endangered species. The taxonomic and conservation status of the Lord Howe Island populations are discussed.

*Additional keywords:* biogeography, New Caledonia, New Zealand, south-west Pacific, systematics.

### Introduction

Oceanic islands typically have high levels of endemism and the origin of their species continues to be an intriguing question for evolutionary biologists. Notable examples among vertebrates of such studies include Darwin's finches of the Galapagos Islands, the honeyeaters of Hawaii (Freed *et al.* 1987) and *Brachylophis* on Pacific Islands (Gibbons 1981, 1985). There are also spectacular examples of radiations among invertebrates, some of the better known being the Pacific Island land snails, where there have also been massive human-induced extinctions (e.g. Solem 1990; Cowie 1992, 1996).

The likelihood of dispersal of individuals and taxa to oceanic islands is dependent on many factors, including the distance from source populations and the size and habitat complexity of the island. However, overriding all these factors is the dispersal ability of the taxon. Dispersal ability can be determined by intrinsic abilities (power of flight, body size and physiology (e.g. resistance to desiccation, tolerance of salt water, habits or habitat preferences; an arboreal species may be more likely to be transported by wind storms than a ground-living or burrowing species)) or extrinsic factors (prevailing winds, presence of suitable dispersal agents etc.). Land snails have no intrinsic means of long-distance dispersal, although small-sized species, in particular, may be dispersed aerially during major storms, accidentally carried by birds (Rees 1965; Vagvolgyi 1975; Kirchner *et al.* 1997) or rafted on floating vegetation. Successful long distance passive dispersal for most taxa is rare, especially once communities are established (Ward and Thornton 2000). The improbability of oversea dispersal for some taxa has led to hypotheses involving sunken continents or land bridges.



**Fig. 1.** The SW Pacific, showing the locations of the species of *Placostylus* included in the present analysis. The ellipse around New Caledonia is intended to show the overall range of the six species of *Placostylus* recognised from that area. The diamond indicates the locality of the New Caledonian specimen of *P. fibratus* used in the analysis.

Lord Howe Island (31°33'S, 159°05'E) lies in the Tasman Sea, is approximately 11 km long and rises to 875 m. It is nearest to eastern Australia, lying 700 km NE of Sydney and 496 km E of Port Macquarie, the nearest point on the coast of New South Wales (NSW). Norfolk Island is 890 km away and Auckland, New Zealand, is 1560 km away (Fig. 1). The highly endemic fauna and flora have elicited much discussion (see Paramonov 1958, 1960, 1963). The island is of volcanic origin, being formed between 6.4 and 6.9 million years ago (McDougall *et al.* 1981), and lies on the eastern edge of the Lord Howe Rise, a submarine fragment of eastern Australia that separated in the Cretaceous (Cook and Belbin 1978). Drill cores show that the Rise itself has never been above water (Van der Lingen 1973). A chain of submarine seamounts runs due north of Lord Howe Island. These are assumed to be progressively older northwards (McDougall *et al.* 1981).

Among the highly endemic fauna and flora is the extinct horned tortoise (*Meiolania platyceps* Owen, 1886) that was apparently terrestrial with poor swimming abilities (Gaffney 1983, 1996) and with close relationships to taxa in eastern Australia and New Caledonia (Walpole Island; Gaffney 1996). Notable among the endemic flora are three endemic genera of palms. There are many indigenous invertebrates, including over 80 species of land snails (Iredale 1944). Prominent among the land snails is *Placostylus bivaricosus* (Gaskoin, 1855), which reaches up to approximately 8 cm in length. Hedley (1892) stated that '... *Placostylus* appears a more fruitful subject of study [for biogeography] than any other molluscan genus inhabiting the same area' and the mystery of the presence of *Placostylus* on Lord Howe was also discussed by Etheridge (1891).

The distribution of *Placostylus* was such a conundrum to Hedley (1892) that he hypothesised that the islands containing these snails were ‘... portions of a shattered continent [which he called the Melanesian Plateau] and are connected by shallow banks formerly dry land’. This hypothesis met strong opposition from at least one notable contemporary, Alfred R. Wallace (see text of letter to Hedley; Wallace 1974).

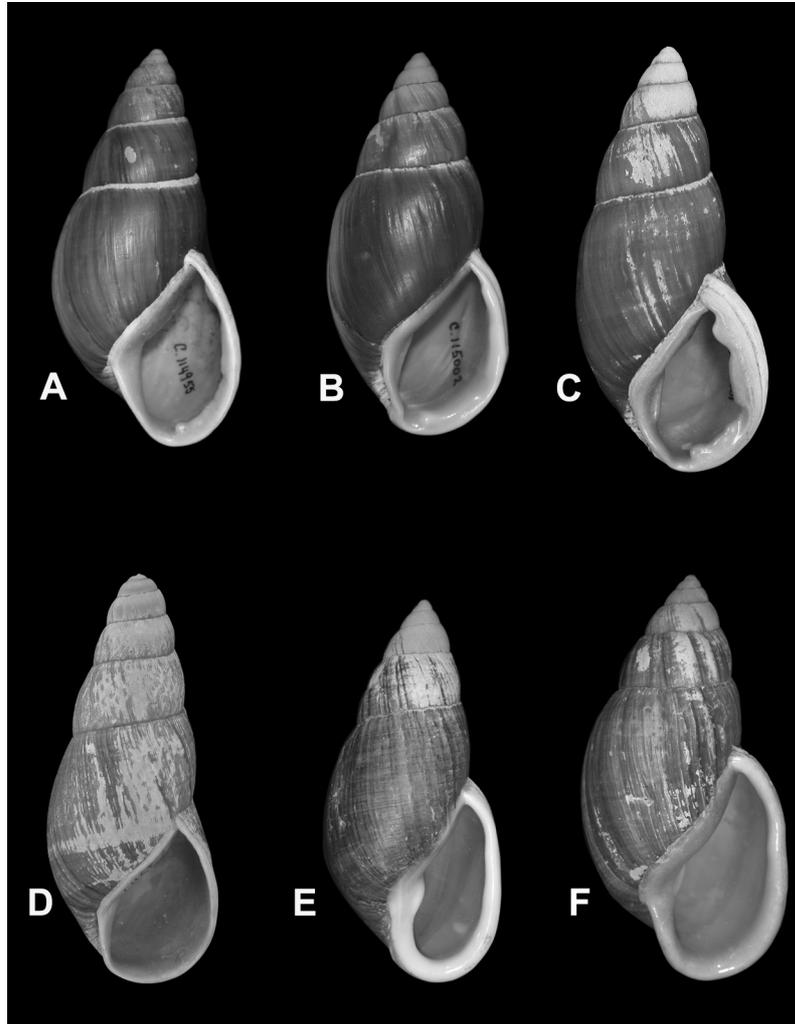
In the most recent taxonomic review of the group (Haas 1935), species attributed to the genus *Placostylus* are found through some of the western Pacific Islands (New Zealand, New Caledonia, Solomon Islands, Fiji and Vanuatu). Haas (1935), who divided the genus into several subgenera, restricted the typical subgenus to New Caledonia (type species *Limax fibratus* Martyn, 1784; ICZN Opinion 1662 (ICZN 1992)). Several ‘subgenera’ are recognised that encompass the Pacific Island and New Zealand taxa, but the New Zealand–Lord Howe and New Caledonian (plus Loyalty Islands) taxa, with their large, solid shells, are more similar to one another than to the taxa further north, as noted by Hedley (1892). Because no other similar species are included in this grouping, it is reasonable to assume that the sister-taxon of the Lord Howe species is from either New Zealand, the Three Kings Islands or New Caledonia.

The New Caledonian (including Loyalty Islands) *Placostylus* are still relatively poorly known, both taxonomically and biologically. There are many names available in the literature, but no modern revisions have been published. Franc (1956) recognised 19 species but, using anatomical characteristics, Chérel-Mora (1982, 1983) reduced these to four, although most of her work remains unpublished. Dr E. Neubert, who is currently investigating the taxonomy and anatomy of the group in New Caledonia and the region, stated that, of approximately 140 available names, six valid species and approximately 20 geographic subspecies can be recognised in New Caledonia (Neubert 2001). The shell morphology of some of the New Caledonian taxa is very similar to that of Lord Howe Island and New Zealand taxa. The taxon included in the present analysis is *P. fibratus* (Fig. 2E,F), the type species of *Placostylus*. Many of the New Caledonian taxa are threatened (Neubert 2001) and some are still used for food. Salas *et al.* (1997) provide some data on the biology of *P. fibratus*.

While the systematics of the Lord Howe species is poorly understood (see Appendix), a recent survey of extant populations has been performed (Ponder and Chapman 1999), as well as a preliminary study of the genetics (Colgan and Ponder 2001).

Species similar to Lord Howe Island *Placostylus* occur in northern New Zealand (Powell 1947, 1951b; Choat and Schiel 1980; Triggs and Sherley 1993; Sherley 1996) and the Three Kings Islands (off northern-most New Zealand; Powell 1951a; Brook and Laurenson 1992). Members of the subgenus *Maoricolpus* Haas, 1935 (type species *Bulimus shongii* (= *hongii*) Lesson, 1830) include the species found in northern New Zealand and Lord Howe Island. The Three Kings Islands species (*P. bollonsi* Suter, 1908) is placed in a monotypic subgenus, namely *Basileostylus* Haas, 1935.

In New Zealand, there are three main taxa. *Placostylus hongii* (Fig. 2A) has been recorded from sites between Whangaroa and Whangarei on the mainland Northland and on offshore islands between Whangaroa and Great Barrier Island (Powell 1979; Browne 1980; Brook and McArdle 1999). *Placostylus ambagiosus* Suter, 1906 (Fig. 2B,C), comprising ten named extant subspecies (Powell 1947, 1951b), is located in the northern-most tip of the Northland Peninsula, where it is confined to tiny remnant populations. The third taxon, *P. bollonsi* (Fig. 2D), is confined to the Three Kings Islands that lie 60 km NW of Cape Reinga. This group is comprised of one large island and three small islands, all of which have colonies of this snail (Brook and Laurenson 1992). The biota of these islands also



**Fig. 2.** Shells of *Placostylus* species (all material from the Australian Museum, Sydney, NSW; dimensions are maximum length). Images not to same scale. *A*, *P. hongii* (Lesson), Tauranga-Kawai Point, 6 km N of Whanaki, Northland, New Zealand, C.114955, 77.6 mm. *B*, *P. ambagiosus paraspiritus* Powell, paratypes, headland 1 mile S of Cape Maria van Diemen, Northland, C.115002, 64.4 mm. *C*, *P. ambagiosus whareana* Powell, paratype, valley to north of Whareana Stream, between Waikuku Beach and Parengarenga, Northland, C.115001, 82 mm. *D*, *P. bollonsi bollonsi* (Suter), paratype, Big King Island, Three Kings Islands, C.29117, 91.5 mm. *E,F*, *P. fibratus* (Martyn), Isle of Pines, New Caledonia, C.409415, 84 mm (*E*) and Bourail, New Caledonia, C.409409, 84 mm (*F*).

includes many other endemics. All the New Zealand species of *Placostylus* have received considerable taxonomic (Powell 1938, 1947, 1948, 1951*a*, 1951*b*; Climo 1973; Sherley 1996) and, more recently, conservation attention (Brook and Laurenson 1992; Triggs and Sherley 1993; Sherley 1996; Sherley *et al.* 1998), including the publication of a Recovery Plan (Parrish *et al.* 1995).

Some of the New Zealand taxa are critically endangered: the Three Kings species, *P. bollonsi*, is confined to two small populations distinguished as separate subspecies

(Powell 1948, 1951a; Climo 1973), one of approximately 130 individuals in a small area of only approximately 1.69 ha and the other of approximately 360 individuals in approximately 2.7 ha (Brook and Laurenson 1992). These populations are restricted by lack of suitable habitat and are not threatened significantly by predators. *Placostylus ambagiosus*, a species found only in far northern New Zealand, consists of a number of named subspecies (Powell 1938, 1947, 1951b) confined to tiny remnant populations that show some degree of genetic structuring using isozyme electrophoresis (Triggs and Sherley 1993). Some of these populations have fewer than 10 living individuals (Parrish *et al.* 1995) and all are threatened by introduced predators (rats, birds and pigs). *Placostylus hongii*, which lives south of *P. ambagiosus* in Northland, is known from a few mainland locations and some offshore islands and is extant from five mainland populations and three offshore island groups (Brook and McArdle 1999). Translocation of the two mainland species has been undertaken for conservation purposes (Sherley 1994).

The broader phylogenetic relationships of *Placostylus* within the Bulimulidae are still not fully understood. Solem (1959) argued for a relationship between *Bothriembryon*, the New Hebridean (i.e. Vanuatu) *Diplomorpha* and *Placostylus* based on anatomical data, with the latter genus having a northern origin. However, Breure (1979) placed *Placostylus* in a separate subfamily that he regarded as the sister-taxon to the rest of the family. He hypothesised that members of the Placostylinae reached New Zealand via east Antarctica and moved north. According to Breure (1979), placostylines show little relationship with the buliminids found in the western and southern parts of Australia (*Bothrembrion*; subfamily Orthalicinae).

The present paper provides evidence from molecular data relating to the question of the origin of the Lord Howe Island *Placostylus*. Because the 'subspecific' taxa (see Appendix) on Lord Howe Island are extinct, their status cannot be assessed using this methodology.

## Material and methods

### Material

A list of the specimens sequenced is given in Table 1. The sequenced specimen of *P. fibratus* came from a population with intermediate characteristics between *P. fibratus fibratus* and *P. fibratus souvillei* Kobelt, 1891 (Dr E. Neubert, personal communication).

### DNA extraction and sequencing

Lord Howe Island and New Caledonia specimens were extracted and sequenced in Sydney, whereas New Zealand specimens were extracted and sequenced at Landcare Research, Auckland.

For New Zealand specimens, foot muscle tissue was homogenised in 500  $\mu$ L extraction buffer (0.01 M EDTA, 0.05 M NaCl, 0.5 M Tris-HCl, pH 8.0, 2% sodium dodecyl sulfate). A 10  $\mu$ L aliquot of 10 mg mL<sup>-1</sup> proteinase K (Boehringer, Mannheim, Germany) was added and the homogenate incubated at 65°C for 1 h. Samples were extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1), followed by extraction with chloroform/isoamyl alcohol (24:1). The DNA was ethanol precipitated and the pellet rehydrated in 50  $\mu$ L buffer (10 mM Tris Cl (pH 7.4), 1 mM EDTA (pH 8.0)) following RNase treatment.

A 655 bp region of the cytochrome *c* oxidase 1 (*COI*) gene was amplified by polymerase chain reaction (PCR) using primers LCO-1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO-2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') designed by Folmer *et al.* (1994). Amplifications were performed in a volume of 50  $\mu$ L and consisted of 10 pmol of each primer, 10 mM Tris-Cl, pH 8.3, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl and 0.2 mM of each dNTP. The addition of 2 units Taq polymerase (Boehringer) followed an initial step of 2 min denaturation at 94°C. Cycling consisted of denaturation at 94°C for 1 min, annealing at 50°C (*COI*) for 1 min and extension at 72°C for 1 min and 30 s, for 35 cycles. A final cycle included a 5 min extension at 72°C.

The PCR products were purified using the QIAquick™ PCR direct purification kit (Qiagen, Venlo, The Netherlands), according to the manufacturer's instructions. Direct sequencing of purified products by the

Table 1. List of specimens sequenced and their localities

Specimen	Museum no.	Locality	Tissues
<i>P. bivaricosus</i>		Lord Howe Island	F
P1	C387658	Forest near Airport, 31°32'29" 159°4'29"	F
P2	C387660	Stephen's Reserve, 31°31'28" 159°3'56"	F
P3, 6	C387650	Lagoon foreshore, 31°31'41" 159°3'47"	F
P4	C387742	Ned's Beach, 31°31'20" 159°3'52"	F
P5	C387675	Hiscox property, 31°31'17" 159°4'04"	F
P7-8	C387687	North Bay, 31°31'08" 159°2'31"	F
<i>P. ambagiosus</i>		Far north, Northland, New Zealand	F
<i>P. a. 'tirikawa'</i>	WG850-853	Near Tirikawa Pa, 34°26'29" 172°44'44"	DG, M
<i>P. a. wattii</i>	Powell GS 6	North Cape, east, 34°24'47" 173°02'47"	DG, M, Ov
<i>P. a. annectens</i>	Powell		
GS 16, 17, 19			
GS18, 20			
<i>P. a. lesleyae</i>	Powell GS 21-25	Te Huka, 34°25'10" 172°55'54"	DG, M
<i>P. hongii</i>		Matirarau, 34°25'17" 172°54'24"	DG, M, Ov
WG 862-865		Te Paki Trig, 34°28'03" 172°46'18"	DG, M
		Northland, New Zealand	
		Whangaruru North Head Scenic Reserve (35°22'20" 174°21'45"),	DG, M
		Northland, New Zealand	
<i>P. bollonsi</i>		Three Kings Islands, New Zealand	DG, M
<i>P. b. bollonsi</i>	GS 26-30	Three Kings, 34°09'06" 172°08'40"	DG, M, Ov
<i>P. b. caperatus</i>	Powell GS 31-35	Three Kings, 34°09'13" 172°08'25"	DG, M, Ov
<i>P. b. arbutus</i>	Powell GS 36-40	Three Kings, 34°09'22" 172°07'46"	DG, M, Ov
<i>P. b. 'northeast'</i>	GS 41-45	Three Kings, 34°07'55" 172°09'59"	DG, M, Ov
<i>P. fibratus</i>		New Caledonia	
	C409417	On the SW slope of Monts Koghis at approximately 500 m, 22°10'S 166°32'E	F

DG, Digestive gland; F, foot; M, mantle; Ov, ovary.

The New Zealand material is lodged in the New Zealand Arthropod Collection at Mt Albert, Auckland; other voucher material is housed in the Australian Museum, Sydney, NSW.

primers used for the original amplification was achieved using the Prism Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Norwalk, CT, USA), following the manufacturer's instructions. Sequences were analysed on an automated DNA sequencer (model 377; Applied Biosystems). Two independent PCR products from each specimen were sequenced in both directions.

For the Sydney experiments, molecular biological procedures generally followed the methods of Colgan *et al.* (2000a, 2000b). The DNA extraction of foot muscle followed the hexadecyltrimethylammonium bromide procedure of Saghai-Marooof *et al.* (1984).

The Sydney primer pair for *COI* is listed below with degeneracies written with standard IUB codes. Numbers in parentheses indicate the 5' end of the sequence in the complete mitochondrial genome of the earthworm *Lumbricus terrestris* (Boore and Brown 1995): COX1AF, (19) CWAATCAYA AAGATATTGGAAC; COX IAR, (726) AATATAWACTTCWGGGTGACC. Polymerase chain reaction cycling used the same basic protocols as Colgan *et al.* (2000a, 2000b), with a general reaction mix of 1.0 U Red Hot™ thermostable DNA polymerase, buffer IV (10×, 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 750 mM Tris-HCL, pH 9.0, 0.1% (w/v) Tween; Advanced Biotechnologies, Leatherhead, Surrey, UK), 0.05 mM dNTPs, 2.5 mM MgCl<sub>2</sub>, 12.5 pmol of each primer and 1 μL of a dilution (usually 1:10) DNA sample in a reaction volume of 50 μL overlaid with 30 μL oil. The usual cycling profile was denaturation at 95°C for 4 min, annealing at 45°C for 45 s and extension at 72°C for 30 s (one cycle), followed by 32 cycles of 95°C for 30 s, 45°C for 45 s and 72°C for 30 s and one cycle of 95°C for 30 s, 45°C for 45 s and 72°C for 5 min. The PCR products were purified using the QIAquick™ PCR Purification Kit and sequenced in both directions with an automated DNA sequencer (ABI® 310; Applied Biosystems) using the BigDye™ version 2.0 sequencing kit.

#### Alignment and sequence composition

Sequences were edited using Sequence Navigator version 1.0.1 (Anonymous 1994). A compilation of all sequences was aligned using the default values for parameters in CLUSTAL W (Thompson *et al.* 1994). It was not necessary to alter this alignment manually. Data were compiled using MacClade (Maddison and Maddison 1992).

Maximum parsimony was conducted in PAUP\* 4.0b9 (Swofford 2000) with the default conditions for parsimony analyses with branch and bound searches guaranteed to find the shortest trees. All characteristics were unordered and unweighted. The steepest descent option was not enforced and accelerated transformation for character optimisation was assumed. Zero length branches were collapsed to give polytomies. Gaps were treated as unknown in all analyses. Bootstrap pseudo-sampling was conducted for 100 replicates with branch and bound searching in each. Shortest trees satisfying alternative hypotheses of relationships were sought with the same strategy but using constraints in PAUP.

For maximum likelihood analyses in PAUP\*, a Hasegawa–Kishino–Yano (HKY) invariant model was used with the following settings. The number of substitution types was two, with the transition transversion ratio estimated by maximum likelihood. Empirical nucleotide frequencies were used. The proportion of invariable sites was estimated via likelihood and the distribution of rates at variable sites was assumed equal. The molecular clock model was not enforced. Other parameters took the default values in PAUP\* 4. Comparison of trees using Kishino–Hasegawa likelihood tests assumed a normal approximation, with a two-tailed test. Shimodaira–Hasegawa tests used a one-tailed resampling of estimated log-likelihoods bootstrap with 1000 replicates.

Outgroup sequences were obtained from the helicoidean pulmonates *Aegista scepasma* (Bradybaenidae) (Genbank Accession AB024900; Shimizu and Ueshima 2000), *Cepaea nemoralis* (Helicidae) (Genbank Accession U23045; Yamazaki *et al.* 1997), *Euhadra herklotsi* (Bradybaenidae) (Genbank Accession Z71701; Yamazaki *et al.* 1997) and *Albinaria caerulea* (Genbank Accession NC\_001761; Hatzoglou *et al.* 1995).

#### Morphometrics

Shell measurements were made using callipers. The discriminant analysis was undertaken using SYSTAT™ version 10 (SPSS, Chicago, IL, USA).

## Results

The locations of samples are shown in Table 1.

GenBank accession numbers for the *COI* sequences used are AY165836–AY165843, AY165852 and AY290737–AY290745. The alignment used in the analysis is available from

the authors. As usual with mitochondrial sequences, there is a major AT bias in nucleotide composition. The mean overall percentages of nucleotides is as follows: A, 26.844; C, 14.653; G, 17.174; T, 41.328 (overall AT average 68.17%).

The probability that the nucleotide composition of the sequences is homogeneous is very close to 1 ( $\chi^2 = 37.600$ , d.f. = 63,  $P = 0.99$ ), suggesting that variation in base composition between taxa has little effect on the analyses.

The levels of pairwise difference of the HKY measure of genetic distance within the *Placostylus* samples vary between 0.0188 (within Lord Howe Island) and 0.6278 (between *P. ambagiosus annectens* and *P. bollonsi* 'north-east') within New Zealand samples. In Table 2, the averages of the Kimura two-parameter distance are given for comparisons within and between various species. Within species, the averages are less than 0.1, but distances between species rise to 0.2014 for the comparison of Lord Howe Island snails with *P. bollonsi*.

The results are illustrated in Figs 3, 4. The consensus of the maximum parsimony trees (Fig. 3) includes the following strongly supported monophyletic clades: Lord Howe Island specimens (with a decay index of 27), *P. ambagiosus*, *P. ambagiosus* + *P. hongii* and *P. bollonsi*. Notably, the New Zealand samples are split into two distinct lineages. Constraining these to be monophyletic requires five more steps than the unconstrained, most parsimonious trees. The Lord Howe Island samples are sister to the *P. ambagiosus* + *P. hongii* clade. This group is, in turn, sister to the New Caledonian sample. Four extra steps are required to make the Lord Howe specimens sister to this to the exclusion of the New Zealand snails. A sister pairing of Lord Howe and New Caledonia specimens is seen in only 6.6% of bootstrap trees compared with 38% of bootstrap replicates showing the pairing of Lord Howe Island with *P. ambagiosus* + *P. hongii*. The sister pairing of New Zealand *P. ambagiosus* + *P. hongii* with New Caledonia or all the New Zealand taxa with New Caledonia requires, respectively, two and five more steps than the maximum parsimony tree. Comparisons of the likelihoods of the trees satisfying various constraints are shown in Table 3 and the maximum likelihood tree is shown in Fig. 4. In particular, the Kishino–Hasegawa tests reject the possibility that there is a single New Zealand radiation that is

**Table 2. Average of pairwise genetic distances within and between *Placostylus* species using the Kimura two-parameter genetic distance**

Comparisons	Average
Within species	
<i>P. ambagiosus</i>	0.0144
<i>P. bivaricosus</i>	0.0630
<i>P. bollonsi</i>	0.0028
Between species	
<i>P. ambagiosus</i> / <i>P. bivaricosus</i>	0.1581
<i>P. ambagiosus</i> / <i>P. bollonsi</i>	0.1755
<i>P. ambagiosus</i> / <i>P. fibratus</i>	0.1345
<i>P. ambagiosus</i> / <i>P. hongii</i>	0.0572
<i>P. bivaricosus</i> / <i>P. bollonsi</i>	0.2014
<i>P. bivaricosus</i> / <i>P. fibratus</i>	0.1717
<i>P. bivaricosus</i> / <i>P. hongii</i>	0.1494
<i>P. bollonsi</i> / <i>P. fibratus</i>	0.1849
<i>P. bollonsi</i> / <i>P. hongii</i>	0.1732
<i>P. fibratus</i> / <i>P. hongii</i>	0.1325



**Table 3. Comparisons of the likelihoods of trees constrained to show various relationships**

Tree	–ln L	Diff –ln L	Kishino– Hasegawa	Shimodaira– Hasegawa
Unconstrained	3019.61928			
NC with NZ	3025.29525	5.67597	0.000*	0.182
NC with LHI	3023.03754	3.41826	0.000*	0.346
LHI with NC + part NZ	3023.02717	3.40789	0.000*	0.339

The first column shows the imposed constraint (if any), the second column shows the negative of the log likelihood of the maximum likelihood (ML) trees, the third column shows the difference between tree likelihoods and the last two columns give the probabilities that the compared trees have the same likelihood under the Kishino–Hasegawa (two-tailed) test and the Shimodaira–Hasegawa (one-tailed) test. The constraints are that: (1) New Caledonia (NC) and all New Zealand (NZ) specimens are a sister pair; (2) New Caledonia and all Lord Howe Island (LHI) snails form a sister pair; and (3) the Lord Howe Island snails are sister to a group comprising the New Caledonian sample and New Zealand *P. hongii* and *P. ambagiosus*.

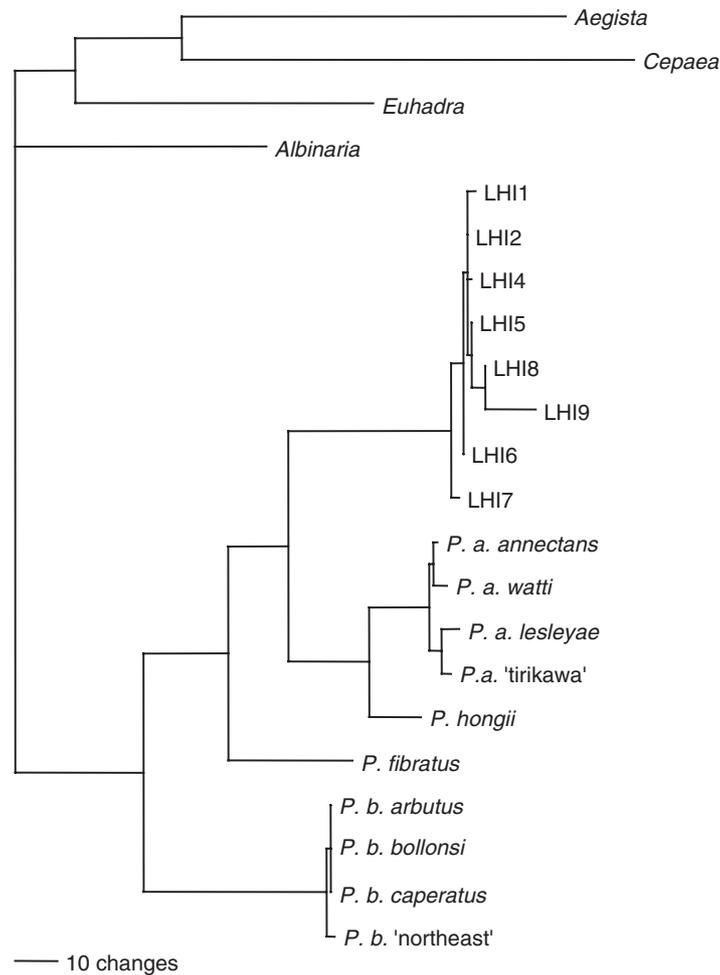
sister to the New Caledonian sample (constraint 1) or that this latter specimen and the Lord Howe Island snails are sister taxa (constraint 2).

### Discussion

New Caledonia and New Zealand were separated by the end of the Cretaceous (Hall 2002), but it is unclear as to how much of the Norfolk Ridge remained emergent through the early Tertiary. The oldest basalts on Lord Howe Island were formed only 6.4 million years ago. Thus, the geological evidence indicates that dispersal, rather than vicariance, must account for the presence of *Placostylus* on Lord Howe Island. While we can only speculate about possible dispersal mechanisms, an analysis such as this can provide evidence for the likely origin of the source for the dispersal event. This does not eliminate the probability that past intermediate populations, for example, on islands that are now submerged seamounts, ridges or plateaus to the north of Lord Howe Island (or the Three Kings Islands), may have facilitated dispersal as ‘stepping stones’.

The Three Kings Islands are comprised of indurated, deformed marine sedimentary and volcanic rocks of probable early Cretaceous age (Haywood and Moore 1987; Brook and Laursen 1992). Brook and Laursen (1992) state that ‘... it is possible that marine conditions persisted in the vicinity of present day Three Kings Islands for much or all of that time’ (Cretaceous to early Cenozoic). The present configuration of northern New Zealand (including the Three Kings) was probably developed during the mid-Cenozoic, with a subaerial peninsula developed from about Kaitiaki/Doubtless Bay to the Three Kings area in the early Miocene (15–20 million years ago), this degenerating to a few islands by the mid-late Miocene (Brook and Thrasher 1991). The northern-most end of Northland (‘North Cape’) was, at that time, a separate island and was connected to the rest of Northland subsequent to the early Pliocene (5–3 million years ago; Pillans *et al.* 1992).

Whereas dispersal must be invoked to account for the presence of *Placostylus* on Lord Howe Island, the Three Kings Islands could, seemingly, have been connected or nearly connected to Northland in the mid-Tertiary and closely adjacent (approximately 10 km) during interglacial periods in the Pleistocene (Brook and Laursen 1992). Given these data, postulating an origin of *P. bollonsi* from Northland seems logical and has been argued for by Brook and Laursen (1992). However, there is little support for this hypothesis from our data, which suggest that the Three Kings taxon and the mainland taxa represent two



**Fig. 4.** Phylogram for the cytochrome *c* oxidase 1 data of the maximum likelihood tree resulting from an heuristic search with 10 replicates of random sequence additions. See Fig. 3 for details of taxa.

separate clades, as reflected in the allocation of different subgeneric names. It appears that the Three Kings taxon is the result of either a long overseas dispersal event or (more probably, in our opinion) it represents a surviving relict of a taxon distributed on now submerged islands along the Norfolk Ridge.

Our results clearly suggest a sister relationship between the northland *P. ambagiosus* and *P. hongii*, separated by the late Pliocene isthmus between what was once a northern island. Whereas there is strong support for the monophyly of each of the main radiations (*P. ambagiosus*, *P. hongii*; *P. bollonsi* and *P. bivaricosus*), regrettably, the analysis is weakened by there being only a single sample from New Caledonia.

Brook and McArdle (1999) have advocated pre-European Maori transport of *Placostylus hongii* (which was used as a food item; Haywood and Brook 1981) to and from offshore islands to account for their present distribution. There is no evidence of pre-European contact with Lord Howe Island, so some other form of overseas dispersal must have occurred. This most likely occurred as a result of an individual being carried in a

severe storm to the island. Whereas this may have come from a nearby population on an island (that has since disappeared) to the north, given that there is a chain of seamounts northwards, the results of our analysis suggest that there was a common origin with the mainland New Zealand taxa. Self-fertilisation is common in pulmonate snails, so even a single juvenile could establish a population. *Placostylus* hatchlings are known to be often arboreal, this habit facilitating such a mode of dispersal of this otherwise ground-dwelling species. Extremely rare aerial dispersal of hatchlings and juveniles, less than 1 cm in length, during major storms seems, to us, to be a more feasible explanation of dispersal than other hypotheses invoking rafting or transport by birds.

The results show low levels of genetic divergence between individual Lord Howe Island sequences (Fig. 3) but, on the available small amount of material, no clear pattern emerges (*16S* rDNA sequences are also available for the Lord Howe Island samples and show substructure between the available populations (Colgan and Ponder 2001)). There is considerable variation in shell morphology within the area containing the material used in the analysis (Ponder and Chapman 1999) but, again, the material sampled is not adequate to address questions relating to whether there is any underlying genetic basis for this variation. The range of the available samples from Lord Howe Island does not cover all the island, with none coming from the hills and mountains in the southern part where *Placostylus* appears to be extinct. While it remains possible that more southerly populations, from the remote areas around Mounts Lidgbird and Gower, may still be extant, it remains untested whether they are genetically distinct, as suggested by their shell morphology, or whether there is a significant geographic barrier to gene flow in this region. Triggs and Sherley (1993) showed that there was a considerable level of genetic variation between populations of *P. ambagiosus*. In these cases, small but overlapping differences in shell morphology led to prior creation of several subspecies (Powell 1938, 1947, 1948, 1951b; Sherley 1996).

The genetic status of New Zealand *Placostylus* species has been assessed by allozyme electrophoresis (Triggs and Sherley 1993). Evidence of distinct lineages (possibly at the species level) was found in one mainland morphospecies, *P. ambagiosus*, over a geographic scale of tens of kilometres, with differentiation apparently fostered by Pleistocene and late Pliocene sea level changes. These authors found no significant variation in allozymes in *P. bollonsi* from the Three Kings Islands, but the results presented herein suggest that at least the north-east population may be distinct.

The results clearly discount the Lord Howe Island taxon being a recent introduction from New Zealand but, because we have only one specimen from New Caledonia, where there are probably six Recent species present, the possibility of a relatively recent introduction from that source cannot be discounted.

#### *Conservation status of Lord Howe Island Placostylus*

Etheridge (1889) noted that *Placostylus* on Lord Howe Island was '... found everywhere under cover, and in immense numbers' and that it '... appears to avoid open spaces as a rule, and prefers shady damp situations and the scrubby hill sides where composed of the Coral-sand rock. It is sparingly represented even at the higher altitudes, being reported as seen under the 'wall' of Mount Ledgbird.' Hedley (1891) noted that it was '... all over the island in sheltered places under stones; abundant'.

However, this previously abundant animal has been the subject of conservation concern for some time. Iredale (1944) noted that there was a '... new difficulty of recent years, the destruction effected by the rat plague, written up by Hindwood'. More recently, Smithers

*et al.* (1977) noted that *Placostylus* is apparently ‘... existing in only four small colonies, whereas from early reports it was dispersed widely from sea-level up to the mountain tops’. Ponder and Chapman (1999) reported that all the living specimens found in their 1999 survey were located on low, rather flat ground with ample litter. In most cases, the samples were taken on calcarenite soils or sandy soils derived, in large part, from calcarenite. Living specimens were found under well-developed litter. *Placostylus* seem to prefer the vicinity of the large native fig trees, but not exclusively so.

*Placostylus bivaricosus* is listed as endangered in NSW under the Threatened Species Conservation Act and a Recovery Plan (NSW National Parks and Wildlife Service 2000) has been prepared.

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### Appendix. The Lord Howe Island *Placostylus* taxa

*Placostylus bivaricosus* (Gaskoin, 1855) is the only currently recognised species found on Lord Howe Island (Smith 1992). The last review of this group was by Iredale (1944), who noted that the: 'variation in this group is worthy of study ... At the present time the various forms may be less visible in the field than in Roy Bell's time, when he indicated no less than six different colonies, separable in the field'.

No critical examination of the taxonomy of the Lord Howe Island *Placostylus* has ever been undertaken, although Smith (1992) catalogued the taxa and details of original names and references can be obtained from that source. Material in the Australian Museum collections appears to support the recognition of at least three, possibly four (see below), Recent and one fossil taxon on the basis of shell morphology (Ponder and Chapman 1999). These can tentatively be treated as 'subspecies' as follows.

#### *Placostylus bivaricosus bivaricosus* (Gaskoin, 1855)

*Bulimus bivaricosus* Gaskoin, 1855. Lord Howe Island (location of type material not known; fide Smith 1992: 108).

*Placostylus bivaricosus belli* Iredale, 1944. Lord Howe Island (holotype Australian Museum C.38973; Fig. 5C).

#### Remarks

The typical subspecies (Fig. 5A,B) is found on the northern end of the island and in the settlement area as far south as Intermediate Hill. *Placostylus bivaricosus belli* (Fig. 5C) is based on a small, narrow (shell length 54.6 mm, width 22.2 mm, aperture length 24.3 mm) specimen that can be matched with other occasional small specimens found within the settlement area.

#### *Placostylus bivaricosus etheridgei* (Hedley, 1891)

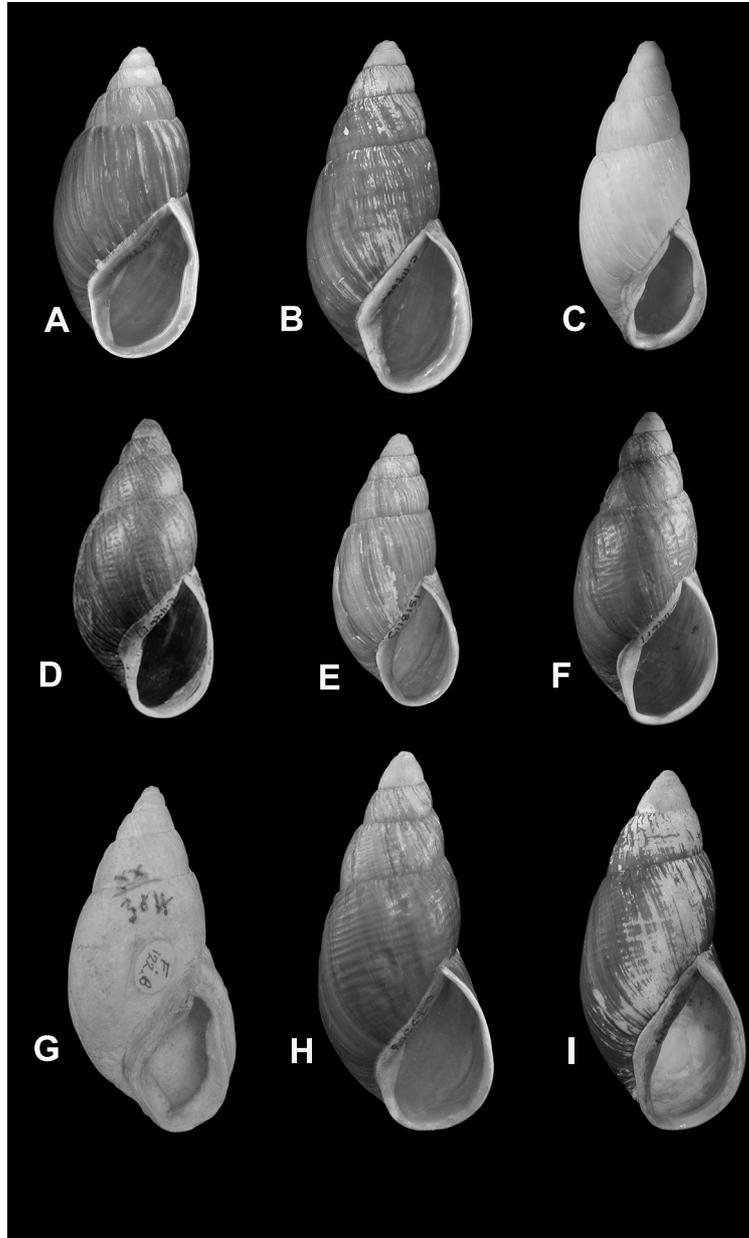
*Bulimus bivaricosus etheridgei* Hedley, 1891. Under the wall of Mt Lidgbird (Hedley, 1891). Iredale (1944) states that this species is 'apparently from the saddle of Mt Lidgbird' (three syntypes, Australian Museum C.33308; Fig. 5H).

*Placostylus etheridgei* 'Brazier', 1889. *Nomen nudum* (introduced as a name on the caption to a figure in Etheridge, 1889).

*Placostylus bivaricosus royi* Iredale, 1944. Little Slope, base of eastern side of Mount Gower (holotype, Australian Museum C.38974; Fig. 5I).

#### Remarks

This taxon is found in the southern mountains. It is variable in size, although often large, and has a thin shell with a weakly developed apertural varix and the surface of the shell is sculptured with oblique rugae. It is probably extinct, no living specimens having been collected for several decades. There is some suggestion from museum material of overlap, or perhaps merging, of typical *bivaricosus* and *etheridgei* in the area around Intermediate Hill. Unfortunately, the populations in this area are now either extremely reduced or, more likely, extinct. Both the available names for this taxon are from populations around the base of Mount Lidgbird and Mount Gower, or in the Erskine Valley, which lies between the two mountains. These have a significantly larger shell than samples from the top of Mount Gower (Fig. 5D,F; see below).



**Fig. 5.** Shells of *Placostylus* from Lord Howe Island (all material from the Australian Museum, Sydney, NSW; dimensions are maximum length). Images not to same scale. *A,B*, *P. bivaricosus bivaricosus* (Gaskoin), Lord Howe Island, C.114669, 56.6 mm (*A*) and C.114666, 60.7 mm (*B*). *C*, Holotype of *P. bivaricosus belli* Iredale, Lord Howe Island, C.38973, 54.6 mm. *D,F*, *P. aff. bivaricosus etheridgei* (Hedley), Mount Gower (C.114677), 54.0 mm (*D*) and 62.2 mm (*F*). *E*, *P. bivaricosus cuniculinsulae* (Cox), Rabbit (= Blackburn) Island, Lord Howe Island, C.118151, 45.6 mm. *G*, *P. bivaricosus solidus* (Etheridge), syntype, Lord Howe Island, C.171111, 74.8 mm. *H,I*, *P. bivaricosus etheridgei* (Hedley), syntype of *Bulinus etheridgei* Hedley, Mt Lidgbird, Lord Howe Island, C.33308, 67.2 mm (*H*) and holotype of *P. bivaricosus royi* Iredale, Little Slope, Lord Howe Island, C.38974, 77.2 mm (*I*).

***Placostylus bivaricosus cuniculinsulae* (Cox, 1872)**

*Bulimus (Placostylus) cuniculinsulae* Cox, 1872. Rabbit Island (= Blackburn Island), Lord Howe Island (holotype, Natural History Museum, London, 1888.12.11.47–8).

**Remarks**

This small form was found only on Blackburn Island and is now extinct (Iredale 1944). Etheridge (1889) noted that there was a great deal of variation in shell morphology and that it seemed ‘... more in keeping with the facts to regard the Rabbit Island shell simply as a variety’. However, the material in the Australian Museum from Blackburn Island all possesses a distinctive, thin shell and is smaller than any adults seen from the main island (Figs 5E, 6; Table 4).

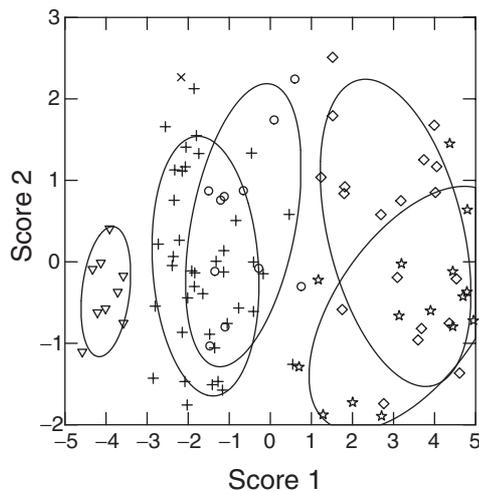
***Placostylus bivaricosus solidus* (Brazier in Etheridge, 1889)**

*Bulimus bivaricosus solida* Brazier in Etheridge, 1889. (Seven syntypes, Australian Museum C.171111 (Fig. 5G), C.171110).

**Remarks**

This large, heavy form is found in calcarenite along the eastern side of the settlement area of the island. Smith (1992) indicates that the original introduction of the name is a *nomen nudum*. However, there is a statement differentiating the taxon in the original introduction of the name (p. 27), so it is valid. In addition, Etheridge (1891) described this taxon in greater detail and provided figures. There are also fossil specimens that appear to be indistinguishable from the Recent material (including one of the syntypes in C.171111), although specimens can usually be readily separated into the *solida* morph or the Recent morph. Unfortunately, there has not yet been any attempt to systematically collect fossil *Placostylus* and relate shell morphology to stratigraphy.

A comparison of three basic shell measurements (Table 4) indicates that the Recent taxa discriminate well (Fig. 6), but mainly on size rather than shape differences. There also appears to be justification for the recognition of an additional unnamed and recently extinct taxon that lived on the top of Mount Gower (Australian Museum C.114677; Fig. 5D,F) and on a razor-back spur to the south of the summit (Australian Museum C.114679). These two lots have similar shell dimensions to typical *P. bivaricosus* (Fig. 6; Table 4), but have the



**Fig. 6.** Plot of the first and second discriminant scores from the measurement data summarised in Table 4. The ellipses have a confidence level of  $P = 0.683$ . (▽), *Placostylus bivaricosus cuniculinsulae*; (+), *P. bivaricosus bivaricosus*; (◇), *P. bivaricosus etheridgei*; (○), *P. bivaricosus* aff. *etheridgei* (top of Mt Gower); (☆), *P. bivaricosus solidus*.

**Table 4. Shell measurements of *Placostylus* from Lord Howe Island**  
Data show the range and mean  $\pm$  s.d.

	Shell length (mm)	Shell width (mm)	Aperture length (mm)
<i>P. bivaricosus bivaricosus</i> (n = 40)	46.0–61.9, 53.4 $\pm$ 3.5	21.3–27.5, 23.8 $\pm$ 1.5	23.9–32.4, 26.9 $\pm$ 2.1
<i>P. bivaricosus etheridgei</i> (n = 19)	65.5–77.1, 71.5 $\pm$ 4.1	28.0–35.3, 31.9 $\pm$ 2.3	31.8–41.1, 35.9 $\pm$ 2.9
<i>P. bivaricosus cuculinsulae</i> (n = 9)	40.0–45.7, 43.3 $\pm$ 1.8	19.4–21.9, 20.3 $\pm$ 0.8	20.3–22.9, 21.4 $\pm$ 0.9
Summit of Mount Gower (n = 11)	51.9–65.1, 57.6 $\pm$ 4.2	23.7–27.8, 25.4 $\pm$ 1.2	26.5–31.9, 28.3 $\pm$ 1.8
<i>P. bivaricosus solidus</i> (n = 15)	61.1–79.0, 71.4 $\pm$ 6.2	26.8–36.6, 32.6 $\pm$ 3.1	31.5–41.0, 37.2 $\pm$ 2.7

Specimens measured: *P. b. bivaricosus* (C118724; C114653; C.78759; C.114656); *P. b. solidus* (C.171111; C.171110; C.114648; C.47513); *P. b. etheridgei* (C.33308; C.38973; C.114672; C.114675); *P. b. cuculinsulae* (C.118151; C.114646; C.114647); Mount Gower (C.114679; C.114677).  
Aperture length is the external, not internal, length.

colour and surface sculpture of *P. bivaricosus etheridgei*. Given their isolation on the summit, they were probably genetically distinct from the populations around the base of the southern mountains and the typical lowland form.