New Zealand Journal of Zoology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tnzz20

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To cite this article: Elahe Parvizi, Angela McGaughran & Mark I. Stevens (2023): Tracking the origins of the introduced terrestrial amphipod, *Puhuruhuru patersoni*, on sub-Antarctic Macquarie Island, New Zealand Journal of Zoology, DOI: 10.1080/03014223.2023.2224580

To link to this article: https://doi.org/10.1080/03014223.2023.2224580

NEW ZEALAND JOURNAL OF ZOOLOGY
https://doi.org/10.1080/03014223.2023.2224580

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Tracking the origins of the introduced terrestrial amphipod, *Puhuruhuru patersoni*, on sub-Antarctic Macquarie Island

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ABSTRACT

The terrestrial amphipod *Puhuruhuru patersoni* (Amphipoda: Talitridae) was discovered on sub-Antarctic Macquarie Island in 1992. The species is only known to naturally occur on New Zealand's South Island and some associated offshore islands. The possible routes by which the species was introduced to Macquarie Island have previously been considered based on morphology and historic activities between New Zealand and Macquarie Island. Here, we sampled across the known range for *P. patersoni* and generated mtDNA COI data for these specimens to investigate the likely origin of the Macquarie Island incursion. Our results showed high genetic diversity across the native range and substantially lower genetic diversity on Macquarie Island. Additionally, our phylogenetic reconstructions suggested that the source of the introduction to Macquarie Island is likely to have been from the southern region of New Zealand's South Island.

ARTICLE HISTORY
Received 5 April 2023
Accepted 7 June 2023

HANDLING EDITOR
Adrian Paterson

KEYWORDS
Amphipoda; invasive species; introduction; Talitridae; terrestrial Crustacea

Introduction

DNA barcoding has become very popular since the early 2000s as a standardised method for species identification (Floyd et al. 2002; Hebert et al. 2003). This diagnostic technique utilises short, standardised DNA sequences, such as the mitochondrial (mtDNA) cytochrome *c* oxidase I (COI) gene, to facilitate species identification. Since its inception, DNA barcoding has also been recognised for its potential in addressing questions related to introduced species (e.g. Armstrong and Ball 2005; Groom et al. 2017), including understanding various aspects of invasion processes such as invasion pathways, dispersal and population dynamics of invasive species (e.g. Comtet et al. 2015).

Macquarie Island is a geographically isolated sub-Antarctic Island that has never been connected to any other land mass and is relatively recent in origin, being above sea-level for less than 700,000 years (Greenslade 2006). It has an area of about 120 km^2, is 34 km long and up to 5 km wide, and is situated at 54°45'S and 159°0'E - around 1000 km south

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/03014223.2023.2224580.

of New Zealand. The climate, topography, flora, and fauna are described in detail by Selkirk et al. (1990) and Greenslade (2006).

The invertebrate fauna of Macquarie Island is well known and includes several hundred native terrestrial invertebrates (Greenslade 2006). Of the introduced macroinvertebrates, four have been studied in detail and all are known from New Zealand's southern islands. While examples exist of likely natural dispersal of arthropod species to sub-Antarctic islands (e.g. Lee et al. 2014; Stevens et al. 2021), the introduction pathways and history of these four species provide strong evidence of human introductions to Macquarie Island.

Two introduced flatworms, *Kontikia andersoni* Jones and *Arthurdendyus vegrandis* Winsor & Stevens originated from New Zealand and have probably been on the island for at least 100 years (Winsor and Stevens 2005; Greenslade et al. 2007). In a 2004 survey, both flatworm species were found to have spread at a rate of around 10 m/year following arrival to the island (Greenslade et al. 2007). More recently, surveys failed to detect *A. vegrandis* but found that *K. andersoni* had continued to spread - at an increased rate of around 500 m/year between 2004 and 2018 (Houghton et al. 2022). The two other introduced macroinvertebrates are both terrestrial Crustcacea - the isopod *Styloniscus otakensis* (Chilton) and the amphipod *Puhuruhuru patersoni* (Stephensen) - and were discovered on Macquarie Island in the early 1990s. Both were probably accidentally introduced to Macquarie Island between 1810 and 1919 with shipping of sealers and supplies from New Zealand (Richardson and Jackson 1995; Greenslade et al. 2008). Population surveys performed in 1992, 2004 and 2018 found *P. patersoni* and *S. otakensis* had not markedly expanded their range since 2004 (Greenslade et al. 2008; Houghton et al. 2022).

Puhuruhuru patersoni has only been recorded south of latitude 45°S in New Zealand, despite extensive previous collections (Duncan 1994). Together, these collections encompass a geographical area approximately 200 km to the north and south of Dunedin, and the Snares, Stewart and South Cape Islands (Duncan 1994). The type locality is not certain, but is believed to be Bench Island, and *P. patersoni* has not previously been recorded from Bluff, the port of Invercargill, or from Auckland Islands or Campbell Island (Duncan 1969, 1994).

The biology and life history of this species has previously been studied in detail by Duncan (1969, 1994). *Puhuruhuru patersoni* is strongly sexually dimorphic, particularly with reference to the adult propodus (part of the gnathopod/claw) (see plate 22 in Duncan 1994). Fully mature Snares Islands males resemble the second gnathopod of young adult South Island/Stewart Island morphs, although the Snares males lack a distinct palm on the propodus of the first gnathopod (Duncan 1994). The Macquarie Island specimens resemble Snares Islands specimens for the second gnathopod, but resemble South Island/Stewart Island morphs by retaining a small palm on the first gnathopod (Figure 1). Although Duncan (1994) found these differences to be fairly profound, on the basis of a clinal variability from north to south within the species in New Zealand, he designated the South Island/Stewart Island forms as *Puhuruhuru patersoni patersoni*, and the Snares Islands population as *Puhuruhuru patersoni snarensis*.

Here, we aim at examining intraspecific diversity and identifying the potential source location of the *P. patersoni* introduction to Macquarie Island. Due to the clinal variation observed in morphology and the potential for cryptic species, we used the mtDNA COI

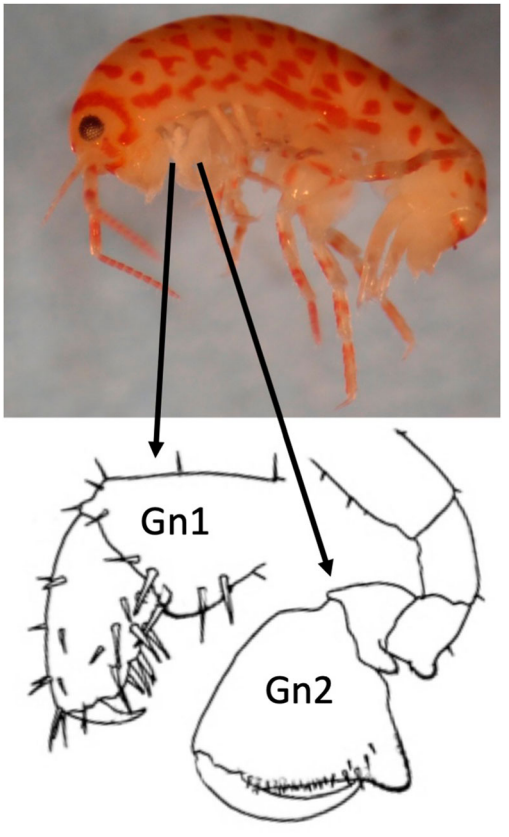

Figure 1. Photo of *Puhuruhuru patersoni* (in ethanol) collected from tussock vegetation in the vicinity of the Biology lab on the Macquarie Island isthmus, shown in Figure 2. The characteristic antenna 2 can be seen in the photo, and the first (Gn1) and second (Gn2) gnathopods of male Macquarie Island specimens shown (modified from Richardson and Jackson 1995).

gene to compare specimens from across the known native distributional range of *P. patersoni* to samples collected from Macquarie Island.

Methods

Sampling

Puhuruhuru patersoni was originally only recorded from a small area near the fuel dump within the Station boundary on the isthmus of Macquarie Island, where it is still contained (Greenslade et al. 2008; Houghton et al. 2022). Individuals were collected from this isthmus from 24 January to 29 February 2004 (see Greenslade et al. 2008 for details).

We also collected samples from across the known native distributional range for *P. patersoni* (New Zealand South Island and Stewart Island including Bench Island, and Snares Islands), as well as additional specimens we identified from Auckland Islands where the species had not previously been recorded (Figure 2). Bench Island lies at the entrance to Paterson Inlet and is collectively grouped under 'Stewart Island' for our analyses (see Suppl. file for all specimen and location details). All individuals

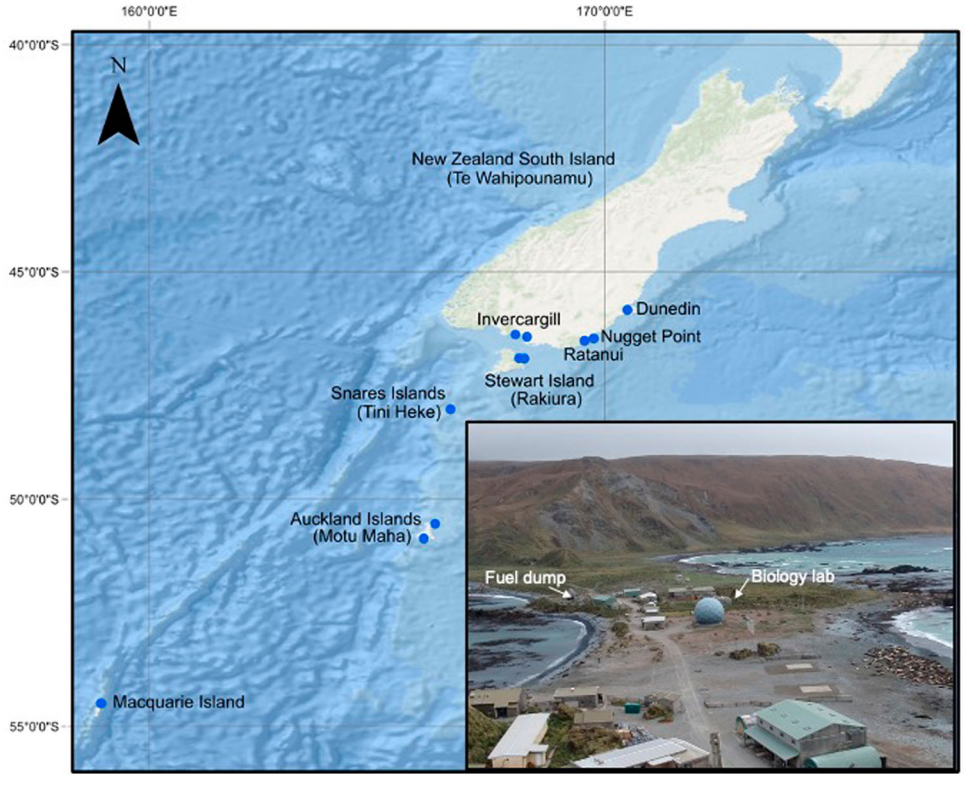

Figure 2. Sampling sites of *Puhuruhuru patersoni* across the native range in New Zealand South Island and sub-Antarctic Islands. The Macquarie Island population is introduced. Inset photo of the station area on the isthmus showing the current distribution of *Puhuruhuru patersoni* in bases of tussock vegetation in the vicinity of the biology lab and fuel dump that remains unchanged since the 2004 survey (compare to figure 3 in Greenslade et al. 2008; see also Houghton et al. 2022).

were collected by hand using forceps, or from litter samples using Berlese funnels and preserved using 95% ethanol. Upon return to the laboratory, all samples were identified using their vestigial pleopods, a feature absent in other terrestrial talitrids, and by the characteristic antenna 2 (Duncan 1994).

Laboratory procedures

We isolated genomic DNA from each specimen using material from approximately five legs. We followed the standard protocol for animal tissues using the DNeasy Tissue Kit (Qiagen, Hilden, Germany), however we extended the lysis step overnight at 56° C. To amplify a 680-bp COI mtDNA fragment, we followed the protocols outlined in McGaughran et al. (2006). Our reaction volume was 50 µL, comprising of 3 µL of DNA (not quantified), 1× PCR buffer (Roche, Penzberg, Germany), 2.2 mm $MgCl_2$, 0.2 mm of each dNTP (Boehringer Mannheim, Mannheim, Germany), 1.0 µm of each primer, and 1.0 unit of Taq DNA polymerase (Roche). We performed the PCR reaction on an Eppendorf Mastercycler gradient thermocycler or a Biometra T1 thermocycler (Whatman Biometra, Göttingen, Germany) using the primers COI-2F (5′-TTY GAY

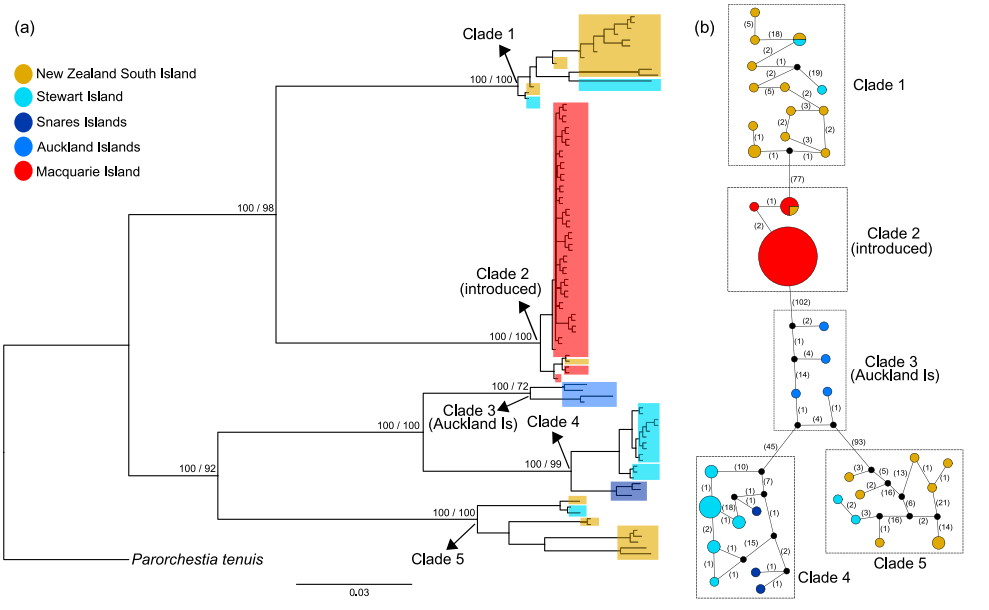

Figure 3. (a) Phylogenetic reconstruction of *Puhuruhuru patersoni* based on the COI mtDNA marker, with *Parorchestia tenuis* used as an outgroup. The values on nodes indicate the posterior probabilities / bootstrap values obtained from Bayesian and maximum likelihood analyses, respectively. See Figure S1 for label details of each specimen. (b) Statistical parsimony haplotype network of 35 unique COI haplotypes of *P. patersoni*. The size of each circle corresponds to the frequency of haplotypes, with the smallest coloured circle representing a single sample. Numbers on lines indicate mutation steps between haplotypes and black circles on lines represent missing intermediate haplotypes.

CCI DYI GGR GGA GGA GAT CC-3′), and COI-2R (5′-GGR TAR TCW GAR TAW CGT NCG WGG TAT-3′) (Otto and Wilson 2001). We followed the thermal cycling conditions of initial denaturation at 94 °C for 60 s; followed by 40 cycles of denaturation at 94 °C for 20 s, annealing at 50 °C for 30 s, and extension at 72 °C for 90 s, with a final extension at 72 °C for 5 min. We then purified PCR products using a Qiaquick PCR Purification Kit (Qiagen) and sequenced them (using the COI-2R primer) on a MegaBACE DNA Analysis System (Amersham Biosciences, Buckinghamshire, England) at the University of Waikato DNA sequencing facility. We used sequencher v. 4.2 (Gene Codes) sequence editor to align sequences. All sequences are available from GenBank (accession numbers OQ734984-OQ735076). All collected specimens are stored in the South Australian Museum, Adelaide.

Phylogenetic analyses

We reconstructed Bayesian and maximum likelihood phylogenetic trees using MrBayes v. 3.2.7 (Ronquist et al. 2012) and IQtree v. 2.0.3 (Minh et al. 2020), respectively. TIM1 + I + G4 was identified as the best-fitting model of nucleotide substitution as determined by ModelTest-NG v. 0.1.7 (Darriba et al. 2020). We retrieved the COI sequence (GenBank accession MT466577) for *Parorchestia tenuis* (Dana) from within the same family Talitridae, as an outgroup in the phylogenetic analyses. We inferred the genealogical relationships among mitochondrial haplotypes of *P. patersoni* by constructing a parsimony-

based haplotype network (Clement et al. 2000) in PopART v. 1.7 (Leigh and Bryant 2015). We also calculated evolutionary divergence among mtDNA clades using the Maximum Composite Likelihood model (Tamura et al. 2004) in MEGA v. 11.0.13 (Tamura et al. 2021).

Population genetic diversity and structure

We calculated the number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π) and segregating sites (S) for each population and each main mtDNA clade (obtained from phylogenetic analysis; see Results) in DnaSP v. 6 (Rozas et al. 2017). Population genetic differentiation among each population and each main mtDNA clade was investigated by computing pairwise F_{ST} with 500 permutations in Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010).

Results

Phylogenetic analysis

Our phylogenetic reconstruction revealed five divergent mtDNA clades for our sampled *P. patersoni* (Figures 3a, S1). Clades 1 and 5 each included haplotypes from New Zealand's South Island and Stewart Island, Clade 4 included haplotypes from Stewart Island and Snares Islands (as a distinct sub-clade), and Clade 3 included only Auckland Islands individuals. Clade 2 consisted of all introduced Macquarie Island samples plus a single specimen from near Ratanui in New Zealand's South Island Catlins region. The native range clades did not show any distinct geographic structure and all (Clades 1, 4, 5) contained haplotypes from Stewart Island (Figure 3a, S1). The haplotype network confirmed these results, showing a substantial number (ranging from 45 to 102) of mutational steps between the main mtDNA clades (Figure 3b).

The average evolutionary divergence between main mtDNA clades using the Maximum Composite Likelihood model in MEGA showed Clade 2 (the introduced clade) to be the most divergent from the other clades, with sequence divergence of 12.6% to Clade 1, 17% to Clade 3, 17.5% to Clade 4 and 18.3% to Clade 5. Clades 3 (Auckland Islands) and 4 (Stewart Island, Snares Islands) were also relatively divergent (8.3%) from each other. Clade 1 was the least divergent from the other clades, with sequence divergence ranging from 12.6% to 19%.

Population genetic diversity and structure

In total, we found 35 COI haplotypes in the native and introduced ranges, representing a substantial haplotype diversity (Hd = 0.781) and nucleotide diversity ($\pi = 0.12$) (Table 1). Among the studied islands, South Island showed the highest haplotype and nucleotide diversity (Hd = 0.98, $\pi = 0.116$) with 19 haplotypes from 22 individuals, while populations on Macquarie Island had the lowest diversity values (Hd = 0.161, $\pi = 0.0007$) and just three haplotypes shared among 47 individuals. Due to the small sample size of Snares Islands and Auckland Islands (n = 3 and n = 4, respectively), we did not calculate diversity indices for these two populations.

Table 1. DNA variation at the COI marker for the native and introduced populations, as well as main mtDNA clades, of Puhuruhuru patersoni. n = number of samples; h = number of haplotypes; Hd = haplotype diversity; π = nucleotide diversity; S = number of segregating sites.

Population / Clade	n	h	Hd	π	S
NZ South Island	22	19	0.982	0.116	176
Stewart Island	17	8	0.852	0.080	171
Snares Islands	3	3	NA	NA	7
Auckland Islands	4	4	NA	NA	27
Macquarie Island (introduced)	47	3	0.161	0.0007	3
Clade 1	15	12	0.961	0.022	38
Clade 2 (introduced)	48	3	0.194	0.0009	3
Clade 3 (Auckland Islands)	4	4	NA	NA	27
Clade 4	16	7	0.833	0.012	23
Clade 5	10	9	0.977	0.052	67

Table 2. Pairwise F_{ST} calculated between each population of *Puhuruhuru patersoni*. * indicates significant F_{ST} *P* values ($P < 0.05$).

Populations	NZ South Island	Stewart Island	Snares Islands	Auckland Islands
NZ South Island	0			
Stewart Island	0.08*	0		
Snares Islands	0.01	0.10	0	
Auckland Islands	0.01	0.09	0	0
Macquarie Island (introduced)	0.50*	0.60*	0.74*	0.71*

Given the potential for mis-identified species within a broader *Puhuruhuru* complex, we also examined diversity within the main mtDNA clades. Clade 1 and Clade 5 showed the highest haplotype diversity and nucleotide diversity (Hd = 0.961 and 0.977 and π = 0.022 and 0.052, respectively). Clade 2, which contained all Macquarie Island individuals and a single South Island individual, showed the lowest haplotype and nucleotide diversity (Hd = 0.194 and π = 0.0009).

Population pairwise F_{ST} showed signatures of gene flow between different native ranges of *P. patersoni*, with values ranging from 0.01–0.10 between New Zealand's South Island and Stewart Island, Snares, and Auckland Islands (Table 2). Pairwise F_{ST} was notably higher for comparisons involving the introduced population on Macquarie Island, which showed genetic differentiation greater than 0.50 (and up to 0.74) from the native populations (Table 2).

Pairwise F_{ST} between the main mtDNA clades showed evidence of gene flow between the native clades (lower F_{ST}, ranging from 0.01–0.10), while the introduced clade (Clade 2) showed high genetic differentiation relative to the native clades (F_{ST} ranging from 0.54–0.67). Clade 3 (Auckland Islands) showed non-significant F_{ST} values when compared to other geographically distant native clades, likely due to the small sample size (n = 4).

Discussion

Phylogenetic relationships

By analysing the mtDNA COI barcode region, we explored the genetic diversity and structure of *Puhuruhuru patersoni* across its entire native geographic range, including the South Island of New Zealand, Stewart Island, and Snares Islands, as well as its

introduced range on Macquarie Island. Our findings revealed a notable level of genetic diversity and spatial structuring of mtDNA clades within the native range. Furthermore, the introduced population on Macquarie Island showed comparatively lower genetic diversity in relation to the native range. The Macquarie Island population formed a distinct mtDNA clade and showed a close phylogenetic relationship with samples from South Island and Stewart Island. Based on these results, we suggest that *Puhuruhuru patersoni* in Southern New Zealand may consist of four distinct taxa, likely new species.

The species varies morphologically with latitude (Duncan 1994). Due to the morphological variations, Duncan (1994) designated the South Island and Stewart Island (and Bench Island) forms as *Puhuruhuru patersoni patersoni* and the Snares Island population as *Puhuruhuru patersoni snarensis*. It is possible that these two morphological subspecies characterised by Duncan (1994) are both contained within our phylogenetic Clade 4, although out of scope for the current study, with *P. p. snarensis* restricted to Snares Islands and *P. p. patersoni* restricted to Bench Island, but no morphological characters were defined for these subspecies. The specimens from Auckland Islands (Clade 3), those from Macquarie Island (including one specimen collected from New Zealand's South Island), and Clades 1 and 5 (both include South Island and Stewart Island specimens), appear to represent four new *Puhuruhuru* species.

We found large sequence divergences (up to 19%) among the five main clades in our phylogenetic analyses and a large number of mutational steps (up to 102) in our haplotype network that almost certainly pertain to the presence of multiple separately evolving lineages that likely represent four new species. The lack of definite types from the original descriptions and subsequent work highlighting the significant morphological variations in characters across the species range (Duncan 1994; Richardson and Jackson 1995), suggests that these authors may have been observing a mix of variation among distinct species that were attributed to *P. patersoni*. To resolve these species assignments further, thorough morphological revision of the genus combined with phylogenomic analysis using high resolution molecular markers, such as single nucleotide polymorphims (SNPs), will be required.

Macquarie Island incursion

Our mtDNA data links Macquarie Island to the Catlins region (near Ratanui) of New Zealand's South Island, suggesting one possible source population for the Macquarie Island incursion. This supports the work of Richardson and Jackson (1995), who, among other resemblances, noted that Macquarie Island specimens resemble South Island specimens from Catlins in the first gnathopod. We only had a single Catlins representative aligning with Macquarie Island in Clade 2, with at least 12.6% divergence and 72–102 mutational steps of this clade to any other clade in our analysis, while other Catlins representatives aligned with other South Island and Stewart Island individuals in Clade 5.

On Macquarie Island, *P. patersoni* occurs only within an area of intensive use that includes the current research station. Prior to the establishment of the station in 1968, the area was used by New Zealand based commercial seal harvesters (Cumpston 1968). The most intense period of activity was from 1874 to 1919. Most voyages

during this period originated from the southern New Zealand ports of Lyttelton (near Christchurch), Port Chalmers (Dunedin), Bluff (Invercargill), and/or Port Paterson (Paterson Inlet) on Stewart Island (Cumpston 1968). Infrastructure building supplies are a known pathway for introduced species to sub-antarctic islands (e.g. Lee et al. 2014), and during this early period on Macquarie Island the isthmus was inhabited and more than a dozen huts were built (Cumpston 1968). Further sampling within New Zealand's South Island would allow confirmation of this hypothesised Macquarie Island incursion pathway.

Conclusions

Despite being present on Macquarie Island for over a century, *P. patersoni* has shown limited range expansion and remains confined to a narrow area (Figure 1; see also Houghton et al. 2022), even though suitable habitats are available outside and adjacent to its current distribution range (Richardson and Jackson 1995) and identifiable barriers to dispersal are absent (Houghton et al. 2022). Additionally, the removal of invasive rodents from the island, which are known to preferentially prey on amphipods on sub-Antarctic Islands (Houghton et al. 2019), does not appear to have led to recent population changes in *P. patersoni* (Houghton et al. 2022). Our analysis of mtDNA data also suggests a small population with limited genetic diversity and the absence of population expansion on Macquarie Island. Such low genetic diversity and limited geographic range on the isthmus may make the species an ideal candidate to eradication efforts. Thus, our findings suggest that targeted management measures could be successful in eradicating *P. patersoni* from Macquarie Island.

Acknowledgements

This work was carried out as part of a survey to map the distribution of introduced terrestrial invertebrates on Macquarie Island (ASAC 2397). We thank the Australian Antarctic Division for financial and logistic support and are grateful for the support of the staff and access to facilities on Macquarie Island. We would also like to thank Penny Greenslade and Paul Sunnucks for valuable discussions, and Robert Edwards, Peter Hudson and Murray Potter for assistance with fieldwork on Macquarie Island. Thanks also to Peter for the images used in Figures 1 and 2. We are also grateful to the Editors and two anonymous reviewers for helpful suggestions.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study received financial and logistic support provided by Australian Antarctic Division (ASAC2397). This manuscript was supported in part from the Australian Research Council (ARC) funding under the SRIEAS (grant agreement no. SR200100005) (Securing Antarctica's Environmental Future).

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