POPULATION GENETIC STRUCTURES AND DISPERSAL PATTERNS OF ARTHROPODS IN NEW ZEALAND AND THE ROSS DEPENDENCY, ANTARCTICA

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ABSTRACT

Climatic and geological changes have been recognised as fundamental mechanisms in the evolution of populations. This thesis assesses population genetic structures and dispersal patterns of arthropods in two geographic regions—Antarctica and New Zealand—using molecular techniques in conjunction with distributional data and dispersal experiments.

Regional genetic divergence was revealed using allozymes and mtDNA (COI) for populations of the endemic Antarctic collembolan *Gomphiocephalus hodgsoni* from southern Victoria Land and Ross Island (Ross Dependency). Genetic discontinuities across geographical barriers (e.g. sea-ice) suggest limited dispersal opportunities and long-term habitat fragmentation throughout the Pleistocene. In addition, the identification of two sympatric and genetically divergent groups throughout one of the continental sites (Taylor Valley) suggests that these mechanisms have been conducive to speciation. The limited mixing of mtDNA haplotypes between one island site (Cape Bird) and one continental site (Granite Harbour) is unlikely to be accounted for by the un-aided dispersal capacity of *G. hodgsoni* and recent human- or bird-mediated dispersal is highly probable. Furthermore, recent localised dispersal of Collembola was identified in two continental sites (Taylor Valley and Granite Harbour) through comparisons with previous distributional studies, and suggests that range expansion of up to 5 km has occurred within the last 40 years.

The endemic estuarine amphipods *Paracorophium lucasi* and *P. excavatum* from North, South and Chatham Islands of New Zealand were examined using allozyme analyses that also identified clear genetic breaks across geographical barriers (e.g. land-bridges) separating biogeographic regions. However, populations of *P. lucasi* were more divergent than populations of *P. excavatum* over similar geographic distances, but in most cases gene flow appears to maintain a homogenous population genetic structure in populations that share a common coastline. These results are congruent with a high rate of female-biased juvenile dispersal that was identified during field experiments with *Paracorophium* spp. in Tauranga Harbour. Such dispersal may be a mechanism to avoid inbreeding and inter- and intraspecific competition. During these experiments, I identified the New Zealand *Paracorophium* species, as well as *P. brisbanensis*, previously recorded only from Australia. I conclude that this latter species is unlikely to be indigenous to New Zealand or the result of natural dispersal from Australia, but rather anthropogenic translocation (i.e. shipping activities).

This research reveals limited dispersal and high levels of genetic divergence for three arthropod taxa from fragmented habitats in Antarctica and New Zealand. I conclude that such levels of cryptic diversity indicate the inadequacies of morphology-based classification schemes. Accordingly, assessments based on genetic diversity (e.g. mtDNA analyses) are required.

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TABLE OF CONTENTS

ABSTRACT ii
ACKNOWLEDGEMENTS iv
LIST OF TABLES x
LIST OF FIGURES xii
LIST OF APPENDICES xiv

THESIS INTRODUCTION 1

CHAPTER I EXPANDED DISTRIBUTIONAL RECORDS OF COLLEMBOLA AND ACARI IN SOUTHERN VICTORIA LAND, ANTARCTICA 12
Abstract 13
Introduction 14
Materials and Methods 15
Study sites 15
Sampling of arthropods 15
Results 18
Beaufort Island and Bratina Island 18
Ross Island 18
Southern Victoria Land 19
Discussion 22
Acknowledgements 25
Literature Cited 26

CHAPTER II LONG-TERM ISOLATION AND RECENT RANGE EXPANSION FOR THE ENDEMIC SPRINGTAIL *GOMPHIOCEPHALUS HODGSONI* FROM VICTORIA LAND, ANTARCTICA 33
Abstract 34
Introduction 35
Materials and Methods 37
Collection of samples 37
Allozyme electrophoresis 37
Mitochondrial DNA analysis 39
Results 42
Allozyme variability 42
mtDNA variability 47
Discussion 51
Acknowledgements 57
Literature Cited 58

CHAPTER III THE COROPHIID AMPHIPODS OF TAURANGA HARBOUR, NEW ZEALAND: EVIDENCE OF AN AUSTRALIAN CRUSTACEAN INVADER 67
Abstract 68
Introduction 69
Materials and Methods 70
Study site and sampling of amphipods 70
Morphological analyses 71
Allozyme analyses 72
Results 74
Discussion 79
Conclusion 81
Acknowledgements 82
Literature Cited 83

CHAPTER IV NEW ZEALAND'S COROPHIID AMPHIPODS: ALLOZYME EVIDENCE FOR SPECIATION DURING THE CENOZOIC 87
Abstract 88
Introduction 89
Materials and Methods 93
Collection of samples 93
Allozyme electrophoresis 95
Data analyses 96
Results 97
Geographic distribution 97
Allozyme variation 97
Population genetic structure 101
Discussion 105
Spatial genetic structure 105
Divergence through allopatric isolation 107
Pleistocene climate change or geological isolation? 109
Conclusion 111
Acknowledgements 112
Literature Cited 113

CHAPTER V JUVENILE FEMALES DOMINATE DISPERSAL OF COROPHIID AMPHIPODS IN A NEW ZEALAND ESTUARY 123
Abstract 124
Introduction 125
Materials and Methods 127
Study area 127
Sampling of amphipods 128
Statistical analyses 130
Results 132
Adult and juvenile transport 132

Adult and juvenile sex-ratios 138
Discussion 139
Acknowledgements 142
Literature Cited 143

THESIS CONCLUSION 148

APPENDIX I Soil Fauna of Antarctic Coastal Landscapes 156

APPENDIX II Synonymy of the New Zealand corophiid amphipod genus, *Chaetocorophium* Karaman 1979, with *Paracorophium* Stebbing, 1899: morphological and genetic evidence 185

LIST OF TABLES

TABLE II.1 Genetic variability at 11 allozyme loci in 18 populations of *Gomphiocephalus hodgsoni*. N = mean sample size per locus, P = percentage of polymorphic loci (using the 95% criterion), A = mean number of alleles per locus, Ap = mean number of alleles per polymorphic locus, and H_{obs} = observed heterozygosity, H_{exp} = expected heterozygosity, and * = a significant ($P<0.05$) deviation at one or more loci (see text). § = samples collected Jan 1999; † = samples collected 2000/2001; all other samples were collected Jan 2000; ‡ = samples used for allozyme analyses only. Allozyme analyses were not performed on Lake Penny or Cape Bird individuals collected in Jan 2002 44

TABLE II.2 Polymorphic nucleotide sites among *Gomphiocephalus hodgsoni* haplotypes. The common continental haplotype (A) is used as a reference sequence. Locations where each haplotype was found is indicated using location codes from Figure 3, and haplotypes are given a single letter code (i.e. A, B, etc.). Identical character states are indicated by dots 49

TABLE III.1 Allele frequencies at 14 polymorphic loci for *Paracorophium lucasi, P. excavatum, P. brisbanensis* and *Corophium* sp., collected from the Waimapu Estuary, Tauranga Harbour, New Zealand 76

TABLE III.2 Measures of genetic variability for *Paracorophium lucasi, P. excavatum, P. brisbanensis* and the *Corophium* sp. and collected from the Waimapu Estuary, Tauranga Harbour, New Zealand. Standard errors in parentheses 77

TABLE IV.1 Genetic variability at 13 loci in all populations of *Paracorophium lucasi*, *P. excavatum* and *P. brisbanensis*. Notation preceding locations refer to those used in the text, figures, tables, and appendix. N = mean sample size per locus, P = percentage of polymorphic loci (95% criterion), A = mean number of alleles per locus, Ap = mean number of alleles per polymorphic locus, H_{obs} = observed heterozygosity and H_{exp} = expected heterozygosity, and * = a significant ($P<0.05$) deviation at one or more loci (see text), [1,2,3,4] = proximate populations pooled for UPGMA analyses only when F_{ST} among populations were not significantly different to zero (determined from bootstrap analyses) 99

TABLE IV.2 *F*-statistics (F_{ST}, F_{IS}) averaged over 13 loci. Linear regression coefficient (R^2) and Spearman's rank correlation Index (R) of log$D$ and logkm used to assess isolation-by-distance (*I-D*) (see methods) for different geographic regions for *Paracorophium lucasi* and *P. excavatum* ($^{**}P < 0.01$, $^{*}P < 0.001$). For populations included in each cluster refer to Figure 3 103

TABLE V.1 Repeated measures ANOVA for each demography (e.g. adult male) for the bed-load and suspended-load traps (performed separately). Date=the ten sampling dates, Site=Site 1 and Site 2, Tide=ebb and flood tides, M=adult male, F=adult female, GF=gravid female, JM=juvenile male, JF=juvenile female 134

TABLE V.2 Mean body size (mm) and standard error (in parentheses) at Site 1 and Site 2 for each demography (e.g. adult male) averaged over all sampling dates. *=when gravid females with stage IV brood were present, and †=when stage V brood was found 136

TABLE V.3 Three-way ANOVA's for each demography (e.g. Adult male) comparing the size distributions averaged over all sampling dates. Non-significant interactions were removed from each model. SITE=Site 1, Site 2; sediment core at site 1; TRAP=bed-load, suspended-load, sediment core at site 1; TIDE=ebb and flood tides 137

LIST OF FIGURES

FIGURE I.1 Locations (solid circles) examined during 1998-2001 in the Ross Sea region 17

FIGURE I.2 The modified aspirator used to collect arthropods 17

FIGURE I.3 Distribution of arthropods in Taylor Valley. Sites indicate where arthropods were present (numbered circles – see text), or absent (solid circles). Site 22 was previously examined by Strandtmann (1967) and site 23 by Schwarz et al. (1993) 21

FIGURE II.1 Sampling localities for *Gomphiocephalus hodgsoni* throughout southern Victoria Land, Antarctica 41

FIGURE II.2 UPGMA dendrogram (Nei's (1978) unbiased genetic distance) of allozyme data (11 loci) for 18 *Gomphiocephalus hodgsoni* populations. Shaded bars correspond to those used in Fig. 3, and population codes correspond to Table 1 46

FIGURE II.3 MtDNA (COI) analyses based on sequence variation using 599 aligned sites for 45 *Gomphiocephalus hodgsoni* individuals. *Hypogastrura tullbergi* and *H. sensilis* were included as outgroup taxa. Analyses included individuals from 4 Ross Island populations, from Beaufort Island and from 6 continental populations (see map inset) (shaded bars correspond to those used in Fig. 2, and for individual codes see methods and Table 1). All individuals were used in: A) UPGMA phylogram; and B) Majority rule consensus of 12 equally parsimonious trees (tree length = 181, C.I. = 0.95, R.I. = 0.95) (50% majority rule appear above branches, bootstrap confidence limits (1000 replicates) greater than 55% below branches) 50

FIGURE III.1 Map of the study site location at Waimapu Estuary, Tauranga Harbour, New Zealand (37°40'S, 176°10'E). Inset: shows the known locations for *P. brisbanensis* on the eastern coast of Australia from Hervey Bay (25°17'S, 152°49'E) to Moreton Bay (27°24'S, 153°07'E) (P. Davie, pers. comm.) 73

FIGURE III.2 The faunal assemblage as a percentage of the total number of individuals (n = 3,803) collected in monthly samples for *Paracorophium lucasi, P. excavatum, P. brisbanensis, Corophium* sp., and unidentified juveniles 75

FIGURE III.3 Dendrogram of Nei's (1978) unbiased genetic identity for individuals of *Paracorophium lucasi, P. excavatum, P. brisbanensis* and *Corophium* sp. collected from the Waimapu Estuary, Tauranga Harbour, New Zealand. All individuals were morphologically identified prior to allozyme analysis 78

FIGURE IV.1 The changing outline of the New Zealand archipelago over the last 12 MY. Land above sea-level shaded grey. Arrows represent prevailing ocean circulation. The Pliocene (6-2 MYA) landmass provided few barriers for aquatic dispersal between the east and west coasts, in contrast to the upper Miocene (12-6 MYA), the last glacial maximum (approx. 17 KYA) and the present. Figures adapted from Fleming (1979) and Stevens et al. (1995) 92

FIGURE IV.2 Distribution of *Paracorophium lucasi* (stars) and *P. excavatum* (solid circles) in New Zealand. Triangles show sympatric occurrences and hatched circles indicate sites where *Paracorophium* was not found. *P. brisbanensis* was found at N8 94

FIGURE IV.3 Phenetic analyses (UPGMA dendrogram) of the genetic distance (Nei 1978) between *Paracorophium brisbanensis* and populations of *P. lucasi* and *P. excavatum* (pooled populations correspond to Table 1) 104

FIGURE V.1 Location of the study site at Waimapu Estuary, Tauranga Harbour, New Zealand (37°40'S, 176°10'E). Inset: location of Site 1 at the mouth of the estuary and Site 2 in the sub-tidal channel running through the estuary 131

FIGURE V.2 Length frequency histograms (body size) of the demographic categories (Males, Females, Gravids) for the *Paracorophium* spp. from October 1999 to October 2000. All individuals from Site 1 and Site 2 were combined and grouped into 0.4 mm size classes (*n*=sample size) 135

LIST OF APPENDICES

APPENDIX I.1. Field sites where arthropods were recorded over the austral summers from 1998 to 2001. Species identified: *Gomphiocephalus hodgsoni* [G], *Neocryptopygus nivicolus* [N], and *Stereotydeus mollis* [S].....30

APPENDIX II.1 Allelic frequencies for 8 polymorphic loci from 18 *Gomphiocephalus hodgsoni* populations. *n* = total number of individuals used for the allozyme analyses in each population. For population codes refer to Table 1 65

APPENDIX II.2 Genetic distance (using the Kimura 2-parameter model) based on sequence variation in the mtDNA COI sequences (599 aligned sites) among the 14 identified *Gomphiocephalus hodgsoni* haplotypes and the two outgroup taxa (*Hypogastrura tullbergi* and *H. sensilis*). The haplotype codes refer to those used in Table 2 66

APPENDIX IV.1 Allele frequencies at 13 loci for all populations of *Paracorophium lucasi*, *P. excavatum* (* = pooled populations corresponding to Fig. 3) and *P. brisbanensis*. Locations refer to those used in the text, figures, and tables. Alleles are labelled according to their relative mobility (see text) 120

THESIS INTRODUCTION

The isolation of populations, both geographical and genetical, has long been recognised as a potential mechanism conducive to speciation (von Buch 1825; Wagner 1868; Darwin 1872; Dobzhansky 1937; Huxley 1942; Kimura 1953; Mayr 1942; Slatkin 1993). In this way, taxa that are geographically isolated with restricted dispersal are susceptible to micro-evolutionary processes (Mayr 1954; Templeton 1980). The study of micro- and macroevolution has enjoyed a resurgence of popularity with many studies providing an empirical and conceptual link between systematics and population genetics (see Bermingham and Moritz 1998). Phylogeography has been suggested as the bridge linking such studies providing an assessment of landscape evolution, including the dispersal of taxa through a region, speciation, adaptive radiation, and extinction (Bermingham and Moritz 1998). Such investigations will further our knowledge of the fundamental links between population processes and regional patterns of diversity and biogeography.

This way of thinking has only a short history, and Mayr (1982, p 560) rightly states that "...population genetics was a major contribution of the naturalists... concerned with the study of natural populations, with variation within populations, and with the changes on geographical gradients from population to population." With the development of ideas on populations came a new concept of races as variable populations, each with a different geographical history, and the development of the biological species concept (Mayr 1942). Over the last 177 years there has been a growing recognition of the importance of population

processes, and the 'revolutionary' thoughts of Mayr (e.g. 1942, 1963, 1982) have been instrumental in the field of evolutionary biology.

However, it was Leopold von Buch (1774-1853) who pioneered this field with his description of the fauna and flora of the Canary Islands. In particular, von Buch (1825) noted that speciation is in most cases "geographical" (Mayr 1963). This led to a unified theory of geographical speciation proposed by Moritz Wagner (1813-1887) following his explorations of Asia, Africa, and America (Mayr 1982, pp 562-564). Wagner found that the closest relatives of a given species were mostly in adjacent areas and generally separated by geographical barriers, and nowhere had he found any evidence for sympatric speciation (Wagner 1868, cited in Mayr 1982). From these experiences he proposed the theory of geographic speciation in 1868 in his *Migrationsgesetz der Organismen.*

Since von Buch (1825) and Wagner (1868), it has been generally accepted that a subdivided population may eventually split into reproductively isolated groups if the nature of the habitat among the subpopulations limits or prevents migration, thus forming an isolating barrier to gene flow. The belief that geographic barriers prevent gene flow was used to justify Mayr's (1942) definition of biological species as populations bounded by genetic barriers. However, in recent years theoretical models of clinal differentiation and parapatric speciation suggest that genetic divergence also occurs within a continuous habitat, and that measured rates of gene flow are often too low to be of evolutionary significance (e.g. Templeton 1980; Slatkin 1993; Gavrilets and Vose 2000).

The consequences of geographical barriers to gene flow affect metapopulation viability, as well as species long-term persistence (Mayr 1963; Pusey 1987; Stenseth and Lidicker1992; Bilton et al. 2001). This has become increasingly

important for conservation biologists (e.g. Moritz and Faith 1998), particularly when movements among habitat patches are hindered, resulting in the isolation of gene pools. For taxa that have limited dispersal capabilities, small or temporary geographical barriers may be sufficient to isolate populations. Knowledge concerning the rates of speciation and extinction, as well as the dispersal processes linking populations will enhance our understanding of the factors responsible for the origin and maintenance of biodiversity.

Molecular techniques have made it possible to investigate the genetic structures of morphologically similar populations (Parker et al. 1998), and in many cases have revealed cryptic species complexes that are often linked to geographic isolation and/or a taxon's dispersal capability (e.g. Avise et al. 1987; Gavrilets and Vose 2000; Trewick 2000; Knowles 2001). However, in spite of a large number of theoretical, and research publications over the last century, there remains a paucity of information on invertebrates from many southern hemisphere regions. Here, I address this gap by examining arthropods in the southern Pacific region. In particular, I focused on terrestrial arthropods from Antarctica and aquatic arthropods from New Zealand. Long-term isolation in these regions suggest that arthropods would provide an ideal model to examine the effect of potentially limited dispersal and habitat fragmentation on regional genetic differentiation.

The thesis consists of five chapters, with the first study ecosystem introduced in Chapter I. This chapter includes a description of collecting methods, and in particular provides accurate locations where Collembola (*Gomphiocephalus hodgsoni* and *Neocryptopygus nivicolus*) and Acari (*Stereotydeus mollis*) were collected from southern Victoria Land, Antarctica, during three austral summers

from 1999 to 2001. Chapter I concludes with a comprehensive comparison to distributional records for these endemic Antarctic arthropods made over the last 40 years to assess possible range expansion/contraction and the potential for human disturbance.

The primary results on Antarctic terrestrial arthropods are presented in Chapter II. The examination of historical geographic change has provided a framework for examining colonisation and dispersal among populations within a phylogeographic context (Avise et al. 1987; Avise 1998). Prior to the commencement of my research in 1998 there were no studies that examined the importance of Pleistocene vicariance events in shaping the distribution of genetic diversity for the terrestrial fauna of Antarctica. However, recent studies have indicated highly fragmented population genetic structures (Courtright et al. 2000; Fanciulli et al. 2001; Frati et al. 2001). Chapter II addresses this issue by examining the phylogeography of *G. hodgsoni* from southern Victoria Land using allozymes and mitochondrial DNA. I predicted that the dispersal capacity of *G. hodgsoni* would be limited due to the extensive Pleistocene glaciations and was likely to have promoted isolation and divergence in populations from fragmented habitats.

Studies that have examined the importance of Pleistocene vicariance events in shaping the distribution of genetic diversity have mostly focussed on northern hemisphere taxa. For example, the biota of North America were exposed to severe range adjustments as species tracked shifting climate regimes caused by recurrent glacial advances and retreats during the Pleistocene (Hewitt 1996; Klicka and Zink 1997; Avise 1998; Cox and Hebert 2001; Knowles 2001). For the southern hemisphere, temperate regions have also been shown to be largely affected by the

lowering of global temperatures during the Pleistocene glaciations and also to much older geological changes (Fleming 1979; Craw 1988; Pole 1989; Trewick 2000; Trewick and Wallis 2001). These studies have assessed terrestrial taxa in New Zealand and have found high levels of diversification most likely driven by: (1) climatic changes over the last two million years; or (2) much older geological changes during the Cenozoic.

However, very little is known about the genetic structures for the aquatic invertebrates of New Zealand (Smith and Collier 2001; Hogg et al. 2002) and even less about the marine and estuarine fauna (Schnabel et al. 2000). New Zealand has a small landmass (270,000 km^2) but wide latitudinal range (ca. 12°) with extensive coastline, making it ideal for investigating patterns of diversification and dispersal of aquatic taxa that use ocean currents to move among habitat patches. Hence, I assessed the population genetic structures and dispersal of the two endemic corophiid amphipods, *Paracorophium excavatum* and *P. lucasi*. Both lack a specific dispersal stage and hence may be exposed to geographical barriers imposed by the broad geographic range of the New Zealand topography, but present-day genetic structure may also be a consequence of landmass alterations over time.

Chapter III introduces this second study ecosystem, the estuarine and freshwater regions of New Zealand. Here, the demographic characteristics of *P. lucasi* and *P. excavatum* were examined at Tauranga Harbour. During this study both morphological and genetic data were used to reveal the presence of a third *Paracorophium* species, *P. brisbanensis*, previously only recorded from the eastern coast of Australia. Both allozyme and geographic locality data for these species were used to infer the introduction of *P. brisbanensis* to Tauranga

Harbour. I conclude by discussing the potential causes of this introduction and possible consequences to other New Zealand endemics.

Chapter IV presents the primary results for the New Zealand study. Data from an allozyme electrophoretic survey were used to estimate the degree of genetic variability and differentiation at inter- and intraspecific levels for *P. lucasi* and *P. excavatum*. Samples were collected during 1998-2000 from 49 coastal and three freshwater habitats in North, South and Chatham Islands. This chapter examines if the population genetic structures are correlated to climatic and geological changes that altered the New Zealand archipelago during the Cenozoic.

Dispersal is often suggested as a key component of an organism's life-history strategy affecting the dynamics of populations, the persistence of species, local adaptation, speciation, and the evolution of other life-history traits (Pusey 1987; Stenseth and Lidicker 1992; Bilton et al. 2001). In this way, dispersal can be advantageous for avoiding predation, competition among kin and for preventing inbreeding (Pusey 1987). Chapter V examines the dispersal dynamics of corophiid amphipods. The genus *Paracorophium* was examined during a 13 month study at Tauranga Harbour to assess dispersal and the ability to retain and recruit individuals. Little is known of the dispersal dynamics of *Paracorophium*, and here I examine whether dispersal is passively controlled in response to the tidal cycles of the estuary, whether the size of an individual will determine its probability to become a potential disperser, and if dispersal is sex-biased.

The thesis concludes with a summary of the main findings of my research, as well as suggestions for future research. Additionally, two appendices are included containing information on Antarctic and New Zealand arthropods. Appendix I provides a review of the soil fauna of the maritime and continental Antarctic with

particular reference to those taxa associated with the continental oases (Windmill Islands and Ongul Island) of East Antarctica and those of the South Shetland Islands, and includes results from Chapter II. Appendix II provides a synonymy of the New Zealand corophiid amphipod genus, *Chaetocorophium,* with *Paracorophium* using morphological and genetic evidence using data from Chapter IV.

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CHAPTER I

EXPANDED DISTRIBUTIONAL RECORDS OF COLLEMBOLA AND ACARI IN SOUTHERN VICTORIA LAND, ANTARCTICA[†]

Key-words: Collembola, Acari, arthropod distribution, dispersal, invertebrate sampling

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We provide new distributional records for the Collembola and Acari from south Victoria Land in the Ross Dependency, Antarctica, including the first extensive survey of Taylor Valley. We also describe the design of a modified device for the rapid sampling of Antarctic terrestrial arthropods. Sampling was carried out during the austral summers from 1998 to 2001. In several instances we found *Gomphiocephalus hodgsoni*, *Neocryptopygus nivicolus* and *Stereotydeus mollis* at sites where they were not reported in comprehensive surveys made some 40 years ago, which may imply recent range expansion (eg: local dispersal). By contrast, at McMurdo Station and at the former North Base (Marble Point) the distribution of *G. hodgsoni* and *S. mollis* was restricted, relative to previous records. We conclude that these latter changes may be the direct result of human activities.

Antarctica has been isolated from other continents by a wide oceanic belt (c. 40°S – 66°S) for at least 10 million years (Schultz 1995; Lawver & Gahagan 1998). The sub-Antarctic islands, southern New Zealand and Tierra del Fuego are now the only terrestrial areas that penetrate this oceanic zone. The isolation and harsh environment of Antarctica have produced a unique polar desert that is now dominated by mosses, lichens and algae (Broady 1996; Seppelt & Green 1998). In polar regions, species diversity of arthropods is extremely low (Vernon et al. 1998), and only those with adaptations to extreme cold temperatures through the use of behavioural and ecophysiological strategies are represented (Sømme & Block 1991). The community composition of free-living terrestrial soil fauna includes Collembola (Wise 1967, 1971; Wise & Shoup 1971; Frati et al. 1997), Acari (Gressitt 1967; Strandtmann 1967; Pugh 1997), rotifers, nematodes and tardigrades (Tilbrook 1967; Jennings 1979; Freckman & Virginia 1989; Wharton & Brown 1989; Schwarz et al. 1993).

Information on the distribution and taxonomy of terrestrial arthropods of the Ross Dependency received considerable attention in the 1960s (Gressitt et al. 1963; Wise & Gressitt 1965; Gressitt 1967; Strandtmann 1967; Wise 1967; Wise & Spain 1967; Wise 1971; Wise & Shoup 1971). However, there has been a paucity of additional information since (eg: Potapov 1991; Frati et al. 1997). Although research has been sporadic, renewed interest in the arthropod fauna has enabled the distribution and species designations of arthropods to be re-examined following earlier studies some 40 years ago (eg: Strandtmann 1967; Wise 1967, 1971).

Accordingly, our aims were threefold. Firstly, to examine the distributional records for Collembola and Acari from south Victoria Land, Ross Dependency; secondly, to contrast this with previous records; and thirdly, to provide a more detailed benchmark for the long-term monitoring of arthropod populations.

MATERIALS AND METHODS

Study sites

We assessed the presence of Collembola (Hypogastruridae and Isotomidae) and free-living Acari (Prostigmata) from Ross Island and throughout the continental region of south Victoria Land (Fig.1). During January 1999, we examined Bratina Island, Cape Bird and Hut Point Peninsula, and Granite Harbour, and in January 2000 we sampled at Beaufort Island, Cape Royds, Cape Evans, Garwood Valley, Miers Valley and re-examined Hut Point Peninsula (Fig. 1). From November 2000 to January 2001 we sampled at Cape Crozier, Granite Harbour, Mt. England, Springtail Point, Marble Point and Taylor Valley (Fig. 1). Complete details are provided in the Appendix.

Sampling of arthropods

We modified the design of the common aspirator to collect from the underside of stones, while minimising environmental disturbance associated with other methods (eg: Berlese Funnel, Tulgren extractor, flotation method). We miniaturised the storage chamber of the aspirator with a Nalgene™ 1.2 ml internal

thread cryo-vial (Fig. 2) and used this vial for storage of specimens. This minimised the possibility of any transfer of specimens between locations as a new vial was used at each site. The center of the cryo-vial lid was drilled leaving a hole with the thread and sides of the lid intact. Two flexible clear plastic tubes with an internal diameter of 3 mm (4.5 mm external) were pushed through the hole in the lid. The collecting tube was cut at 45 degrees to minimise potential blockage by specimens (Fig. 2). The tube leading to the mouthpiece was covered with 0.05 mm mesh. After the tubes were in place, silicon sealant was used to seal around the tubes to prevent air and specimens from escaping.

Specimens were identified using the original authorities for *Neocryptopygus nivicolus* Salmon 1965 and *Stereotydeus mollis* Womersley & Strandtmann 1963. *Gomphiocephalus hodgsoni* Carpenter 1908 was identified using Salmon (1962). All specimens were stored in either liquid nitrogen or 95% ethanol.

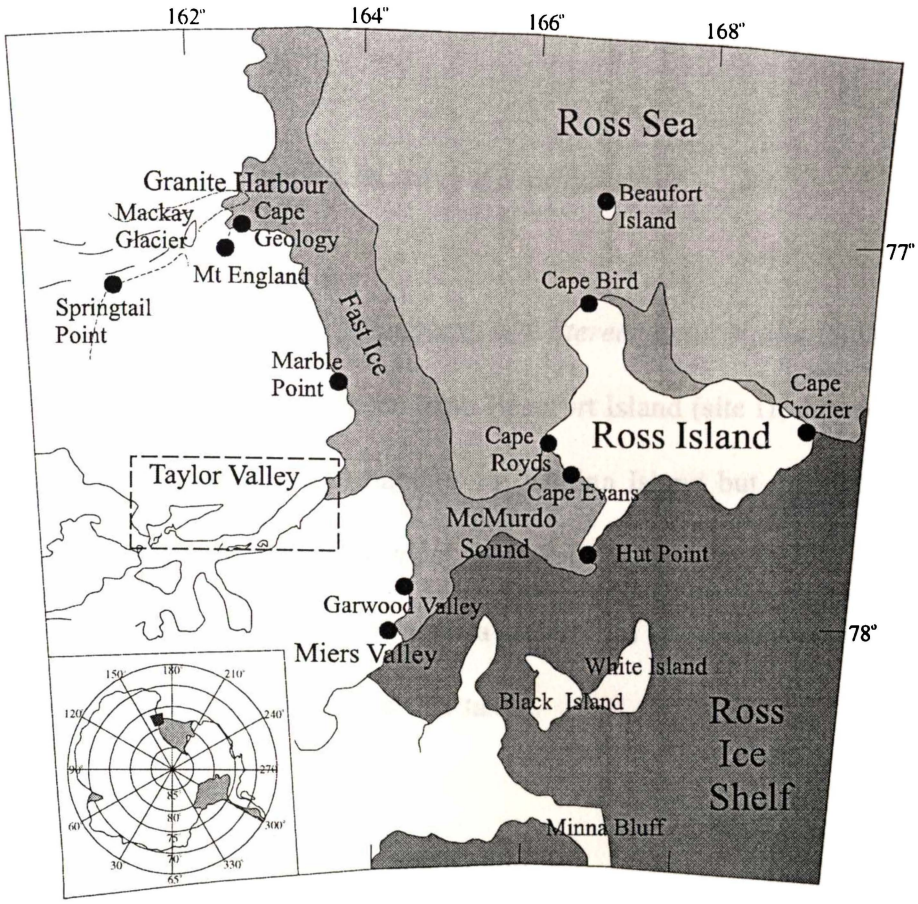

FIGURE 1. Locations (solid circles) examined during 1998-2001 in the Ross Sea region.

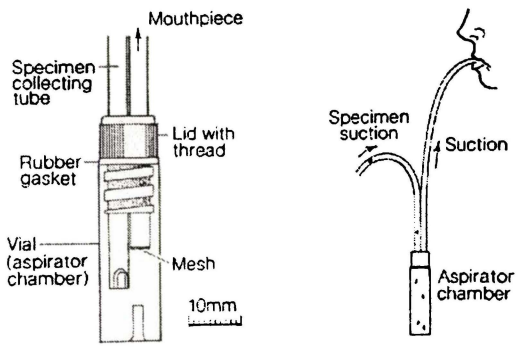

FIGURE 2. The modified aspirator used to collect arthropods.

Beaufort Island and Bratina Island

Gomphiocephalus hodgsoni (Collembola) and *Stereotydeus mollis* (Acari) were very abundant in moss samples taken from Beaufort Island (site I1, Appendix). By contrast, we found similar moss habitats on Bratina Island but did not find any evidence (eg: live specimens or exuviae) that would suggest either Collembola or Acari were present. Further sampling in a number of other habitats on Bratina Island also revealed no evidence of either taxon.

Ross Island

G. hodgsoni and *S. mollis* were both found at Cape Bird (site B1), at two sites from Cape Crozier (sites C1-2), at Cape Royds (sites R1-7), and at sites south of Cape Royds towards Cape Barne (sites R8-13). At Cape Evans *S. mollis* was found to be quite extensive throughout the region (sites E1-3), but *G. hodgsoni* was only found at two sites (sites E2-3), where site E2 was 220 m above sea level and was particularly abundant.

On two occasions (January 1999, 2000) sites were examined around the McMurdo Station area on Hut Point Peninsula, Ross Island. *S. mollis* was collected from Observation Hill at Cape Armitage (site O1), but we did not find any specimens on Hut Point where it had been previously recorded by Strandtmann & George (1973). Likewise, *G. hodgsoni* had been previously recorded from Hut Point and above McMurdo Station (Wise 1971), but we could

find no evidence (either live specimens or exuviae) to indicate its presence.

Southern Victoria Land

Sampling at Cape Geology, Granite Harbour (Fig. 1), revealed two collembolan species, *G. hodgsoni* and *Neocryptopygus nivicolus* as well as *S. mollis*. *G. hodgsoni* was found at 24 sites in the Granite Harbour region, and at ten of these *N. nivicolus* was also found (sites G4-7, G16-19, G21). In the Mt. England region (Fig. 1), *G. hodgsoni*, *N. nivicolus* and *S. mollis* were found. All three species were found at various sites on the lower northern and eastern slopes of Mt. England (sites G25-28), and *G. hodgsoni* was also found with *S. mollis* between Mt. England and New Glacier (site G24).

At the site of the former North Base (Marble Point, Fig. 1), which was bulldozed into the ground between 1957-1959 (Broadbent 1994), *S. mollis* was found (site P2), but there was no evidence of *G. hodgsoni*, despite previous records (Wise et al. 1964; Wise 1967; Wise & Spain 1967). Only at less disturbed sites between Gneiss Point and Marble Point (sites P1, P4-6) and near the Wilson Piedmont Glacier (site P3) was *G. hodgsoni* found. At the current Marble Point Station site there was no evidence of either species.

S. mollis was present at all locations examined around Lake Fryxell in the Taylor Valley (sites T1-6, Fig. 3), and *G. hodgsoni* was only found from streambeds on the southern side of the lake (sites T4, 5). Below all glaciers examined on the south side of the valley (Fig. 3), from Howard Glacier (site T7) to Borns Glacier (site T19) we found both *G. hodgsoni* and *S. mollis*. These species were also found on the southeast side of Lake Chad, 3-4 m from the lake

edge (site T21). The distribution of *G. hodgsoni* and *S. mollis* extended the entire length of the Delta Stream from Howard Glacier (site T7) to Lake Fryxel (site T5). Both species were found in a streambed area (site T17) that flows from a small glacier situated between Hughes and Calkin Glaciers, and were also found along several dry streambed areas from the south side of the valley (sites T8, T9, T15, T16, T20).

Both *G. hodgsoni* and *S. mollis* were extensively distributed throughout Miers Valley (sites M1-8). Regions adjacent to Miers Valley (sites M9-13) and Garwood Valley (site G1) were also found to harbour both species.

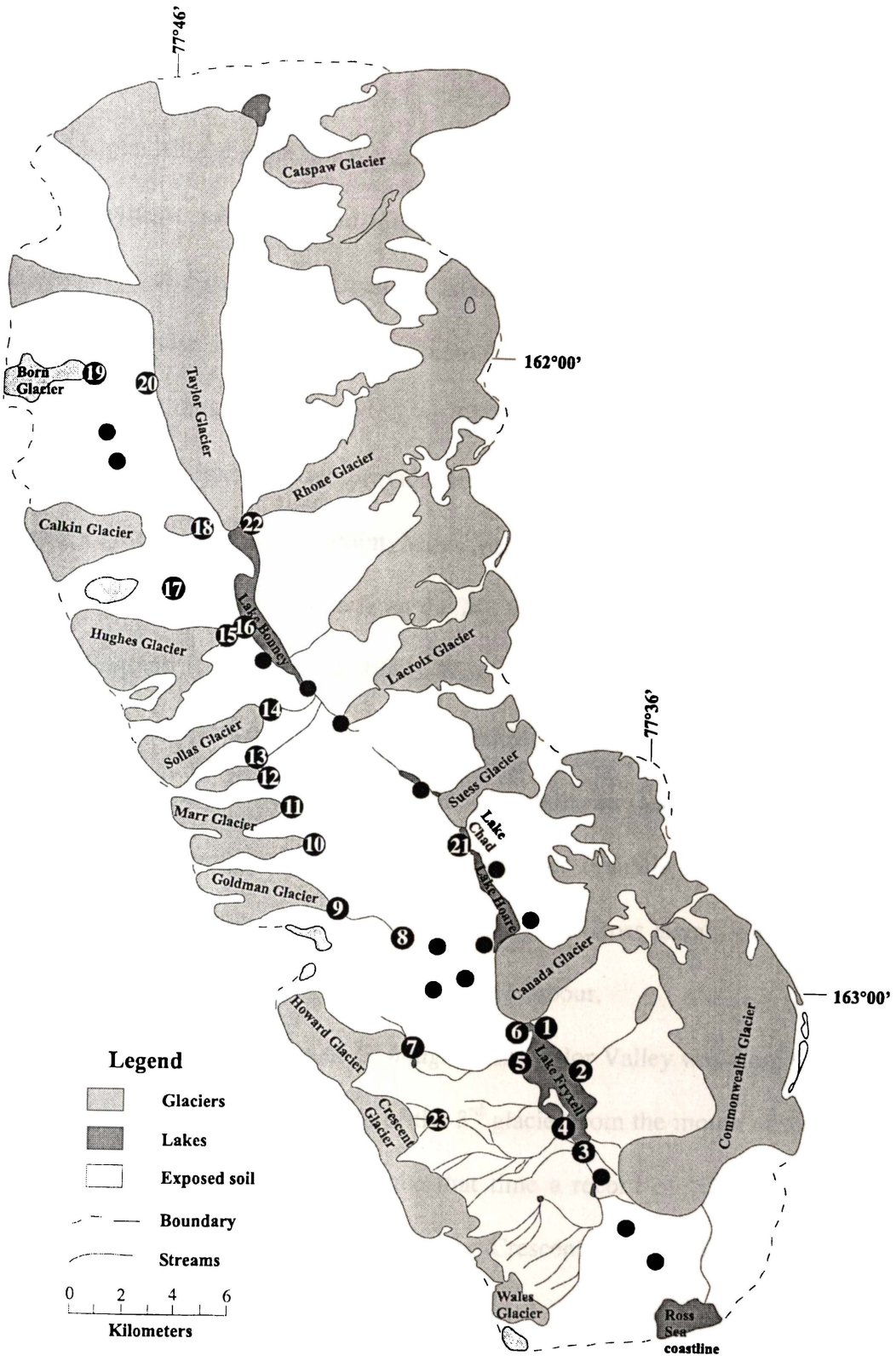

FIGURE 3. Distribution of arthropods in Taylor Valley. Sites indicate where arthropods were present (numbered circles – see text), or absent (solid circles). Site 22 was previously examined by Strandtmann (1967) and site 23 by Schwarz et al. (1993).

The expanded distributional records for *Gomphiocephalus hodgsoni*, *Neocryptopygus nivicolus* and *Stereotydeus mollis* follow an extensive re-examination of Ross Island, Beaufort Island and south Victoria Land. Previous records (eg: Wise 1967, 1971) have found *G. hodgsoni* from Mt. George Murray (75°55'S) to Minna Bluff (78°28'S), on Black, White, Ross and Beaufort Islands in the Ross Sea, and now we provide detailed distributional records from Ross Island, Taylor Valley, Marble Point, Miers Valley and Granite Harbour regions. *N. nivicolus* was previously known from the Mt. Gran-Willett Range, Mt. Seuss, and on Mt. England (Gressitt et al. 1963; Wise 1971), and in the present study we report on its occurrence at Cape Geology and Mt. England, Granite Harbour. The distribution of *S. mollis* (Strandtmann 1967) is similar to *G. hodgsoni* but extends further north to Terra Nova Bay and is also found on Franklin Island, and here we provide detailed records of its distribution throughout Ross Island, Taylor Valley, Marble Point, Miers Valley region and Granite Harbour.

Previously, the only record of *G. hodgsoni* in Taylor Valley was from the south side with Acari below the terminus of the 3rd glacier from the mouth of the valley (77°40'S) (Gressitt et al. 1963). Since that time a record of 'Collembola' was made by Schwarz et al. (1993) between the Crescent and Howard Glaciers (see Fig. 3). *S. mollis* has previously been reported from Rhone Glacier, Canada Glacier and Lake Chad (Strandtmann 1967; Spain 1971; Block 1985; Schwarz et al. 1993), though its distribution throughout the remainder of the valley was unknown. At Lake Chad we found *S. mollis*, but also found *G. hodgsoni* (site T21, Fig. 3), and it is unlikely that *G. hodgsoni* would have been overlooked by Spain

(1971) and Strandtmann & George (1973) if it was present at that time. Previous studies (Wise & Spain 1967; Spain 1971; Strandtmann & George 1973) also make reference to a total absence of any life around Lake Bonney, but we found both species in two streambeds near the Lake (site T16, Fig. 3). More recent studies in Taylor Valley have also provided no new records of Collembola or Acari (Powers et al. 1998; Treonis et al. 1999; McKnight et al. 1999; Priscu 1999; Virginia & Wall 1999). By contrast, we have found them to be in high abundance near streams, particularly in the vicinity of glacial melt-water, throughout Taylor Valley (Fig. 3). One possible explanation is that differences in sampling strategies (eg: soil extraction techniques vs examination on site) may have biased studies for specific taxa. However, this is uncertain.

Previous studies in Granite Harbour made no record of *N. nivicolus* at Cape Geology nor were there any records of *G. hodgsoni* in the Mt. England region (Wise et al. 1964; Wise 1967; Wise & Shoup 1971; Davidson & Broady 1996). Furthermore, the only locations where *N. nivicolus* and *G. hodgsoni* have previously been found together were at Mt. Seuss and Convoy Range (see Wise 1967 for review). However, we also found them to co-exist at Cape Geology—a new record for *N. nivicolus,* and on Mt. England—a new record for *G. hodgsoni. N. nivicolus* had also supposedly been recorded from Cape Crozier (Salmon 1965). However, we did not find *N. nivicolus* in any of our samples, and we concur that this record is likely to have been made in error (Janetschek 1967; Wise 1971).

In contrast to the possible range expansions observed at the above sites, records from around McMurdo Station and at the site of the former North Base (Marble Point) seem to suggest that human activities over a period of less than 40 years

have caused the reduction in distribution for these taxa. Such human influence was previously implicated by Strandtmann & George (1973) when reporting on the limited distribution and local abundance of *S. mollis* in the McMurdo Station region in the late 1960s. Previous studies (Wise et al. 1964; Wise 1967; Wise & Spain 1967) suggest that the distribution of *G. hodgsoni* was extensive from Marble Point to Gneiss Point and extended throughout the site of the former North Base to the Wilson Piedmont Glacier. In recent years, soils from both sites have been shown to have greater heavy metal concentrations compared to undisturbed sites (Claridge et al. 1995; Sheppard et al. 1997), and Campbell et al. (1994) showed that there has been no significant re-establishment of icy permafrost in the soils since land disturbance occurred at Marble Point. The absence of arthropods at these sites appears to be associated with the extensive human disturbance (see Broadbent 1994), and we also found moss communities to be damaged and desiccated, even when surface moisture appeared adequate (eg: melt-water).

Comparing previous records to our own from Lakes Chad and Bonney in Taylor Valley, and Cape Geology and Mt. England at Granite Harbour suggest local dispersal within the last 40 years for these taxa. The effects of human disturbance appears to be restricted to McMurdo Station and the former North Base, where the distribution of these arthropods was limited in comparison to previous records. The Ross Dependency is an ideal region to examine dispersal and re-colonisation of Antarctic habitats with both Collembola and Acari found from the most northern regions (c. 70°S) to the most southern recorded for any terrestrial animal (85°32'S, Wise & Gressitt 1965). It is only with accurate distributional records that species ranges can be examined, particularly with an

increasing human presence in Antarctica, as well as the possibility of new habitats becoming available as a result of climatic shifts.

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APPENDIX 1. Field sites where arthropods were recorded over the austral summers from 1998 to 2001. Species identified: *Gomphiocephalus hodgsoni* [G], *Neocryptopygus nivicolus* [N], and *Stereot ydeus mollis* [S]

LOCATION	SITE	SPECIES*	LAT (S)	LONG (E)
Beaufort Island	I1	G,S	76°55.906'	166°54.808'
Cape Bird	B1	G,S	77°13.266'	166°26.810'
Cape Royds	R1	G	77°32.760'	166°09.779'
	R2	S	77°32.712'	166°09.538'
	R3	S	77°32.949'	166°09.429'
	R4	G	77°32.783'	166°09.130'
	R5	G	77°32.555'	166°09.312'
	R6	G	77°32.421'	166°09.190'
	R7	G	77°32.339'	166°09.400'
	R8	G	77°33.074'	166°12.454'
	R9	S	77°33.705'	166°14.325'
	R10	G,S	77°33.905'	166°13.748'
	R11	G,S	77°33.933'	166°13.822'
	R12	G,S	77°34.009'	166°13.736'
	R13	G	77°34.187'	166°14.204'
Cape Evans	E1	S	77°38.119'	166°26.315'
	E2	G,S	77°38.033'	166°26.551'
	E3	G,S	77°34.187'	166°14.204'
Observation Hill	O1	S	77°51.174'	166°40.804'
Cape Crozier	C1	G,S	77°27.795'	169°11.812'
Igloo Spur	C2	G,S	77°31.748'	169°17.208'
Granite Harbour				
Cape Geology	G1	G,S	77°00.863'	162°36.086'
	G2	G,S	77°00.882'	162°36.025'
	G3	G,S	77°00.920'	162°35.914'
	G4	G,N,S	77°00.957'	162°35.819'
	G5	G,N,S	77°00.942'	162°35.767'
	G6	G,N,S	77°00.834'	162°34.580'
	G7	G,N,S	77°00.738'	162°34.267'
	G8	G,S	77°00.635'	162°33.771'
	G9	G,S	77°00.534'	162°33.464'
	G10	G,S	77°00.470'	162°33.450'

continued,

APPENDIX 1 *continued,*

LOCATION	SITE	SPECIES*	LAT (S)	LONG (E)
	G11	G,S	77°00.500'	162°33.274'
	G12	G,S	77°00.511'	162°33.058'
	G13	G,S	77°00.534'	162°32.776'
	G14	G,S	77°00.548'	162°32.614'
	G15	G,S	77°00.562'	162°32.469'
	G16	G,N,S	77°00.591'	162°32.317'
	G17	G,N,S	77°00.579'	162°32.246'
	G18	G,N,S	77°00.562'	162°32.204'
	G19	G,N,S	77°00.552'	162°32.161'
	G20	G,S	77°00.525'	162°32.105'
	G21	G,N,S	77°00.742'	162°33.597'
Botany Bay	G22	G,S	77°00.402'	162°39.310'
Flatiron	G23	G,S	77°00.938'	162°22.069'
New Glacier	G24	G,S	77°01.339'	162°27.000'
Mt. England	G25	G,N,S	77°02.298'	162°28.180'
	G26	G,S	77°01.942'	162°26.400'
	G27	G,S	77°02.251'	162°26.100'
	G28	G,N,S	77°02.277'	162°25.800'
Springtail Point	W1	N	77°10.117'	160°43.433'
Marble Point	P1	G,S	77°26.122'	163°49.569'
	P2	S	77°25.328'	163°41.572'
	P3	G,S	77°25.297'	163°40.467'
	P4	G,S	77°25.020'	163°43.466'
	P5	G,S	77°25.328'	163°41.572'
Gneiss Point	P6	G,S	77°24.527'	163°44.249'
Taylor Valley				
Canada Glacier	T1	S	77°36.924'	163°02.505'
Huey Creek	T2	S	77°36.429'	163°07.539'
Lake Fryxell	T3	S	77°36.161'	163°15.701'
Von Guerard Stream	T4	G,S	77°36.570'	163°14.052'
Delta Stream	T5	G,S	77°37.428'	163°06.560'
Green Stream	T6	S	77°37.217'	163°05.102'
Howard Glacier	T7	G,S	77°39.735'	163°05.835'
	T8	G,S	77°39.985'	162°54.627'
Goldman Glacier	T9	G,S	77°41.083'	162°52.600'
Marr Glacier east lobe	T10	G,S	77°41.757'	162°46.737'

continued,

APPENDIX 1 *continued,*

LOCATION	SITE	SPECIES*	LAT (S)	LONG (E)
Marr Gl. central lobe	T11	G,S	77°42.140'	162°43.315'
Marr Gl. west lobe	T12	G,S	77°42.780'	162°39.365'
	T13	G,S	77°43.022'	162°38.082'
Sollas Glacier	T14	G,S	77°42.742'	162°35.580'
Hughes Glacier	T15	G,S	77°43.745'	162°30.327'
	T16	G,S	77°43.311'	162°26.349'
	T17	G,S	77°44.167'	162°20.712'
Calkin Glacier	T18	G,S	77°44.195'	162°16.728'
Borns Glacier	T19	G,S	77°45.833'	162°02.240'
	T20	G	77°45.536'	162°01.277'
Lake Chad	T21	G,S	77°38.571'	162°46.492'
Garwood Valley	G1	G,S	78°01.179'	164°03.404'
Miers Valley	M1	G,S	78°05.762'	163°45.540'
	M2	S	78°05.889'	163°47.828'
	M3	G	78°05.853'	163°46.810'
	M4	G	78°06.086'	163°49.436'
	M5	S	78°05.950'	163°53.354'
	M6	G,S	78°04.408'	163°47.791'
	M7	S	78°04.635'	163°46.736'
	M8	G	78°06.953'	163°41.645'
	M9	G	78°03.990'	163°52.049'
	M10	G,S	78°07.187'	163°41.373'
	M11	G,S	78°07.600'	163°40.777'
	M12	G	78°07.465'	163°42.341'
Marshall Valley	M13	G,S	78°03.944'	163°52.662'

CHAPTER II

LONG-TERM ISOLATION AND RECENT RANGE EXPANSION FROM GLACIAL REFUGIA REVEALED FOR THE ENDEMIC SPRINGTAIL *GOMPHIOCEPHALUS HODGSONI* FROM VICTORIA LAND, ANTARCTICA

Key-words: allozymes, Collembola, habitat fragmentation, mitochondrial DNA, phylogeography

ABSTRACT

We examined the phylogeography of the endemic Antarctic collembolan *Gomphiocephalus hodgsoni* using allozymes and mtDNA (COI) to determine if potentially limited dispersal and long-term habitat fragmentation have promoted regional genetic differentiation. Allozyme analyses showed that differentiation among 18 populations within the Ross Dependency was high (F_{ST} = 0.52) with two main groups each representing a distinct geographical region: (1) Ross Island and Beaufort Island; and (2) all continental sites. Ross Island populations showed low levels of divergence (D = 0.001-0.008) and no correlation with geographic distance, suggesting their derivation from a single glacial refuge. By contrast, divergence among continental regions (D = 0.07-0.31) and moderate levels of geographic differentiation indicate a much older history with several refugia likely. Two sympatric allozyme genotypes were found at one continental site (Taylor Valley) and were congruent with two mtDNA haplotypes, implying non-random breeding groups. In addition, haplotype sharing between one Ross Island site (Cape Bird) and one continental site (Granite Harbour) was identified. Otherwise, the clades showed mostly fragmented allopatric distributions. The extensive Pleistocene glaciations, in conjunction with limited dispersal opportunities appear to have promoted isolation and divergence among the fragmented habitats. Furthermore, the McMurdo Sound appears to be an effective isolating barrier to dispersal. However, we suggest that the un-aided dispersal capacity of *G. hodgsoni* is unlikely to account for the limited mixing of haplotypes across the McMurdo Sound and recent human or bird mediated dispersal is highly probable.

The examination of historical geographic change has provided a framework for examining vicariance events among populations. In recent years, genealogical studies of organisms have utilised this information for the interpretation of the evolutionary relationships among taxa, and also to examine colonisation and dispersal among populations within a phylogeographic context (Avise 1998). Phylogeography as a field of study has been growing in popularity with an increasing number of recent publications (e.g. Masta 2000; Knowles 2001; Roslin 2001), including a special issue of *Molecular Ecology* (Bermingham & Moritz 1998). However, few studies have employed a phylogeographic approach to examine the importance of Pleistocene vicariance events in shaping the distribution of genetic diversity for the terrestrial fauna of Antarctica (Courtright *et al.* 2000; Fanciulli *et al.* 2001; Frati *et al.* 2001). Antarctica is an ideal location for phylogeographic studies, where the spreading of the continental plates may be responsible for much of the faunal and floral distributions (e.g. Wise 1967; Broady 1996; Pugh 1997; McInnes & Pugh 1998; Skotnicki *et al.* 2000).

In addition, frequent shifts in species range during glacial cycles have been used to test the role of the Pleistocene glaciations in speciation (e.g. Hewitt 1996; Klicka & Zink 1997; Avise *et al.* 1998; Knowles 2001). Such studies have generally examined two opposing views: (1) that habitat fragmentation during glacial advances promoted divergence; and (2) cycles of population contraction and expansion acted as a homogenising force. Although some studies have found no link between the effect of glacial cycles and speciation (e.g. Cracraft & Prum 1988; Riddle 1996; Klicka & Zink 1997), many studies have tested these

hypotheses in North America and found evidence to support Pleistocene speciation (e.g. Rising & Avise 1993; Avise *et al.* 1998; Knowles 2001).

In contrast to northern hemisphere taxa, there are two inherent differences in assessing Pleistocene speciation theory on Antarctic terrestrial taxa. Firstly, Antarctica has been isolated from other continents by a wide oceanic belt (ca. 40°S – 66°S) for at least 10 million years (Schultz 1995; Lawver & Gahagan 1998). Secondly, the Antarctic terrestrial landscape is dominated by long-term habitat fragmentation, with little more than 1% of the 14 million km^2 of the continent ice-free today, and more than ten major glacial cycles over the last one million years (Hays *et al.* 1976). Accordingly, Antarctic terrestrial life has evolved as a unique collection of endemic fauna and flora (see Beyer & Bölter 2002 for review).

In Antarctica, prolonged low temperatures and increased glacial activity have meant that many regions became isolated and the survival of taxa, particularly the terrestrial invertebrates, could only be possible in ice-free refugia, such as nunataks (Wise 1967; Hogg & Stevens 2002). Nunataks allow for possible refuges during the last glaciation, but many terrestrial habitats have only become available for (re-)colonisation within the current inter-glacial (<17,000 years). For these reasons Victoria Land, in the Ross Sea region, represents the ideal model system to examine hypotheses related to Pleistocene speciation and the evolutionary persistence of Antarctic taxa relative to long-term perturbations. Here we present the first population genetic study of *Gomphiocephalus hodgsoni* Carpenter 1908 (Collembola: Hypogastruridae) from the most southern terrestrial habitats in Victoria Land, Antarctica. *G. hodgsoni* is endemic, and restricted to southern Victoria Land between Mt. George Murray (75°55'S) and Minna Bluff (78°28'S),

and to adjacent nearshore islands (Wise 1967, 1971; Stevens & Hogg 2002). We tested two hypotheses: firstly, that long-term habitat fragmentation has resulted in high levels of divergence among populations; and secondly, that levels of divergence among populations is consistent with Pleistocene speciation theory.

MATERIALS AND METHODS

Collection of samples

A total of 19 populations were sampled from 1999 to 2002 (Table 1, Fig. 1). At two locations (Taylor Valley and Granite Harbour) we collected individuals from several sub-populations, and from Granite Harbour and Cape Bird individuals were each obtained from two austral summers (Table 1). From these 19 populations sampled, we used ten main localities for allozyme analyses, and eleven for the mtDNA analyses (see Table 1). Approximately 60 individuals were collected at each location from the underside of stones using a modified aspirator (see Stevens & Hogg 2002 for complete details). Individuals were flash-frozen in liquid nitrogen and stored at -75°C until needed for molecular analyses.

Allozyme electrophoresis

Individual specimens were homogenised in 6 µl of distilled water and analysed using cellulose acetate electrophoresis (Richardson *et al.* 1986; Hebert & Beaton 1993). In total 22 enzyme systems were assayed and the following 11 revealed sufficient activity, resolution and could be reliably scored: Adenylate Kinase (AK,

EC 2.7.4.3); Aldehyde Oxidase (AO, EC 1.2.3.1); Arginine Kinase (ARK, EC 2.7.3.3); Fumarate Hydratase (FUMH, EC 4.2.1.2); Glucose-6-Phosphate Isomerase (GPI, EC 5.3.1.9); Hexokinase (HK, EC 2.7.1.1); Isocitrate Dehydrogenase (IDH, EC 1.1.1.42); Malate Dehydrogenase (MDH, EC 1.1.1.37); Mannose-6-Phosphate Isomerase (MPI, EC 5.3.1.8); Phosphoglucomutase (PGM, EC 5.4.2.2); Pyruvate Kinase (PK, EC 2.7.1.40). We were able to compare the relative mobility of alleles between gels by running a control (amphipod) of known electrophoretic mobility. Alleles were designated by the relative difference in anodal mobility of respective gene products, i.e. the 'fastest' allele was designated "A", the next fastest allele "B", and so on. In some cases it was not possible to score all loci for each individual. Accordingly, sample sizes vary slightly among loci.

The program Genetic Data Analysis version 1.1 (Lewis & Zaykin 2001) was used to calculate descriptive and hierarchical population statistics. Genotypic frequencies were determined for each population, and polymorphic loci (95% criterion) were examined for agreement of genotypes with Hardy-Weinberg equilibrium using Fisher's exact test, followed by sequential Bonferroni corrections (Rice 1989). Divergence was assessed among populations using Wright's (1978) F_{ST} and among individuals in a single population (F_{IS}). Hierarchical cluster analysis was performed using the UPGMA algorithm (Sneath and Sokal 1973) calculated using Nei's (1978) unbiased genetic distance.

Isolation-by-distance (*I-D*) analyses (Wright 1943; Slatkin 1993) were performed to examine geographic differentiation among populations according to the island model (Wright 1931, 1943) and the stepping stone model (Kimura 1953; Kimura & Weiss 1964; Ibrahim *et al.* 1996). To test for the existence of *I-D*

we performed a regression of log transformed pairwise genetic distance (log*D*) and geographic distances (log*km*) (as the crow flies) and calculated the regression coefficients (R^2) (Slatkin 1993). Spearman's rank correlation index (*R*) was used to test how much of the allelic variance among populations was explained by geographic distance alone. We used SPSS for Windows version 10.7 (SPSS Inc.) for these analyses.

Mitochondrial DNA analysis

Total DNA was extracted from 1-8 individuals from each of the 19 localities (Table 1). Extractions consisted of homogenising an entire individual in 20 µl of extraction buffer (1×PCR buffer + $MgCl_2$, 1% Tween 20, 100 µg/ml proteinase K), freezing at -76°C for 15 min, incubating at 55°C for eight hours, followed by 15 min at 94°C to denature the proteinase K (Cox & Hebert 2001; Frati *et al.* 2001).

PCR amplification (Saiki *et al.* 1988) was carried out using a 50 µl reaction volume consisting of 3 µl of DNA (not quantified), 1×PCR buffer (Roche), 2.2 mM $MgCl_2$, 0.2 mM of each dNTP (Boehringer Mannheim), 1.0 µM of each primer, and 1.0 unit of *Taq* DNA polymerase (Roche) on a Eppendorf Mastercycler gradient thermocycler. Amplification (via PCR) of a 710 base pair fragment of the mitochondrial (mt) cytochrome *c* oxidase I (COI) gene was amplified using the universal primers LCO1490 (5'- ggt caa caa atc ata aag ata ttg g -3') and HCO2198 (5'- taa act tca ggg tga cca aaa aat ca -3') (Folmer *et al.* 1994). The thermal cycling conditions were: 94°C for 1 min followed by 5 cycles of

denaturation and polymerase amplification (94°C for 1 min, 45°C for 1.5 min and then 1 min at 72°C) and followed by 35 cycles of 94°C for 1 min, 51°C for 1.5 min and then 1 min at 72°C, followed by 5 min at 72°C (Witt & Hebert 2000). All reaction products were purified by 20% Polyethylene Glycol (PEG 8000/2.5M NaCl) extraction and ethanol precipitation. Sequencing was performed using the same primers as those used for PCR amplification on an ABI 377XL automated sequencer (Applied Biosystems Inc.) at the University of Waikato DNA sequencing facility.

Sequences were aligned using Sequencer (Gene Codes ver. 4.1.2 for Macintosh) sequence editor. Sequences were verified as being derived from arthropod DNA using the GenBank BLAST algorithm, and these data were analysed using PAUP* 4.0b10 (Swofford 2002). Phylogenetic analyses used two Arctic collembolans as outgroup taxa: *Hypogastrura tullbergi* (Schäffer) and *H. sensilis* (Folsom). All sequences are accessible from GenBank. We used χ^2-tests, as implemented in PAUP* to determine whether the assumption of equal base frequencies among sequences was violated on all sites and parsimony-informative sites only. A distance matrix of pairwise nucleotide sequence divergence was calculated using the Kimura 2-parameter model (Kimura 1980), and used to estimate a neighbour-joining (NJ) phylogram. Maximum parsimony heuristic searches were conducted on unweighted data with the steepest descent and tree bisection reconnection options invoked after repeated analyses did not improve support for the nodes using various transitions and transversions weighting regimes. Confidence in the cladistic analyses was assessed by estimation of the g_1 skewness statistic from 100,000 random tree length distributions (Hillis &

Huelsenbeck 1992), and by bootstrap analysis with 1000 pseudoreplicates (Felsenstein 1976).

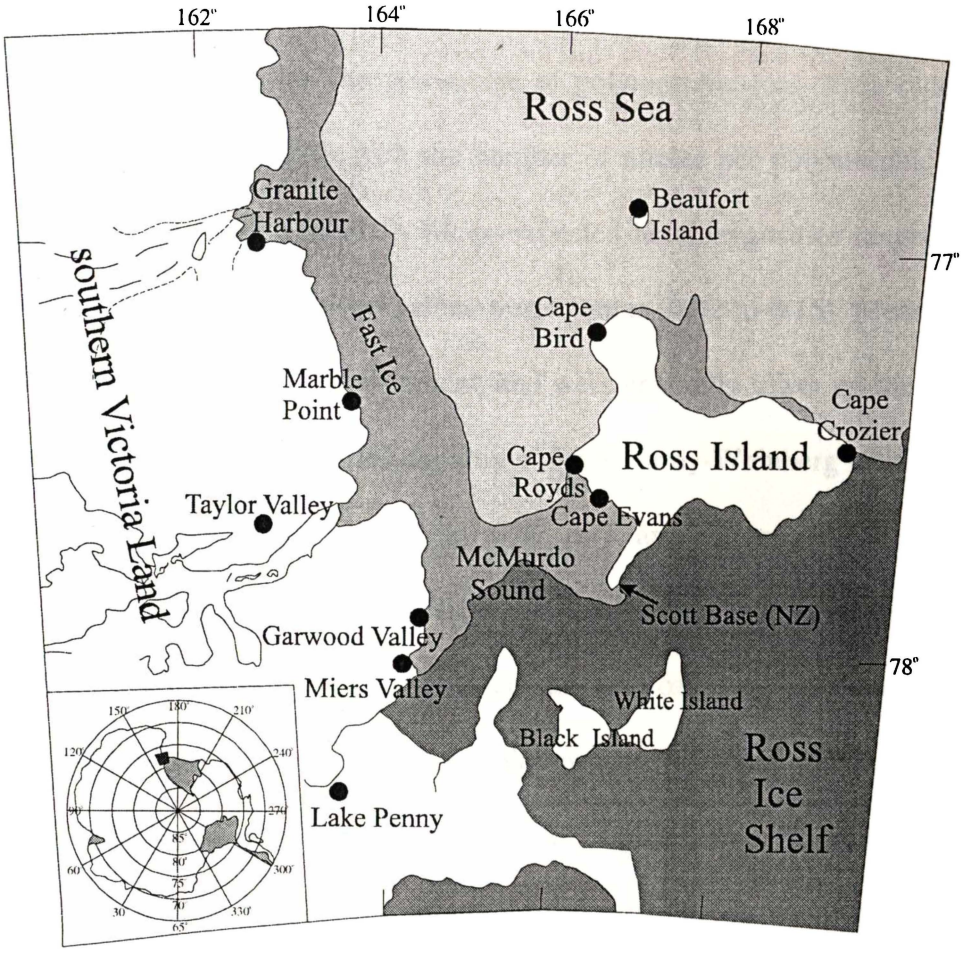

FIGURE 1. Sampling localities for *Gomphiocephalus hodgsoni* throughout southern Victoria Land, Antarctica.

Allozyme variability

Of the eleven scorable loci, eight showed allelic variability among the 18 populations (Appendix 1). The percentage of polymorphic loci (95% criterion) ranged from 9.1% to 54.5%, with the number of alleles per polymorphic locus ranging from 2.0 to 5.0 (Table 1). Mean expected heterozygosities ranged from 0.03 to 0.18, and mean observed values were similar (0.02 to 0.15). Measures of variability were highest on the continent, and were generally lower on the islands (Table 1). Significant ($P < 0.05$) departures from Hardy-Weinberg expectations were found for some populations at the *IDH*, *ARK* and *AK* loci and in all cases were associated with heterozygote deficiencies due to the detection of one or two novel alleles. In particular, we did not detect any heterozygotes between alleles A and B for the *AO* locus in the three Taylor Valley sites (Appendix 1).

Wright's (1978) F_{ST} (0.53) averaged over all populations revealed high levels of genetic differentiation. High levels of intraspecific substructuring were also found (F_{IS} = 0.34), indicating deviation from Hardy-Weinberg assumptions in some populations. Among the Ross Island and Beaufort Island populations F_{ST} = 0.06, and among the continental populations F_{ST} = 0.23.

UPGMA analysis revealed two well differentiated groups (D = 0.31), represented by: (1) all continental populations, and; (2) all island populations (Fig. 2). In comparison to the continental populations, Ross Island and Beaufort Island were fixed for allele C at the *AO* locus and possessed allelic frequency shifts at the *PGM* and *IDH* loci (Appendix 1). On the continent, Taylor Valley contained the

genotype found in all other continental sites. However, we also found a second genotype fixed for a third allele (A) at the *AO* locus. We found the highest proportion of this genotype at Lake Chad (TV3), which was genetically distinct from all other continental sites in the UPGMA analysis (D = 0.12). Both genotypes were also found sympatrically in two other locations from the valley (TV1, TV2). For the continental populations, Marble Point (MP) and Taylor Valley (excluding TV3) south to Miers Valley (MV) and Garwood Valley (GV) formed a separate cluster to all Granite Harbour populations (D = 0.07), including those collected from different austral summers (GH1-GH7) (Table 1, Fig. 2). Ross Island and Beaufort Island showed very little genetic structure (D = 0.001-0.008), with the island populations subdivided into two groups: (1) R2 and R3; and (2) BI, R1 and R4 (Fig. 2).

Genetic distance was correlated to geographic distance as the spatial genetic structuring among all populations using *I-D* analyses was positive and significant (R^2 = 0.4, $P < 0.001$; R = 0.69, $P < 0.01$). Geographic differentiation was found to increase when only continental sites were used (R^2 = 0.59, $P < 0.001$). About 64% (R = 0.64, $P < 0.01$) of the variance in allele frequencies for this geographic region could be explained by genetic drift alone. However, by removing the single Lake Chad population (TV3) the relationship among the continental sites became much stronger (R^2 = 0.67, $P < 0.001$; R = 0.82, $P < 0.01$). By contrast, the Ross and Beaufort Island sites did not show any geographic differentiation (R^2 = 0.23, P = 0.16; R = 0.19, P = 0.59).

TABLE 1. Genetic variability at 11 allozyme loci in 18 populations of *Gomphiocephalus hodgsoni*. N = mean sample size per locus, P = percentage of polymorphic loci (using the 95% criterion), A = mean number of alleles per locus, Ap = mean number of alleles per polymorphic locus, and H_{obs} = observed heterozygosity, H_{exp} = expected heterozygosity, and * = a significant ($P<0.05$) deviation at one or more loci (see text). [§]= samples collected Jan 1999; [†]= samples collected 2000/2001; all other samples were collected Jan 2000; [‡]= samples used for allozyme analyses only. Allozyme analyses were not performed on Lake Penny or Cape Bird individuals collected in Jan 2002.

LOCATION	Lat (S)	Long (E)	N	P	A	Ap	H_{obs}	H_{exp}
ISLANDS								
BEAUFORT I. (BI)	76°55.906'	166°54.808'	35.1	27.3	1.6	2.7	0.05	0.07
ROSS ISLAND								
Cape Bird[§] (R1)	77°13.266'	166°26.810'	53.8	36.4	1.8	2.3	0.05	0.08*
Cape Royds (R2)	77°32.760'	166°09.779'	41.2	27.3	1.6	2.7	0.04	0.07*
Cape Evans (R3)	77°38.033'	166°26.551'	24.2	9.1	1.2	2.0	0.02	0.03
Cape Crozier[†](R4)	77°27.795'	169°11.812'	37.9	27.3	1.5	2.0	0.07	0.05*
	Island mean		38.4	25.5	1.5	2.3	0.04	0.07
CONTINENT								
GRANITE HARBOUR								
Cape Geology[§] (GH1)	77°00.863'	162°36.086'	34.5	45.5	2.5	4.2	0.15	0.19
Botany Bay[§‡] (GH2)	77°00.402'	162°39.310'	36.6	36.4	2.5	5.0	0.14	0.20*
Cape Geology[§‡] (GH3)	77°00.957'	162°35.819'	32.1	45.5	2.3	3.4	0.14	0.16
Cape Geology[§‡] (GH4)	77°00.738'	162°34.267'	39.7	36.4	2.4	4.5	0.08	0.16*
Botany Bay[‡] (GH5)	77°00.402'	162°39.310'	29.9	36.4	1.9	3.3	0.07	0.14*
Cape Geology[†] (GH6)	77°00.863'	162°36.086'	28.5	36.4	1.8	3.3	0.08	0.11*
Mt England[†‡] (GH7)	77°02.298'	162°28.180'	24.6	36.4	2.0	3.5	0.09	0.15*
	Granite Harbour mean		32.3	39.0	2.2	3.9	0.11	0.16
MARBLE POINT[†] (MP)	77°26.122'	163°49.569'	23.3	27.3	1.8	3.7	0.06	0.13*

continued,

TABLE 1 *continued,*

LOCATION	Lat (S)	Long (E)	N	P	A	Ap	H_{obs}	H_{exp}
TAYLOR VALLEY[†]								
Howard Glacier (TV1)	77°39.735'	163°05.835'	23.8	54.5	2.2	3.2	0.10	0.18*
Borns Glacier (TV2)	77°45.833'	162°02.240'	15.6	36.4	1.6	2.5	0.09	0.17*
Lake Chad (TV3)	77°38.571'	162°46.492'	34.0	27.3	1.5	2.7	0.10	0.11*
	Taylor Valley mean		24.5	39.4	1.8	2.8	0.10	0.15
GARWOOD V. (GV)	78°01.179'	164°03.404'	10.0	9.1	1.2	3.0	0.04	0.04
MIERS V. (MV)	78°05.762'	163°45.540'	36.7	27.3	1.8	3.7	0.05	0.12*
LAKE PENNY (LP)	78°18.595'	163°24.473'	—	—	—	—	—	—
	Continental mean		28.4	35.0	2.0	3.5	0.09	0.14
	All populations		31.2	32.3	1.8	3.2	0.08	0.12

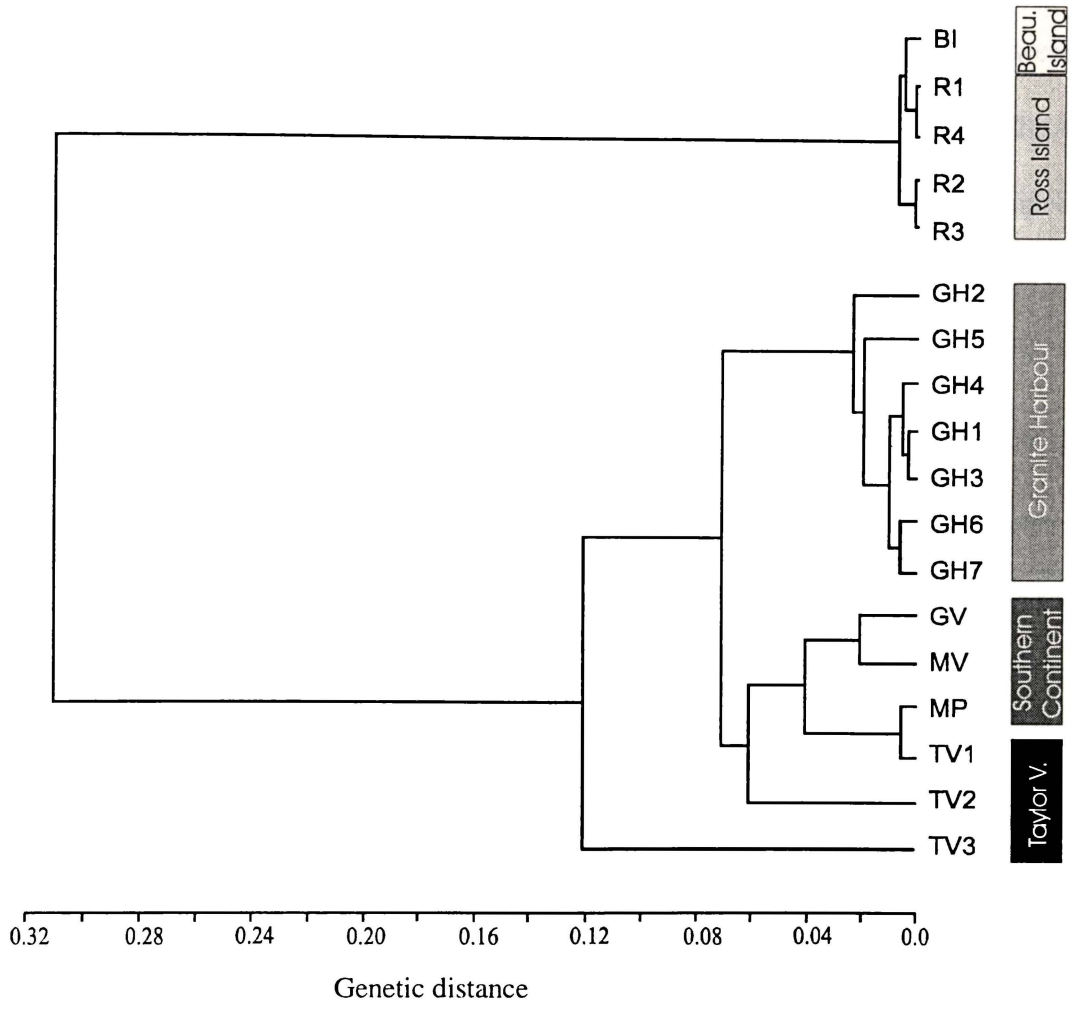

FIGURE 2. UPGMA dendrogram (Nei's (1978) unbiased genetic distance) of allozyme data (11 loci) for 18 *Gomphiocephalus hodgsoni* populations. Shaded bars correspond to those used in Fig. 3, and population codes correspond to Table 1.

A 599-bp fragment was used in these analyses and no insertions or deletions were detected. The nucleotide composition of the sequences was biased for A and T (A = 36%, T = 27%, C = 17%, G = 19%), a common feature of arthropod mitochondrial DNA (Frati *et al.* 2001). We were unable to reject the hypothesis of homogeneity across base frequencies among sequences for all sites (χ^2_{132}= 2.69, $P > 0.05$) and for parsimony-informative sites only (χ^2_{132}= 66.74, $P > 0.05$). Across all 45 *G. hodgsoni* sequences, there were 22 variable sites and 17 parsimony-informative nucleotide substitutions. Table 2 shows the polymorphic nucleotide sites among the 14 different haplotypes identified. The distribution of these haplotypes was mostly geographic with most locations containing 1-4 haplotypes (Table 2). The number of nucleotide substitutions between each haplotype ranged from 1 to 12, and sequence divergence ranged from 0.2% (1 substitution) to 2% (12 substitutions) (Appendix 2).

Six haplotypes were identified from Ross Island (F-K), one from Beaufort Island (L), and three from Granite Harbour (E, F, J) with six from the southern continental sites (A-D, M, N) (Table 2). From Taylor Valley we sequenced eight individuals from three populations, one individual (TV1-1) had the 'A' haplotype found in other nearby continental populations, the other seven (three from TV1, three from TV2 and one from TV3) were found to have two unique haplotypes (M, N) that differed from each other by a single nucleotide (Table 2). Shared haplotypes were identified in only three instances: (1) two Cape Royds individuals (R2-4,5) shared the 'K' haplotype with all three Cape Evans individuals (R3-1,2,3); (2) a Taylor Valley individual (TV1-1) was identified with the Miers

Valley and Lake Penny haplotype (A), and these sequences differed by a single nucleotide to Marble Point; and (3) the only instance of haplotype sharing between Ross Island and the continent was between Cape Bird (R1) and Granite Harbour (haplotypes F, J) (Table 2).

The parsimony analysis found 12 equally parsimonious trees (tree length = 181, C.I. = 0.95, R.I. = 0.95). The 50% majority rule consensus was found to have identical topology to the NJ analysis, which is shown in Figure 3 (tree topologies were similar using several methods). Both strong phylogenetic signal (g_1 = -3.75, g_{crit} = -0.09, $P < 0.01$) and bootstrap values greater than 55% support the parsimony analysis and the tree topologies in Figure 3. The outgroup taxa were found to be very distinct (19-23% sequence divergence) from *G. hodgsoni*. The *G. hodgsoni* clades show mostly fragmented allopatric distributions, and the phylogenetic analysis revealed clear breaks among the sequences from Taylor Valley, Beaufort Island, Ross Island and Granite Harbour (Fig. 3). Taylor Valley was the most divergent with genetic distance (Kimura 2-parameter model) ranging from 0.014 to 0.02, and formed a well supported (100% bootstrap) basal clade to all other *G. hodgsoni* individuals in the parsimony analysis (Fig. 3). Beaufort Island individuals also show high levels of divergence, with genetic distance ranging from 0.01 to 0.015, and formed a monophyletic clade and sister group to a second, larger clade containing all Ross Island and the remaining continental sites. This second clade was further subdivided into an unresolved southern continental clade with two minor phylogeographic groups: (1) MP; and (2) GV, MV, LP and a single individual from TV1 forming an unresolved polytomy (Fig. 3). The southern continental clade formed a sister group to a poorly resolved (bootstrap support <50%) clade containing all Ross Island sites and Granite Harbour, which

could be subdivided into three subclades: (1) R2 and R3; (2) R1, R2, R4, and a single GH6 individual (unresolved polytomy); and (3) GH1 and three R1 individuals (Fig. 3).

TABLE 2. Polymorphic nucleotide sites among *Gomphiocephalus hodgsoni* haplotypes. The common continental haplotype (A) is used as a reference sequence. Locations where each haplotype was found is indicated using location codes from Figure 3, and haplotypes are given a single letter code (i.e. A, B, etc.). Identical character states are indicated by dots.

		1	1	1	3	8	1	2	2	2	2	2	2	2	3	3	4	4	4	4	4	5	5
		1	1	1	3	8	1	0	1	4	5	7	7	9	2	9	0	2	5	5	7	2	7
	LOCATION	2	5	8	4	3	1	4	0	3	5	0	9	6	1	9	8	9	0	9	7	2	6
A	LP-2,3;MV-1,2,3,4; TV1-2	G	T	T	A	C	G	T	C	G	C	A	G	G	T	C	G	G	G	C	C	A	G
B	LP-1					.	T	.															
C	GV								.	T	.												
D	MP-1,3						.	C	.														
E	GH1-1									.	T	G	.	.	C	.						.	T
F	GH1-2,3,4,6;R1-5,6									.	T	.		.	C	.						.	C
G	R1-4									.	T	.		.	C	.							
H	R1-1	.	C	.										.	C	.							
I	R1-3											.	A	.	C	.							
J	R1-2;R2-1,2,3;R4-1,2,3;GH6-5													.	C	.							
K	R2-4,5;R3-1,2,3							.	T	.				.	C	.	A	.					
L	BI-1,2,3	A	.	C	T												.	A	.	T	.	.	T
M	TV1-2;TV2-1,3							.	T			.	A	.	T	.	A	A	.	T	G	C	
N	TV1-3,4;TV2-2;TV3	.	.	.	.	T	.	.	T	.	.	.	.	A	.	T	.	A	A	.	T	G	C

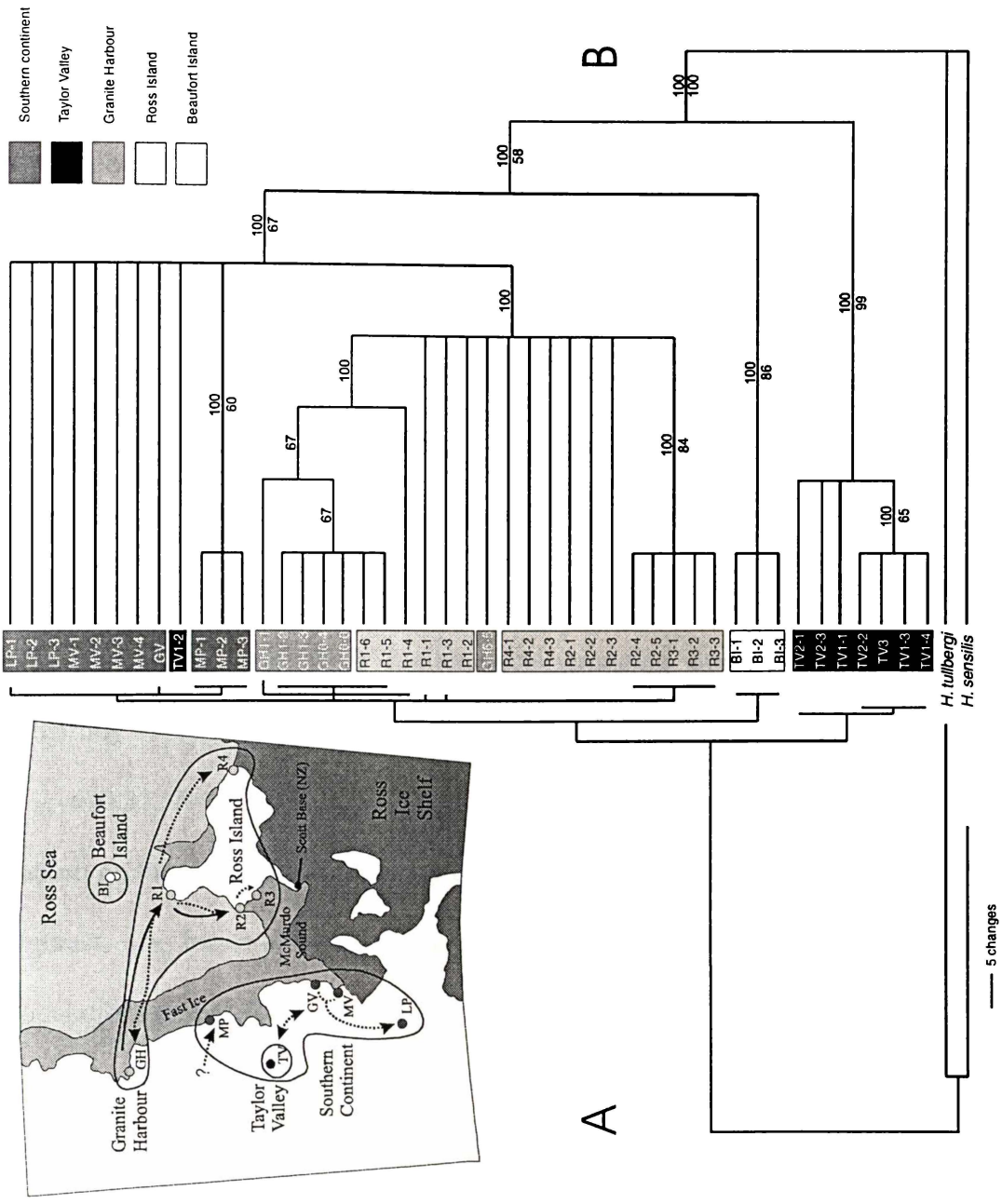

FIGURE 3. MtDNA (COI) analyses based on sequence variation using 599 aligned sites for 45 *Gomphiocephalus hodgsoni* individuals. *Hypogastrura tullbergi* and *H. sensilis* were included as outgroup taxa. Analyses included individuals from 4 Ross Island populations, from Beaufort Island and from 6 continental populations (see map inset) (shaded bars correspond to those used in Fig. 2, and for individual codes see methods and Table 1). All individuals were used in: A) NJ phylogram; and B) Majority rule consensus of 12 equally parsimonious trees (tree length = 181, C.I. = 0.95, R.I. = 0.95) (50% majority rule appear above branches, bootstrap confidence limits (1000 replicates) greater than 55% below branches).

Based on both allozyme and mtDNA data, *Gomphiocephalus hodgsoni* populations were characterised by high levels of genetic diversity throughout the continental sites but lower levels for populations on Ross Island (Figs. 2, 3). The relationship between geographic and genetic distance found on the continent, and moderate levels of differentiation (F_{ST} = 0.23), suggests that populations have been diverging along a cline conducive to allopatric or parapatric isolation (Templeton 1980). Fanciulli *et al.* (2001) found a similar pattern with high levels of differentiation and allele combinations grouping mostly with geographic proximity for the northern Victoria Land collembolan *Gressittacantha terranova* (F_{ST} = 0.31; R = 0.5, $P < 0.001$). For both *G. hodgsoni* and *G. terranova* the pattern on the continent is indicative of a stepping-stone model (Slatkin 1993). By contrast, we found a near homogenous genetic structure (F_{ST} = 0.06) for populations across Ross Island and Beaufort Island based on the allozyme data (Fig. 2). For these populations there was no pattern of isolation-by-distance. Such a lack of geographic differentiation resembles Wright's Island model (Wright 1954) with populations that have not had sufficient time to reach equilibrium between gene flow and genetic drift (*sensu* Slatkin 1993).

The Ross Island populations also have lower allelic variability, but high genetic similarity (in contrast to the continental populations), which may indicate that the consequences of bottleneck and/or founder effects have been more pronounced for the island populations (Templeton 1980). For example, if genetic drift was the major force modifying the structure of these geographically isolated populations, then we should have detected comparable levels of genetic differentiation among

populations on both the continent and among the islands. Furthermore, genetic drift should have led to the fixation of alleles and therefore a reduction of allelic diversity (Futuyma 1998), which we observed for Ross Island and Beaufort Island populations (Table 1). This may imply that many Ross Island populations have been colonised since the last glacial maximum (LGM) by few individuals, or that successive bottleneck events are now reflected by lower heterozygosity, polymorphism and strong genetic similarity among the island populations. Alternatively, a lack of population differentiation on these island populations may be due to asexual reproduction, which has been identified in three collembolan species from Europe (Simonsen & Christensen 2001). However, both male and female *G. hodgsoni* were found from these populations in the present study. It is likely that repeated fragmentation of a once widespread species, with a high rate of localised population extinctions, have resulted in regions of homogenous genetic structure with range expansion from few source populations since the LGM. The genetic divergence between the island populations and the continent for *G. hodgsoni* could be as recent as the last glaciation if we assume recent founder and/or bottleneck effects for the island populations (Nei 1987).

The mtDNA (COI) analyses revealed only 22 positions with nucleotide variability in one or more sequences for *G. hodgsoni.* Sequence divergence among different haplotypes ranged from 0.2 % to 2.0 %, and is comparable to rates reported for other arthropods with a Pleistocene coalescence. Using a molecular clock rate of 2-2.3% sequence divergence per million years (Brower 1994; Folmer *et al.* 1994; Roslin 2001), suggests that all groups in the present study diverged within the last one million years. Isolation among populations is reflected in the estimates of sequence divergence among *G. hodgsoni* individuals and the

heterogeneous population structure. The sequence divergence found for the Taylor Valley (1.7%) and Beaufort Island clades (1.3%) was remarkably high considering that less than 0.6% separated all other clades. Such patterns of isolation among fragmented habitats may be a common feature for Antarctic terrestrial arthropods. For example, considerable haplotype diversity was identified for the northern Victoria Land collembolan *Isotoma klovstadi*, with 18 different haplotypes from 40 individuals, ranging from three to seven haplotypes among each of four continental populations surveyed with only a single mtDNA (COII) haplotype found in more than one population (Frati *et al.* 2001). Genetic structure has also been examined for the nematode *Scottnema lindsayae* from southern Victoria Land but the phylogenetic pattern showed no geographic grouping of haplotypes (Courtright *et al.* 2000). For taxa that have a anhydrophobic life-stage (e.g. nematodes, tardigrades, and rotifers) dispersal via wind and water may act as a homogenising force over larger distances. Interestingly, for the mosses Skotnicki *et al.* (2000) also found no mixing of *Sarconeurum glaciale* isolates (using RAPDs) between Taylor Valley and Ross Island. Furthermore, in Taylor Valley two distinct isolates of *Bryum pseudotriquetrum* were identified (Skotnicki *et al.* 2000). The pattern found for the mosses have striking similarities to *G. hodgsoni* in the present study and may reflect historical patterns of isolation and re-colonisation for these terrestrial flora and fauna throughout southern Victoria Land.

The identification of two sympatric *G. hodgsoni* haplotypes (with a genetic distance of 0.015) in Taylor Valley is congruent with the allozyme analyses and suggests the presence of two distinct species (Figs. 2, 3). Such patterns are likely due to isolation followed by more recent range expansion, although we are unable

to rule out sympatric speciation. This unique haplotype may have been sourced from a single refugium surviving throughout the glacial cycles and is now found throughout the valley. Interestingly, the allozyme analysis was congruent with the mtDNA that showed Beaufort Island as being genetically distinct with a unique haplotype array that had a mean genetic distance of 0.014 from populations on the continent (Figs. 2, 3). However, in contrast to the allozyme data Beaufort Island also had a mean genetic distance of 0.014 from populations on Ross Island, populations which were found to have a closer affinity to Granite Harbour on the continent (Fig. 3). This might reflect greater impact of founder events and genetic drift on maternally inherited haploid mtDNA (i.e. smaller effective population size and/or founding by maternal individuals), lower detectable mutation rate of allozyme loci (and/or selection on specific nuclear alleles), and differential dispersal (and/or loss due to localised population extinction) of mtDNA and nuclear alleles. The maternal mode of transmission of mtDNA may also counteract a reduction of variability (Frati *et al.* 2001), where haplotype diversity may reflect long-term habitat shift due to climatic and/or geologic perturbation.

Common nucleotides between the sequences (Table 2) may suggest colonisation routes where populations survived in isolated refugia and have been the source for colonisation since the LGM (see map inset, Fig. 3). The similarity among populations from Marble Point south to Lake Penny on the continent, and between Granite Harbour and Ross Island, suggest that multiple refuges were likely during the last glaciation. For example, on Ross Island one of the five haplotypes from Cape Bird (J) was the only haplotype identified from Cape Crozier and one of two haplotypes found at Cape Royds, suggesting radiation from Cape Bird since the LGM. A single haplotype (K) identified from Cape

Evans (R3) was also found in two individuals from Cape Royds (R2), although these locations are only 12 km apart and may indicate colonisation from Cape Royds since the LGM (see Table 2, Fig. 3). Colonisation routes, with Cape Bird as a main source population on Ross Island prior to, and since the LGM (Fig. 3), is supported by a decrease in both allelic and haplotype diversity from Cape Bird through to Cape Crozier and Cape Evans (Tables 1, 2). On the continent we also identified the haplotype common to Miers Valley and Lake Penny in one individual from Taylor Valley—these populations are separated by 50-70 km. However, we also found haplotype sharing between Cape Bird and Granite Harbour, populations separated by around 100 km across McMurdo Sound (Fig. 3). The similarity of Granite Harbour to Ross Island in the phylogenetic analyses (Fig. 3), and with all Granite Harbour populations forming a distinct cluster in the phenetic analysis (Fig. 2), suggest one possible colonisation source for Ross Island prior to the beginning of the last glaciation, some 130 thousand years ago (Hays *et al.* 1976). Individuals used for mtDNA analyses from Granite Harbour (GH1-1,2,3,4,6) were identified with a distinctive nucleotide substitution (T) at position 255 (Table 2). This distinct nucleotide was identified in two sequences from Cape Bird (R1-5,6), and we also identified a haplotype identical to those found on Ross Island (J) at Granite Harbour (GH6-5) (Table 2). Such haplotype mixing may suggest very recent translocation.

One possible explanation for the disjunct mixing of identical haplotypes is passive dispersal (e.g. wind, ocean or animal vectors). Passive dispersal has been claimed for the introduction of collembolan species in Eastern Antarctica (Greenslade & Wise 1984). However, passive transport over large distances by ocean or winds is unlikely for Collembola, which lack an effective way to avoid

desiccation during dispersal in Antarctica (Gressitt *et al.* 1960). Alternatively, dispersal via birds (e.g. the skua *Catharacta maccormicki*) may provide a possible means of translocation and moulting Adéle penguins (*Pygoscelis adeliae*) from Ross Island have been observed at Granite Harbour prior to migrating north for the winter (T.G.A. Green pers. comm.). Similar translocation has been suggested as an explanation for the disjunct occurrence of a population of *G. terranova* in northern Victoria Land (Fanciulli *et al.* 2001). In addition, Strong (1967) observed individuals of *Alaskozetes* (Acari) on the feathers of a freshly killed skua, and likewise Tilbrook (1967) collected several species from both bird colonies and nesting areas. However, human-mediated transport of terrestrial invertebrates among sites is also possible.

In addition to recent dispersal, populations such as Cape Bird (R1), Beaufort I. (BI), Granite Harbour (GH), and Taylor Valley (TV) were likely refugia during the Pleistocene glaciations. Volcanic activity on Ross Island has been estimated between 0.68-1.3 MY, with rocks from Cape Bird dated between 3.0-3.7 MY, which is also possibly the least affected coastal region on Ross Island during the LGM (Treves 1968; Armstrong 1978). By contrast, geomorphic evidence indicates that rates of landscape modification in the McMurdo Dry Valleys have been extremely low over the past few million years (Denton *et al.* 1993; Wilch *et al.* 1993; Marchant & Denton 1996), and throughout southern Victoria Land dating of volcanic rocks indicates a much older origin (ca. 18 MY) (Armstrong 1978). The entire area, including the nearby islands, were subjected to many small localised eruptions occurring intermittently over at least 4.6 MY (Treves 1968; Armstrong 1978). Models supported by ice-cores also reveal that the domes on the ice sheet were not higher, but in fact lower during glacials than during

interglacials because of lower precipitation (Barrett 1996). Thus it is unlikely that mass extinctions over a large geographic region would occur.

We suggest that *G. hodgsoni* is the remnant of a once widespread taxon, which is supported by the branching patterns within the phenetic and phylogenetic reconstructions, suggesting that all extant populations may have originated during two or three geographical isolating events within the last one million years (Figs. 2, 3). It is likely that the intraspecific divergence observed for *G. hodgsoni* originated during the Pleistocene and we conclude that divergence was not inhibited by the frequent shifts in species distributions associated with the glacial-interglacial cycles. Comparative phylogeography using other endemic Antarctic terrestrial taxa, particularly nematodes, tardigrades and free-living mites from southern Victoria Land will enable further examination of colonisation routes, range expansion and long-term persistence in this extremely fragmented habitat.

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APPENDIX 1. Allelic frequencies for 8 polymorphic loci from 18 *Gomphiocephalus hodgsoni* populations. *n* = total number of individuals used for the allozyme analyses in each population. For population codes refer to Table 1.

		Ross Island				Granite Harbour							Southern continent					
	BI	R1	R3	R2	R4	GH1	GH2	GH3	GH4	GH5	GH6	GH7	MP	TV1	TV2	TV3	GV	MV
(*n*)	37	63	29	44	41	38	40	37	53	33	29	25	24	25	16	34	10	40
AO																		
A														0.12	0.19	0.82		
B						1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	0.81	0.18	1.00	1.00
C	1.00	1.00	1.00	1.00	1.00													
IDH																		
A	0.03	0.08			0.03									0.02				0.03
B	0.97	0.89	0.80	0.85	0.97	0.05	0.09	0.10	0.09	0.38	0.02			0.04				0.15
C		0.03	0.20	0.15		0.95	0.90	0.90	0.91	0.62	0.87	0.76	1.00	0.94	1.00	1.00	1.00	0.82
D							0.01				0.11	0.24						
ARK																		
A						0.01			0.02					0.02				
B	0.99	0.84	1.00	0.99	0.77	0.24	0.04	0.10	0.39	0.12	0.19	0.44	0.56	0.56	0.81	0.69	1.00	0.99
C		0.16			0.23	0.57	0.56	0.61	0.47	0.88	0.81	0.54	0.41	0.40	0.19	0.31		
D						0.01	0.01		0.03			0.02	0.03	0.02				
E							0.01	0.03	0.02									
F						0.10	0.18	0.16	0.03									
G				0.01		0.06	0.13	0.05	0.02									0.01
H	0.01					0.03	0.07	0.05	0.02									
MPI																		
A	1.00	0.95	1.00	1.00	0.96	1.00	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
B		0.05			0.04			0.03										
PGM																		
A												0.02		0.02				
B	0.90	0.97	1.00	0.85	1.00		0.01			0.09	0.02	0.08						0.13
C	0.05	0.02		0.04		0.60	0.45	0.69	0.78	0.59	0.62	0.60	0.02	0.23	0.41	0.24	0.20	0.49
D						0.18	0.15	0.08	0.06	0.02	0.04	0.10	0.19	0.32	0.38	0.03	0.75	0.26
E	0.05	0.01		0.02		0.11	0.20	0.04	0.04	0.16	0.16	0.06	0.35	0.09	0.09	0.70	0.05	0.07
F				0.09		0.11	0.19	0.19	0.12	0.14	0.16	0.14	0.44	0.34	0.12	0.03		0.05
GPI																		
A								0.01		0.02		0.02	0.02	0.06	0.66			
B	0.76	0.81	0.98	0.92	0.90	1.00	1.00	0.99	1.00	0.98	1.00	0.98	0.98	0.94	0.34	1.00	1.00	1.00
C	0.24	0.19	0.02	0.08	0.10													
AK																		
A		0.02			0.06	0.02	0.02			0.02	0.03	0.04						
B	0.94	0.98	1.00	0.97	0.94	0.69	0.48	0.87	0.67	0.91	0.95	0.94	0.88	0.87	0.96	1.00	1.00	0.85
C	0.03					0.06	0.05		0.11	0.02			0.06	0.10	0.04			0.06
D	0.03					0.13	0.07	0.02	0.11	0.05	0.02		0.02					0.09
E				0.03		0.10	0.38	0.11	0.11			0.02	0.04	0.03				
PK																		
A		0.01				0.08	0.04	0.08	0.02									
B	1.00	0.99	1.00	1.00	1.00	0.85	0.96	0.92	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
C						0.07												

APPENDIX 2. Genetic distance (using the Kimura 2-parameter model) based on sequence variation in the mtDNA COI sequences (599 aligned sites) among the 14 identified *Gomphiocephalus hodgsoni* haplotypes and the two outgroup taxa (*Hypogastrura tullbergi* and *H. sensilis*). The haplotype codes refer to those used in Table 2.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	*H. tull*
B	0.002														
C	0.002	0.003													
D	0.002	0.003	0.003												
E	0.007	0.008	0.008	0.008											
F	0.005	0.007	0.007	0.007	0.003										
G	0.003	0.005	0.005	0.005	0.003	0.002									
H	0.003	0.005	0.005	0.005	0.007	0.005	0.003								
I	0.003	0.005	0.005	0.005	0.007	0.005	0.003	0.003							
J	0.002	0.003	0.003	0.003	0.005	0.003	0.002	0.002	0.002						
K	0.005	0.007	0.007	0.007	0.008	0.007	0.005	0.005	0.005	0.003					
L	0.010	0.012	0.012	0.012	0.014	0.014	0.013	0.013	0.013	0.012	0.015				
M	0.014	0.015	0.015	0.015	0.019	0.015	0.017	0.017	0.017	0.015	0.015	0.019			
N	0.015	0.017	0.017	0.017	0.020	0.017	0.019	0.019	0.019	0.017	0.017	0.020	0.002		
H. tull	0.228	0.228	0.231	0.228	0.228	0.228	0.228	0.231	0.231	0.228	0.228	0.226	0.226	0.228	
H. sen	0.188	0.188	0.186	0.186	0.186	0.188	0.188	0.191	0.191	0.188	0.193	0.186	0.191	0.193	0.169

CHAPTER III

THE COROPHIID AMPHIPODS OF TAURANGA HARBOUR, NEW ZEALAND: EVIDENCE OF AN AUSTRALIAN CRUSTACEAN INVADER[†]

Key-words: Amphipoda, *Paracorophium*, allozymes, ballast water, shipping; species introductions

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Using morphological and molecular techniques we examined the corophiid amphipods in the Waimapu Estuary, Tauranga Harbour in the North Island of New Zealand. Based on morphology we identified two New Zealand endemic species, *Paracorophium lucasi* and *P. excavatum*, as well as *P. brisbanensis*, previously recorded only from the eastern coast of Australia and *Corophium* sp. which has not been previously recorded from New Zealand. Allozyme analyses confirmed the morphological diagnoses of three distinct species within *Paracorophium* and of the single *Corophium* species. The presence of reproductive females and juvenile *P. brisbanensis* suggests a viable, breeding population in Tauranga Harbour. We conclude that the species is likely to have been introduced to New Zealand waters via shipping activities (e.g., ballast water). The possibility that *P. brisbanensis* may now spread to other New Zealand ports as well as the consequences of this introduction for other New Zealand taxa need to be urgently examined.

Shipping activities have led to many species translocations (e.g., Den Hartog et al., 1992; Pinkster et al., 1992; Mills et al., 1993), particularly for taxa that are readily picked up in ballast water (Hebert et al., 1989; Carlton & Geller, 1993; Mills et al., 1993a). The incidence of non-indigenous amphipods has been increasing world-wide since the early 1900s and for those taxa exhibiting euryhaline habits, ballast water has provided an effective means at dispersal (Crawford, 1937; Hurley, 1954; Carlton & Geller, 1993; Mills et al., 1993a).

Of particular concern are the effects that non-indigenous species have on native taxa and resulting changes in energy flow within ecosystems. For example the introduction of two exotic *Gammarus* species in Northern Ireland appeared to have negative effects on the native fauna due to direct predation and species displacement (Costello, 1992; Dick, 1996). The colonisation of the Netherlands by the exotic *Gammarus tigrinus*, and more recently by *Crangonyx pseudigracilis* and *Corophium curvispinum*, has been rapid and coincided with a decrease in the population densities of the resident *Gammarus* species (Den Hartog et al., 1992; Pinkster et al., 1992). In the most extreme case of species displacement, Pinkster et al. (1992) reported the complete disappearance of *Echinogammarus berilloni* from Dutch inland waters since the establishment of these exotic species. However, it is not always clear whether such introductions are causal or coincidental with other environmental perturbations.

Molecular techniques have also made it possible to detect introductions and their source of origin. Using a combined molecular and morphological approach, Witt et al. (1997) recorded the first known amphipod introduction into the lower

Great Lakes of North America. Pinkster et al. (1992) used molecular techniques to identify a second introduction of *G. tigrinus* into the Netherlands and identified a population in Germany as the source of this introduction. The use of molecular techniques may be particularly useful in identifying species that lack morphological distinctiveness (i.e., species that are morphologically cryptic) and hence may be overlooked as exotic species because of limited taxonomic knowledge.

Here, using a combined morphological and molecular approach, we report on the corophiid amphipods of Tauranga Harbour, North Island, New Zealand. Tauranga Harbour was chosen for study because it is one of the largest shipping harbours in New Zealand, processing over 1,100 vessels per year. Accordingly, we considered the port to be a likely location for potential introductions facilitated by shipping activities. As we show, one taxon is likely to be a recent Australian introduction.

MATERIALS AND METHODS

Study site and sampling of amphipods

The Waimapu Estuary is a semi-exposed intertidal sandflat situated approximately 8 km south of the Port of Tauranga (Figure 1). The study site was situated at the mouth of the main riverine channel into the estuary (37°40'S, 176°10'E) (Figure 1). Sampling was carried out over a 24 hour period at monthly intervals from October 1999 to October 2000. We used bed-load and suspended-load traps to collect amphipods. Bed-load traps (3 cm diameter, 60 cm depth) were pushed into

the soft sediment so that the top of the trap lay flush with the sediment surface. Suspended-load traps consisting of a plastic container (10 cm x 15 cm) with small openings (2 cm diameter) at the top were suspended 50 cm above the substrate by a pole. Such sampling is useful in examining active and passive dispersal of biota (Emerson & Grant, 1991; Commito et al., 1995). As part of an ongoing study to examine the dispersal of invertebrates within the estuary, twenty traps of each type were positioned across the estuary to obtain a representative sample. Traps were initially set at the end of an ebb tide and sampling proceeded over two tidal cycles (24 hours). The first sample was obtained at the end of the flood tide (after 6 hours) and the second sample was taken at the end of the following ebb tide (after 12 hours). This procedure was repeated for the following tidal cycle. For the purposes of this study, all samples were pooled for each month to estimate relative abundances of each taxon.

Morphological analyses

We identified four corophiid amphipod species from Tauranga Harbour: *Paracorophium lucasi* (Hurley, 1954) Chapman, 2002, *Paracorophium excavatum* (Thomson, 1884) Stebbing, 1899, *Paracorophium brisbanensis* Chapman, 2002, and an unidentified species of *Corophium*. The latter did not correspond to any of three *Corophium* species reported from New Zealand by Hurley (1954). To identify mature *Paracorophium* we used the diagnostic characters suggested by Chapman (2002) and Chapman et al. (2002). However, juvenile *Paracorophium* could not be identified using morphological characters and instead were identified

using molecular analyses (below). All individuals for allozyme analyses were stored at -75°C.

Allozyme analyses

Individual specimens were homogenised in 14 µl of distilled water and analysed using cellulose acetate electrophoresis (Richardson et al., 1986; Hebert & Beaton, 1993). An initial screening of 24 enzymes revealed the following ten enzyme systems that had sufficient activity, resolution and could be reliably scored: aldehyde oxidase (AO: EC 1.2.3.1); peptidase (PEP: EC 3.4.11/13); isocitrate dehydrogenase (IDH: EC 1.1.1.42); lactate dehydrogenase (LDH: EC 1.1.1.27); glyceraldehyde-3-phosphate dehydrogenase (G3PDH: EC 1.2.1.12); 6-phosphogluconate dehydrogenase (6PGDH: EC 1.1.1.44); phosphoglucomutase (PGM: EC 5.4.2.2); arginine kinase (ARK: EC 2.7.3.3); mannose-6-phosphate isomerase (MPI: EC 5.3.1.8); malate dehydrogenase $NADP^+$ (ME: EC 1.1.1.40). Four enzymes (AO, PEP, IDH and LDH) were coded by two loci each for a total of 14 loci and were designated numerically in order of increasing electrophoretic mobility (e.g., AO-1, AO-2). Alleles were designated by the relative difference in anodal mobility of respective gene products, i.e. the 'fastest' allele was designated "A", the next fastest allele "B", and so on. There were 1-6 detectable alleles for all loci examined. Two individuals from previous runs were re-run to control for any variation in mobility between gel plates from separate runs.

BIOSYS-1 (Swofford & Selander, 1981) was employed to compute statistics for each taxon including: 1) allelic frequencies; 2) agreement of genotypes with Hardy-Weinberg equilibrium (Fisher's exact test); 3) Nei (1978) unbiased genetic

identity; and, 4) H_{obs}/H_{exp}, the proportion of heterozygotes observed/expected (based on Hardy-Weinberg equilibrium).

In addition, using a diagnostic locus (AO-1) we assessed a sub-sample of juvenile individuals to determine the proportion of each species present in samples from July and October 2000.

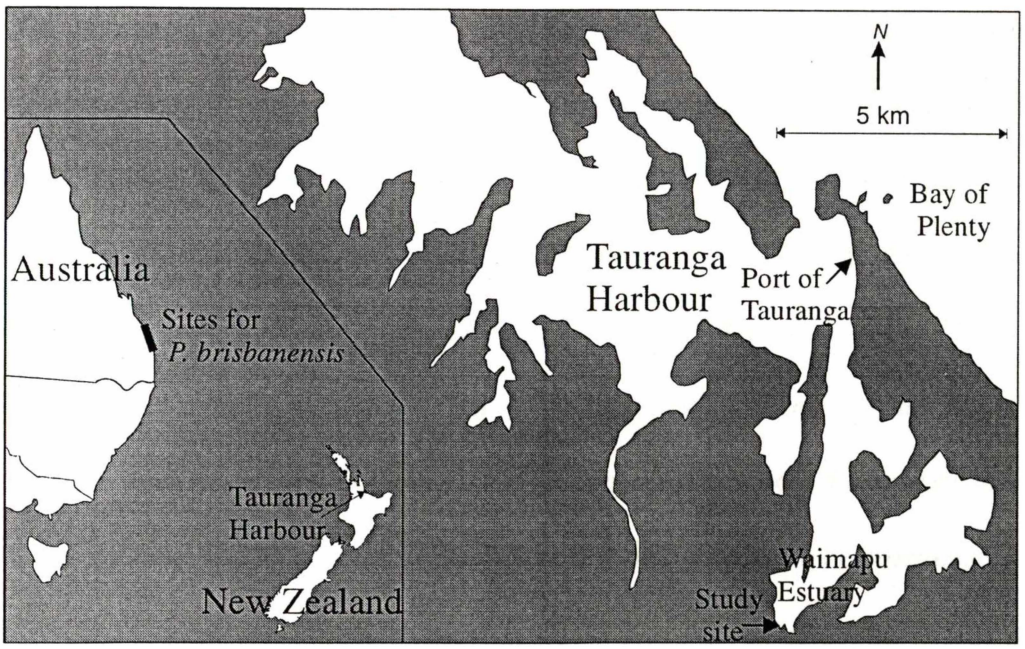

FIGURE 1. Map of the study site location at Waimapu Estuary, Tauranga Harbour, New Zealand (37°40'S, 176°10'E). Inset: shows the known locations for *P. brisbanensis* on the eastern coast of Australia from Hervey Bay (25°17'S, 152°49'E) to Moreton Bay (27°24'S, 153°07'E) (P. Davie, pers. comm.).

The corophiid amphipods *Paracorophium lucasi*, *P. excavatum* and *P. brisbanensis* were present in all monthly samples, and *Corophium* sp. was present in all except October 1999 and September 2000 samples (Figure 2). Combining all monthly samples, juveniles were most abundant (76.9%) with adults representing only 23.1% of the total abundance.

The allozyme analyses confirmed morphological designations in every case. For *P. lucasi* and *P. excavatum* there were 8 and 6 loci, respectively, polymorphic with 2-3 alleles. By contrast, *Corophium* sp. was fixed for all loci examined and *P. brisbanensis* was fixed for all loci except the MPI locus. Allele frequencies for all loci are shown in Table 1. Significant ($P < 0.05$) departures from Hardy-Weinberg expectations were found for some loci among *P. excavatum* and *P. lucasi* and were associated with heterozygote deficiencies. Both *P. brisbanensis* and *Corophium* sp. had much lower observed and expected heterozygosities relative to *P. lucasi* and *P. excavatum* (Table 2). The percentage of polymorphic (variable) loci for *P. lucasi* was 57.1% and *P. excavatum* was 42.9%. This contrasts to *P. brisbanensis* with 7.1% and *Corophium* sp. with no polymorphic loci. The mean number of alleles per locus was 1.6 for *P. lucasi* and *P. excavatum*, 1.1 for *P. brisbanensis* and 1.0 for *Corophium* sp. (Table 2).

Hierarchical cluster analysis (using Nei (1978) unbiased identity) was performed using the unweighted pair-group method with arithmetic averaging (UPGMA) (Sneath & Sokal, 1973). Two major groupings are distinguished in Figure 3. The first was morphologically confirmed as *Corophium* sp., and is basal to the *Paracorophium* clade. The second grouping contains the three

Paracorophium species and shows that *P. excavatum* is basal to both *P. lucasi* and *P. brisbanensis*, which form a sister group.

Juveniles represented 85% of the total individuals trapped during July 2000 and 79% during October 2000 (Figure 2). Allozyme analyses of these individuals identified 45 *P. brisbanensis*, four *P. excavatum* and no *P. lucasi* or *Corophium* sp. in July. In the October sample we found 24 *P. brisbanensis*, four *P. excavatum*, four *P. lucasi* and no *Corophium* sp.

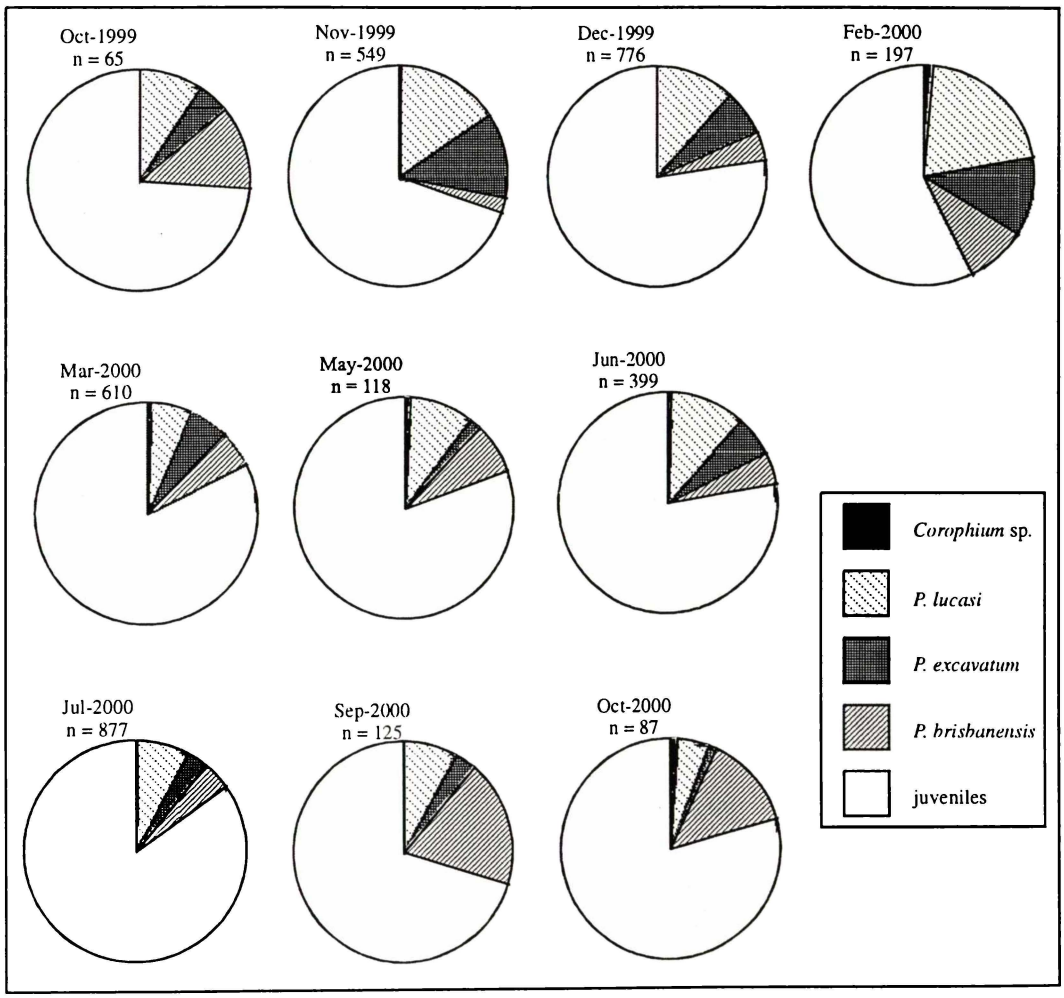

FIGURE 2. The faunal assemblage as a percentage of the total number of individuals (n = 3,803) collected in monthly samples for *Paracorophium lucasi, P. excavatum, P. brisbanensis, Corophium* sp., and unidentified juveniles.

TABLE 1. Allele frequencies at 14 polymorphic loci for *Paracorophium lucasi, P. excavatum, P. brisbanensis* and *Corophium* sp., collected from the Waimapu Estuary, Tauranga Harbour, New Zealand.

Locus	allele	*P. lucasi*	*P. excavatum*	*P. brisbanensis*	*Corophium* sp.
	n	26	32	38	8
AO-1	A				1.00
	B	1.00			
	C		1.00		
	D			1.00	
AO-2	A	1.00			
	B		1.00		
	C			1.00	
	D				1.00
PEP-1	A			1.00	
	B	1.00			
	C		1.00		
	D				1.00
PEP-2	A				1.00
	B	0.04	0.02		
	C		0.98		
	D	0.96			
	E			1.00	
IDH-1	A	0.02	1.00		1.00
	B	0.98			
	C			1.00	
IDH-2	A		0.03		
	B	1.00	0.95		
	C		0.02	1.00	
	D				1.00
LDH-1	A		1.00		
	B	0.96		1.00	1.00
	C	0.04			
LDH-2	A	1.00	1.00		1.00
	B			1.00	
G3PDH	A				1.00
	B			1.00	
	C	1.00	1.00		
6PGDH	A				1.00
	B	0.87			
	C		1.00		
	D	0.13		1.00	
ARK	A		0.05		
	B	0.12	0.75		1.00
	C	0.88	0.20	1.00	
MPI	A				1.00
	B	0.05		0.11	
	C	0.86		0.89	
	D	0.09	0.98		
	E		0.02		

continued,

TABLE 1 *continued,*

Locus	allele	*P. lucasi*	*P. excavatum*	*P. brisbanensis*	*Corophium* sp.
PGM	A				1.00
	B	0.52			
	C	0.48	0.12		
	D		0.02		
	E		0.86		
	F			1.00	
ME	A	0.98			
	B	0.02			1.00
	C			1.00	
	D		0.89		
	E		0.11		

TABLE 2. Measures of genetic variability for *Paracorophium lucasi, P. excavatum, P. brisbanensis* and the *Corophium* sp. and collected from the Waimapu Estuary, Tauranga Harbour, New Zealand. Standard errors in parentheses.

Species	Mean sample size per locus	Mean No. of alleles	% polymorphic loci	Observed heterozygosity (direct count)	Expected heterozygosity
P. lucasi	25.9 (0.5)	1.6 (0.2)	57.1	0.08 (0.04)	0.11 (0.04)
P. excavatum	29.3 (1.0)	1.6 (0.2)	42.9	0.05 (0.02)	0.07 (0.03)
P. brisbanensis	31.8 (2.4)	1.1 (0.1)	7.1	0.01 (0.01)	0.01 (0.01)
Corophium sp.	7.4 (0.3)	1.0 (0.0)	0.0	0.00 (0.00)	0.00 (0.00)

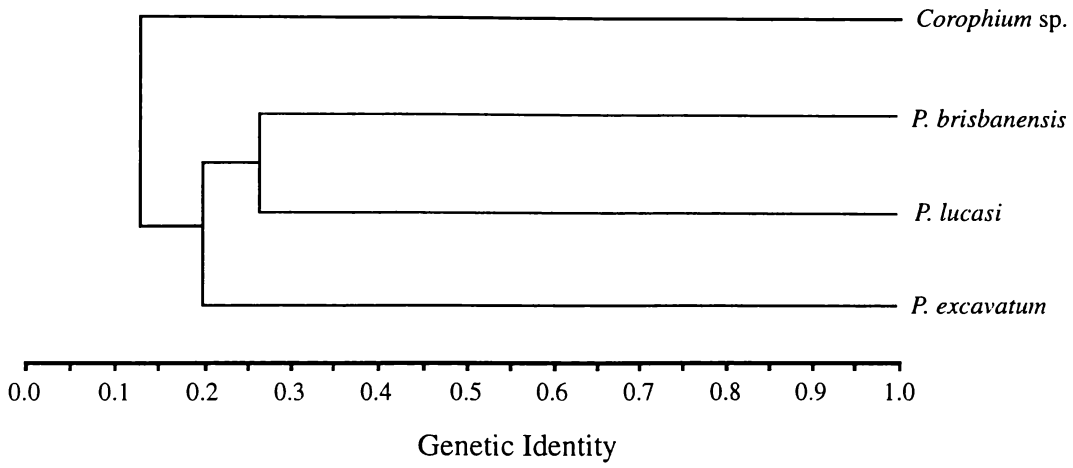

FIGURE 3. Dendrogram of Nei's (1978) unbiased genetic identity for individuals of *Paracorophium lucasi*, *P. excavatum*, *P. brisbanensis* and *Corophium* sp. collected from the Waimapu Estuary, Tauranga Harbour, New Zealand. All individuals were morphologically identified prior to allozyme analysis.

The ability to accurately identify species, particularly the presence of invasive non-indigenous species, is essential in efforts to properly monitor natural systems. Here, using a combined morphological and molecular approach, we provide the first record of *Paracorophium brisbanensis* in New Zealand, previously only recorded from the south-east coast of Australia (Chapman, 2002) from Hervey Bay (25.17°S, 153.03°E) to Serpentine Creek (27.24°S, 153.05°E) (P. Davies, unpubl. data). By contrast, the two New Zealand endemics, *P. lucasi* and *P. excavatum*, are widely distributed throughout New Zealand (Schnabel et al., 2000; Chapman et al., 2002; M. Stevens & I. Hogg, unpubl. data). *Corophium* sp. was not consistent with any previously described species from New Zealand (Hurley, 1954) and is currently undergoing clarification (M. A. Chapman unpubl. data).

The allozyme analyses confirmed morphological identities of the three *Paracorophium* species and supported the specific status recently given to *P. brisbanensis* by Chapman (2002) (Figure 3). Further, these analyses illustrate the utility of these techniques for identifying morphologically similar species, particularly in the juvenile stages when species are often difficult to distinguish. The presence of adult *P. brisbanensis* females with broods indicates that this is a breeding population in the Waimapu Estuary. This was also revealed by allozyme analyses of juvenile individuals (using the AO-1 locus) showing that *P. brisbanensis* made up 92% of animals analysed from July and 75% from October 2000 samples.

One possible explanation for the presence of *P. brisbanensis* in Tauranga Harbour is that it represents a previously unrecorded New Zealand taxon.

However, we suggest three lines of evidence to indicate that *P. brisbanensis* is likely to be an introduction to New Zealand waters. Firstly, in contrast to the known New Zealand *Paracorophium* species and other endemic amphipod taxa (e.g., *Paracalliope fluviatilis*, I. Hogg; K. Schnabel & M. A. Chapman, unpubl. data), *P. brisbanensis* has remarkably limited genetic variability (although there is no genetic comparison of this species from its native distribution) (Table 2). However, this feature is common in exotic species as a result of genetic bottlenecks, resulting from the founding of populations by relatively few individuals (e.g., Smith et al., 1979; Nei, 1987; Spidle et al., 1994; Stepien et al., 1998). *Corophium* sp. also lacked genetic variability in the present study (Table 2), but the limited number of specimens collected suggests that it was sampled outside its habitat range and/or that this species is also a recent introduction. Secondly, in a comprehensive survey of 93 coastal sites throughout New Zealand, 48 contained corophiid amphipods but none was identified as *P. brisbanensis* (Schnabel et al., 2000; Chapman et al., 2002; M. Stevens & I. Hogg, unpubl. data). Furthermore, this first known record is in a shipping harbour on the east coast of New Zealand, rather than on the west coast where colonisation by oceanic drift across the Tasman Sea from the Queensland coast might seem possible. Thirdly, the high proportion of *P. brisbanensis* juveniles identified is common for invading species, often as a result of limited competition or absence of natural predators (Hebert et al., 1989; Pinkster et al., 1992; Mills et al., 1993b; Spidle et al., 1994).

Assuming that juveniles disperse passively, model simulations (C. A. Pilditch, pers. comm.) predict that individuals could spread throughout the harbour within 2-3 tidal cycles, and that a considerable fraction of individuals are likely to be exported into coastal waters. This suggests that the potential for *P. brisbanensis*

and other invasive taxa to spread to nearby harbours is high. Of particular concern, however, is that *P. brisbanensis* could now be transported around New Zealand by shipping activities. The Port of Tauranga is a major shipping port for New Zealand. A total of 2,494 vessels arrived between January 1999 and April 2001, with 8% of these coming from Australia (17% of these from Brisbane). In contrast, 44% of vessels leave Tauranga Harbour for other New Zealand ports. Thus, if *P. brisbanensis* was introduced from Australia then the probability of it now being introduced to other New Zealand harbours is extremely high.

The introduction into New Zealand waters of the Australian *P. brisbanensis* may have serious implications for New Zealand endemics. Examples from North America (Witt et al., 1997) and Europe (Costello, 1992; Pinkster et al., 1992; Dick, 1996) illustrate that competitive displacement and resource competition may have serious consequences for New Zealand's indigenous species. Many introductions are identified after they have become established. By contrast, the discovery of *P. brisbanensis* here may provide the opportunity to assess the impact relatively soon after its arrival.

Conclusion

One of the major sources of recent biological introductions world-wide has been through the use of ballast water in shipping (Mills et al., 1993a), and crustaceans have been identified amongst the most abundant taxa in ballast water (e.g., Carlton & Geller, 1993). By enabling movement of organisms by these means, bays, estuaries and inland waters have become some of the most threatened ecosystems in the world (Carlton & Geller, 1993). Ecological information on

species introductions is necessary to provide a good basis for predicting the impact of exotic species. In order to assess the biological impacts of the introduction of *P. brisbanensis* to New Zealand, studies of their effects at all trophic levels are urgently required.

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CHAPTER IV

NEW ZEALAND'S COROPHIID AMPHIPODS: ALLOZYME EVIDENCE FOR SPECIATION DURING THE CENOZOIC

Key-words: Population genetics, sibling species, *Paracorophium*, glaciation, Pliocene, Miocene, isolation-by-distance

Allozyme electrophoresis was used to examine population genetic structure at inter- and intraspecific levels for the New Zealand endemic corophiid amphipods, *Paracorophium lucasi* and *P. excavatum.* Individuals were collected from estuarine and freshwater habitats from North, South and Chatham Islands. Analyses of genetic structure among interspecific populations indicated clear allelic differentiation between the two *Paracorophium* species (Nei's genetic distance, $D = 1.72$), as well as considerable intraspecific substructuring ($D = 0.11$-0.67). These deep divergences are similar to interspecific levels of divergence for other amphipods and it is proposed that at least two groups from the *P. lucasi* complex and three groups from the *P. excavatum* complex correspond to sibling species. In most cases geographic barriers can account for the allopatric isolation among the suggested sibling species. For populations that share a common coastline we found low levels of differentiation and no correlation with geographic distance, suggesting that gene flow is adequate to maintain homogenous population genetic structure. By contrast, for populations isolated by a land-bridge, moderate levels of geographic differentiation were revealed indicating restricted gene flow. These patterns are consistent with the suggested colonisation and dispersal pathways for these taxa within New Zealand. The juxtaposition of population genetic and biogeographical data for *Paracorophium* in conjunction with the geological record infers past histories of glacial extirpation and possible isolating effects of landmass alterations that have occurred throughout the late Cenozoic.

The isolation of populations, both geographically and genetically, has long been recognised as a potential mechanism conducive to speciation (Kimura 1953; Mayr 1954). In this way, taxa with limited dispersal capabilities and geographic isolation are particularly susceptible to micro-evolutionary processes, such as allopatric speciation (Mayr 1954; Templeton 1980). This is especially evident on islands where populations tend to become isolated from the main distributions, both in terrestrial and aquatic systems (Slatkin 1993). Taylor et al. (1998) emphasised that many aquatic invertebrates often exhibit little morphological variation over vast distances, and many of these also display a positive relationship between geographic distance and genetic distance (Kimura 1953; Templeton 1980; Slatkin 1993). In such a setting, cryptic or sibling species appear to result from the prevailing pattern of morphological conservatism coupled with large genetic divergences that have been observed for many invertebrates (e.g., Knowlton et al. 1993; Stewart 1993; Thorpe and Solé-Cava 1994; Väinölä 1995).

For taxa that exhibit limited dispersal capabilities, small or temporary geographical barriers may be sufficient to isolate populations. Molecular techniques have made it possible to investigate how distributions of morphologically similar populations may be linked to geographic isolation and/or a taxon's dispersal capability (Avise 1992; Parker et al. 1998). For example, the emergence of the Isthmus of Panama has been considered a major isolating barrier for the marine shrimp *Alpheus*, leading to the evolution of sibling species by the isolation of populations between the Caribbean and eastern Pacific (Knowlton et al. 1993). In addition, high levels of genetic substructuring among populations of

the amphipod *Talitrus saltator* in the Mediterranean Sea have been interpreted as morphologically cryptic species (De Matthaeis et al. 2000). The inventory of such instances of cryptic speciation within North America is also increasing with examples including *Daphnia* (Colbourne et al. 1997; Taylor et al. 1998; Černý and Hebert 1999), *Hyalella* (Hogg et al. 1998; Witt and Hebert 2000), cladocerans (Cox and Hebert 2001) and copepods (Boileau 1991; Lee 2000). In New Zealand, Schnabel et al. (2000) suggested the presence of morphologically cryptic species among populations of *Paracorophium excavatum* and implied that coastal currents were the most likely barriers to gene flow. In addition, the turbulent geologic history of New Zealand has also been implicated as a potential agent for geographic, as well as genetic differentiation of taxa (Fleming 1979; Craw 1988; Pole 1989; Trewick 2000a; Wallis et al. 2001; Trewick and Wallis 2001).

The New Zealand archipelago has undergone considerable geologic change during the Cenozoic (ca. 65 MY-present) since its separation from the Gondwanan landmass some 70-80 MY ago (Stevens et al. 1995). For example the Oligocene and Miocene (ca. 6-37 MY) included marine intrusions, volcanism and seismic activity (Cooper and Millener 1993; Stevens et al. 1995) (Fig. 1). During the Pliocene (ca. 2-6 MY), uplift of the landmass separated the east and west coasts of North Island, until its present landmass was attained towards the beginning of the Pleistocene (ca. 2 MY) (Fleming 1979; Stevens et al. 1995). In the Pleistocene the isolation of island-like habitats has been particularly influenced by the advance and retreat of glaciers (Fleming 1979; Stevens et al. 1995). Over the last two million years there have been at least ten glacial cycles, and it has been approximately 17 thousand years since the last glacial maximum (Fleming 1979; Stevens et al. 1995). Such geological and climatic effects have been associated

with the inability of poorly dispersing organisms to recolonise denuded regions (Craw 1988; Main 1989; Pole 1989; McDowall 1997; Trewick and Wallis 2001).

New Zealand has a small landmass (270,000 km^2) but wide latitudinal range (ca. 12°) with extensive coastline, making it ideal for investigating patterns of diversification and dispersal of aquatic taxa that use ocean currents to move among habitat patches. Here, we assessed the population genetic structures of the two endemic corophiid amphipods, *Paracorophium excavatum* and *P. lucasi*. Both lack a specific dispersal stage and hence may be exposed to present-day geographical barriers imposed by the broad geographic range of the New Zealand topography, but may also be a consequence of landmass alterations over time. Accordingly, we tested the hypothesis that the two closely related species would exhibit similar intraspecific population genetic structures where their ranges overlap due to common historical vicariance. We also examined whether these patterns of divergence would correspond to the geological changes and climatic fluctuations occurring throughout the Cenozoic.

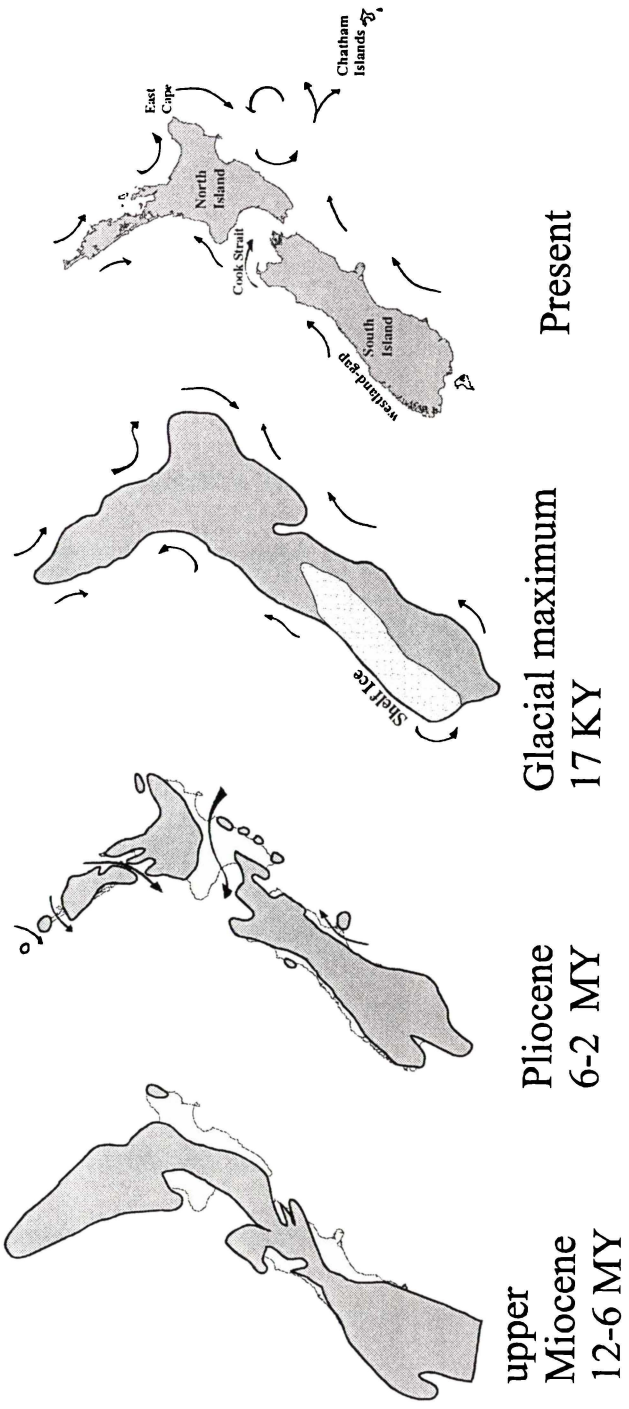

FIGURE 1. The changing outline of the New Zealand archipelago over the last 12 MY. Land above sea-level shaded grey. Arrows represent prevailing ocean circulation. The Pliocene (6-2 MYA) landmass provided few barriers for aquatic dispersal between the east and west coasts, in contrast to the upper Miocene (12-6 MYA), the last glacial maximum (approx. 17 KYA) and the present. Figures adapted from Fleming (1979) and Stevens et al. (1995).

Collection of samples

From September 1998 to August 2000 we examined 53 sites throughout New Zealand that appeared appropriate habitat for *P. lucasi* and *P. excavatum* (Chapman et al. 2002; Stevens et al. 2002). At each site we sampled fine mud and sand by passing a meshed (2 mm) net through the superficial sediment (upper 30-50 mm) and live-sorting on site. Although *Paracorophium* was not found at all sites (presence or absence at each site was standardised by limiting initial sampled to one hour), we generally found *P. lucasi* and *P. excavatum* from intertidal habitats and *P. lucasi* was also found in three inland lakes of North Island, and the exotic *P. brisbanensis* (initially included as an outgroup taxon) from a single site in Tauranga Harbour (N8) (Fig. 2). To identify *P. lucasi* and *P. excavatum* we used the diagnostic characters suggested by Chapman et al. (2002), and for *P. brisbanensis* we used Chapman (2002) and Stevens et al. (2002). All individuals for allozyme analyses were flash-frozen in liquid nitrogen and stored at -76°C. Sites were coded according to geographic location to indicate common coastline or habitat type, for example NE = North Island, east coast; SW = South Island, west coast; CS = Cook Strait; and L = Lake.

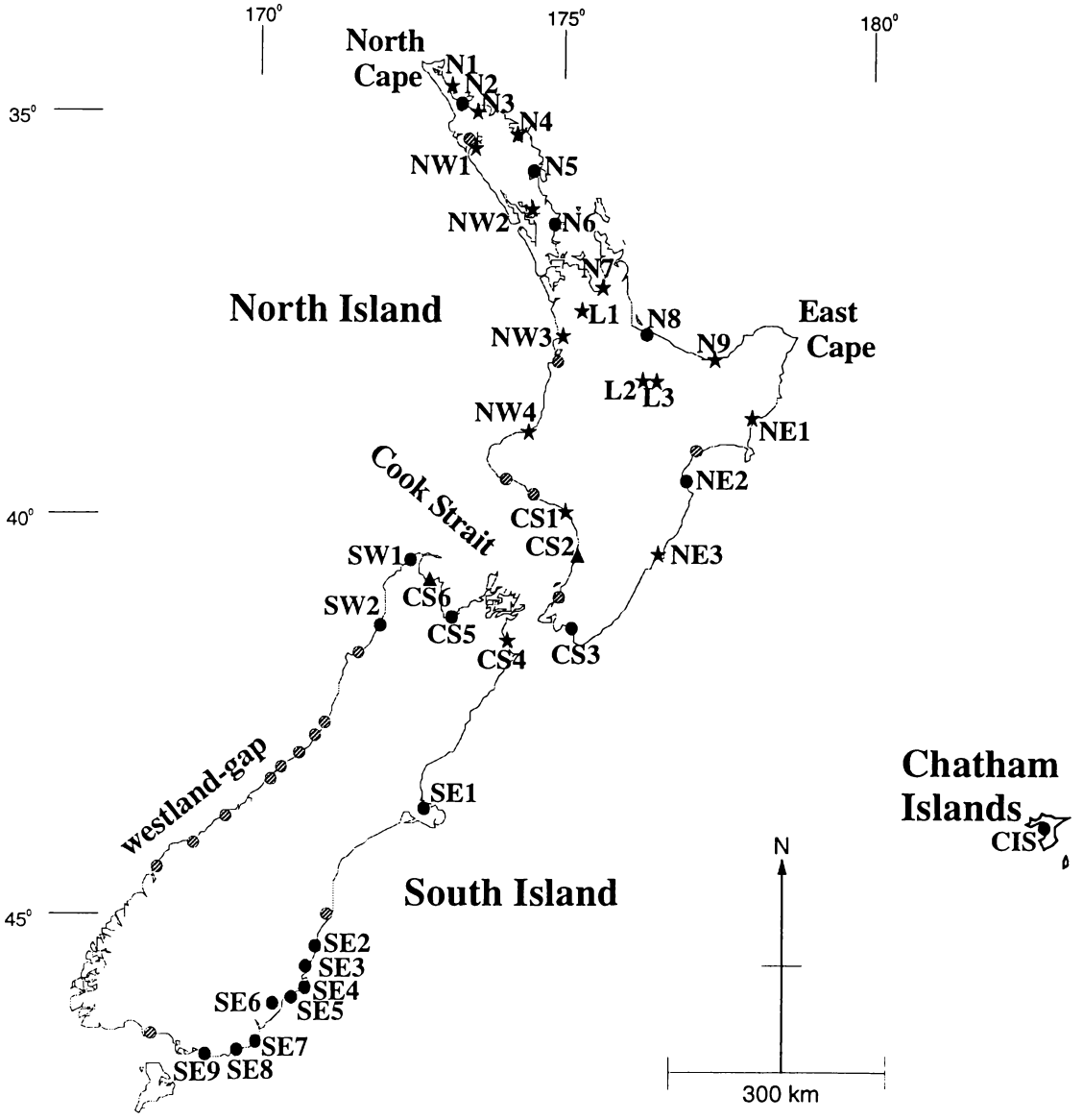

FIGURE 2. Distribution of *Paracorophium lucasi* (stars) and *P. excavatum* (solid circles) in New Zealand. Triangles show sympatric occurrences and hatched circles indicate sites where *Paracorophium* was not found. *P. brisbanensis* was found at N8.

We used cellulose acetate electrophoresis, and adapted stain recipes, buffers and running conditions from Richardson et al. (1986), Hebert and Beaton (1993) and Larose and Hogg (1998). Individual specimens were homogenised in 14 µl of distilled water. We used 10 enzyme systems for *Paracorophium* (Stevens et al. 2002) that revealed sufficient activity and resolution to be reliably scored: aldehyde oxidase (AO: EC 1.2.3.1); arginine kinase (ARK: EC 2.7.3.3); glyceraldehyde-3-phosphate dehydrogenase (G3PDH: EC 1.2.1.12); isocitrate dehydrogenase (IDH: EC 1.1.1.42); lactate dehydrogenase (LDH: EC 1.1.1.27); mannose-6-phosphate isomerase (MPI: EC 5.3.1.8); malate dehydrogenase $NADP^+$ (ME: EC 1.1.1.40); peptidase (PEP: EC 3.4.11/13); phosphoglucomutase (PGM: EC 5.4.2.2); 6-phosphogluconate dehydrogenase (6PGDH: EC 1.1.1.44). Three enzymes (AO, IDH, LDH) were coded by two loci designated numerically in order of increasing electrophoretic mobility (e.g., *AO-1*, *AO-2*). Alleles were designated by the relative differences in anodal mobility of the respective gene products, i.e., the 'fastest' allele was designated "A", the next fastest allele "B", and so on. There were 2-9 detectable alleles for all loci examined. Two individuals from previous runs were re-run to control for any variation in mobility between gel plates, and putative novel alleles were verified using gel line-ups (*sensu* Richardson et al. 1986).

The program Genetic Data Analysis (Lewis and Zaykin 2001) was used to calculate descriptive and hierarchical population statistics. Genotypic frequencies were determined for each population, and polymorphic loci (95% criterion) were examined for agreement of genotypes with Hardy-Weinberg equilibrium using Fisher's exact test, followed by sequential Bonferroni corrections (Rice 1989). Divergence was assessed among populations using Wright's (1978) F_{ST}, and among individuals in a single population (F_{IS}). Significance of pairwise comparisons of F_{ST} (bootstrapping across loci with 5000 replicates) were used to pool some populations for *P. excavatum* only when homogenous population genetic structure could be inferred. Hierarchical cluster analysis was performed using the UPGMA algorithm (Sneath and Sokal 1973) calculated using Nei (1978) unbiased genetic distance (*D*).

Isolation-by-distance (*I-D*) analyses (Wright 1943; Slatkin 1993) were performed to examine geographic differentiation among populations according to the island model (Wright 1931, 1943) and the stepping stone model (Kimura 1953; Kimura and Weiss 1964; Ibrahim et al. 1996). We can discriminate between these because the island model assumes that all populations are of equal size and that migration is equal among them (Wright 1943), whereas the stepping stone model assumes leptokurtic dispersal (Ibrahim et al. 1996). To test for the existence of *I-D* we performed a regression of log transformed pairwise genetic distances and geographic (aquatic) distances (log*D* and log*km*, respectively) and calculated the regression coefficients (R^2) (Kimura and Weiss 1964; Felsenstein 1976; Slatkin 1993). Spearman's rank correlation index (*R*) was used to test how much

of the allelic variance among populations is explained by geographic distance alone (De Matthaeis et al. 2000). SPSS (ver. 10) for Windows was used for these analyses.

RESULTS

Geographic distribution

The genus *Paracorophium* was found throughout New Zealand waters (Fig.2). *P. lucasi* was collected from 18 sites, *P. excavatum* from 21 sites and we were unable to find *Paracorophium* at 17 sites. Both species were found along the eastern coast of North Island and throughout the coastal regions of Cook Strait. *P. lucasi* was found on the west coast of North Island, and *P. excavatum* on the west and east coasts of South Island, as well as being the only species of this genus collected from Chatham Island (Fig. 2). We did not find *P. excavatum* from the northwest coast of North Island, nor did we find *P. lucasi* south of Cook Strait, and neither species were found from the majority of the west coast of South Island (Fig. 2).

Allozyme variation

Allozyme variability was detected at all 13 loci among the three *Paracorophium* species. We also suspected a null allele at an additional locus, *PEP-2*, as no individuals from N1 and N3 populations showed any activity at this locus. These individuals showed normal activity at all other loci, and we detected this putative

null allele in a few individuals from NE1 and CS6. Because it is highly unlikely that N1 and N3 populations were fixed for a null allele due to a potentially large selection coefficient against recessive alleles we have excluded *PEP-2* from all analyses.

Allozyme variability for all populations are summarised in Table 1. The mean number of alleles per locus was 1.4 for *P. lucasi*, and 1.6 for *P. excavatum*. The mean number of alleles per polymorphic locus was 2.6 for *P. lucasi*, and 2.8 for *P. excavatum*. The percentage of polymorphic loci (95% criterion) for *P. lucasi* was 17.1% and for *P. excavatum* was 23.6% and mean heterozygosities were similar between *P. lucasi* and *P. excavatum* (Table 1). Significant deviations were detected at the *ARK* locus from N9, for the *6PGDH* locus from NE1 and NE2, and for the *PEP* locus from NE1 and L2 populations (in all cases the result of heterozygote deficiencies). For the *P. excavatum* genotypic frequencies deviated from H-W equilibrium caused by heterozygote deficiencies at the *ARK* locus from N8, the *MPI* locus for N6, NE2, CS3, SL2 and CIS, and at the *LDH-1* locus in four populations of the lower North Island and South Island (CS6, SO2, SW1, SW2), but not for any of the populations in the upper North Island (N2, 5, 6, 8) or Chatham Island (CIS). CIS had genotypic frequencies deviating from H-W equilibrium at the *LDH-2* and *6PGDH* loci, and the Nelson population (CS5) at the *IDH-1* locus.

TABLE 1. Genetic variability at 13 loci in all populations of *Paracorophium lucasi*, *P. excavatum* and *P. brisbanensis*. Notation preceding locations refer to those used in the text, figures, tables, and appendix. N = mean sample size per locus, P = percentage of polymorphic loci (95% criterion), A = mean number of alleles per locus, Ap = mean number of alleles per polymorphic locus, H_{obs} = observed heterozygosity and H_{exp} = expected heterozygosity, and * = a significant ($P<0.05$) deviation at one or more loci (see text), [1,2,3,4] = proximate populations pooled for UPGMA analyses only when F_{ST} among populations were not significantly different to zero (determined from bootstrap analyses).

LOCATION	Lat (S),Long (E)	N	P	A	Ap	H_{obs}	H_{exp}
Paracorophium lucasi							
N1-Houhora Harbour	34°48',173°06'	23.5	15.4	1.4	3.5	0.07	0.06
N3-Awanui River	35°01',173°17'	26.7	15.4	1.3	3.0	0.06	0.06
N4-Taumarere	35°20',174°06'	24.8	15.4	1.4	3.0	0.04	0.05
N7-Thames	37°05',175°30'	25.8	7.7	1.4	3.0	0.04	0.05
N9-Whakatane	38°00',177°06'	25.8	30.8	1.5	2.3	0.09	0.10*
NW1-Rawene	35°26',173°31'	21.9	7.7	1.2	3.0	0.04	0.04
NW2-Topuni River	36°13',174°28'	16.9	7.7	1.2	2.0	0.04	0.04
NW3-Raglan Harbour	37°48',174°57'	37.4	23.1	1.6	3.0	0.06	0.06
NW4-Waitara	39°04',174°03'	27.0	15.4	1.4	2.0	0.06	0.05
L1-Lake Waikare	37°26',175°13'	37.0	7.7	1.1	2.0	0.03	0.04
L2-Lake Rotorua	38°02',176°17'	30.1	23.1	1.2	2.0	0.05	0.06*
L3-Lake Rotoiti	38°01',176°21'	26.0	7.7	1.2	2.0	0.04	0.04
CS1-Whanganui River	39°55',175°02'	22.5	30.8	1.5	2.5	0.07	0.08
CS2-Foxton	40°18',175°15'	11.0	15.4	1.4	2.5	0.04	0.05
CS4-Wairau River	41°29',174°02'	24.5	23.1	1.5	3.3	0.04	0.04
CS6-Collingwood	40°41',172°40'	23.5	23.1	1.8	3.3	0.06	0.07*
NE1-Gisborne	38°34',177°56'	28.0	23.1	1.3	2.0	0.05	0.07*
NE3-Porangahau	40°38',176°22'	18.0	15.4	1.2	2.0	0.06	0.05*
	Mean	25.0	17.1	1.4	2.6	0.05	0.06

continued,

TABLE 1 *continued,*

LOCATION	Lat (S),Long (E)	N	P	A	Ap	H_{obs}	H_{exp}
Paracorophium excavatum							
N2-Rangauna Harbour	35°01',173°15'	24.9	7.7	1.4	3.0	0.04	0.05
N5-Whangarei Harbour	35°43',174°19'	23.5	15.4	1.5	3.0	0.04	0.05
N6-Omaha Bay	36°35',174°76'	25.2	7.7	1.2	2.0	0.01	0.04*
N8-Tauranga Harbour	37°40',176°10'	29.1	15.4	1.5	3.0	0.04	0.05*
NE2-Napier	39°30',176°48'	30.0	30.8	1.4	2.3	0.04	0.07*
CS2-Foxton[1]	40°18',175°15'	13.0	30.8	1.7	2.3	0.04	0.04
CS3-Lake Onoke[1]	41°25',175°09'	29.7	38.5	2.0	3.0	0.07	0.04*
CS5-Nelson	41°17',173°14'	23.3	23.1	1.6	3.0	0.02	0.09*
CS6-Collingwood[1]	40°41',172°40'	13.1	38.5	1.6	2.4	0.07	0.04*
SW1-Whanganui Inlet	40°34',172°38'	27.2	23.1	1.6	2.7	0.05	0.08*
SW2-Little Wanganui	41°23',172°03'	24.6	30.8	1.5	2.0	0.05	0.13*
SE1-Christchurch	43°32',172°43'	25.0	23.1	1.6	2.7	0.08	0.09
SC1-Shag River[2]	45°29',170°47'	21.5	53.8	2.2	3.0	0.11	0.09*
SC2-Karitane[2]	45°38',170°38'	11.5	38.5	1.5	2.0	0.07	0.04*
SO1-Tomahawk Lagoon[3]	45°51',170°32'	5.7	46.2	1.7	2.5	0.07	0.06
SO2-Brighton River[3]	45°57',170°20'	22.8	38.5	1.6	2.4	0.09	0.07*
SO3-Lake Waihola[3]	46°01',170°05'	5.0	30.8	1.3	2.0	0.09	0.08
SL1-Waikawa Harbour[4]	46°38',169°07'	5.0	38.5	1.4	2.0	0.08	0.07
SL2-Catlins Lake[4]	46°28',169°38'	22.5	30.8	1.8	2.8	0.08	0.06*
SL3-Fortrose[4]	46°34',168°47'	22.6	53.8	1.8	2.1	0.08	0.07
CIS-Chatham Island	43°57',176°33'	73.9	30.8	1.9	3.3	0.08	0.04*
	Mean	34.3	23.6	1.6	2.8	0.05	0.08*
Paracorophium brisbanensis							
N8-Tauranga Harbour	37°40',176°10'	31.3	0.0	1.1	—	0.01	0.01

Genetic differentiation (Wright's (1978) F_{ST}) averaged among all *P. lucasi* populations was 0.72 and among *P. excavatum* was 0.74, indicating low levels of gene flow among populations for both species. High levels of intraspecific substructuring were indicated by large F_{IS} values for *P. excavatum* (mean = 0.48), but were lower for *P. lucasi* (mean = 0.15) (Table 2).

The UPGMA analysis showed that all *P. excavatum* populations formed a distinct cluster (D = 1.72) to *P. brisbanensis* and *P. lucasi*, which form a sister group (D = 1.31; Fig. 3). The two most northern North Island *P. lucasi* populations (N1, N3) were fixed at *PEP* and *ME* loci, and were found to be genetically distinct in the UPGMA analysis (D = 0.32, Cluster I; Fig. 3). We identified two *P. lucasi* populations (Cluster IV: NE1, NE3) on the southeast coast of North Island fixed at *AO-1* and *AO-2* loci, resulting in considerable genetic distance (D = 0.47) from the other *P. lucasi* clusters (Fig. 3). *P. lucasi* Clusters II and III included populations throughout North Island, and Cook Strait (Fig. 3) and were fixed for alternate alleles at either *AO-1*, *AO-2*, *ME* or *PEP* loci when compared to populations from Clusters I and IV. In addition, Clusters II and III were separated genetically (D = 0.11) by a fixed allelic difference at the *LDH-2* locus, although NW3 possessed alleles common to both clusters (Appendix). Deep divergence was also found among the *P. excavatum* clusters with Cluster VII most divergent (D = 0.67; Fig. 3). Chatham Island (CIS) grouped with the northern North Island populations in Cluster VI with a genetic distance of 0.27, with common alleles at three loci (*AO-1*, *AO-2*, *PEP*) with the populations in Cluster VI, but also had common alleles with three loci (*IDH-2*, *LDH-1*, *LDH-2*) with the

populations in Cluster VII (Fig. 3). The population at Nelson (CS5) was morphologically identified as *P. excavatum*, but was also found to be genetically distinct (D = 0.61). By comparing common alleles among the loci we found that the CS5 population had alleles common at 11 loci with *P. lucasi*, 10 with *P. excavatum* and three with *P. brisbanensis*. This contrasts with 10 loci with common alleles between *P. excavatum* and *P. lucasi*, seven between *P. excavatum* and *P. brisbanensis*, and eight between *P. lucasi* and *P. brisbanensis* (Appendix).

Isolation-by-distance analyses among all *P. lucasi* populations showed a significant relationship, but only 35% of the variance in allele frequencies can be explained by genetic drift alone (see Table 2). A similar pattern was found among all *P. excavatum* populations with a significant relationship, although R^2 was small (0.24), but 47% of the variance in allele frequencies can be explained by genetic drift. R^2 and R values revealed varying degrees of genetic correlation with geographic distance using the clusters from Figure 3 that corresponded to fixed allelic differences. For *P. lucasi* we removed the four most divergent populations (Cluster I and IV), and analysed Clusters II and III only, which resulted in a small increase in the relationship. However, analysing populations from Cluster II revealed a highly significant relationship, but without N4 there was no support for this association. Cluster III showed a marginally significant relationship among populations that were geographically proximate for *P. lucasi*, but only 20% of the variance in allele frequencies could be explained by genetic drift alone (Table 2). For *P. excavatum* we found that by removing CS5 (Cluster V) and analysing the populations of Cluster VII we found no significant relationship. By contrast, Cluster VI reveals strong geographic differentiation, but this relationship was not significant when CIS was removed from the analysis (Table 2).

TABLE 2. F-statistics (F_{ST}, F_{IS}) averaged over 13 loci. Linear regression coefficient (R^2) and Spearman's rank correlation Index (R) of log$D$ and logkm used to assess isolation-by-distance (*I-D*) (see methods) for different geographic regions for *Paracorophium lucasi* and *P. excavatum* ($^{**}P < 0.01$, $^{*}P < 0.001$). For populations included in each cluster refer to Figure 3.

	F_{ST}	F_{IS}	R^2	R	*I-D*
Paracorophium lucasi					
Overall	0.72	0.15	0.20^{*}	0.35^{**}	Stepping Stone
Cluster II	0.24	0.10	0.54^{*}	0.76^{**}	Stepping Stone
Cluster II without N4	0.04	0.07	0.19	0.43	Island model
Cluster III	0.27	0.15	0.37^{**}	0.20	Stepping Stone
Cluster II and III	0.51	0.13	0.20^{*}	0.36^{**}	Stepping Stone
Paracorophium excavatum					
Overall	0.74	0.48	0.24^{*}	0.47^{**}	Stepping Stone
Cluster VI	0.69	0.51	0.87^{*}	0.89^{**}	Stepping Stone
Cluster VI without CIS	0.09	0.42	0.15	0.49	Island model
Cluster VII	0.20	0.45	0.04	0.30^{**}	Island model

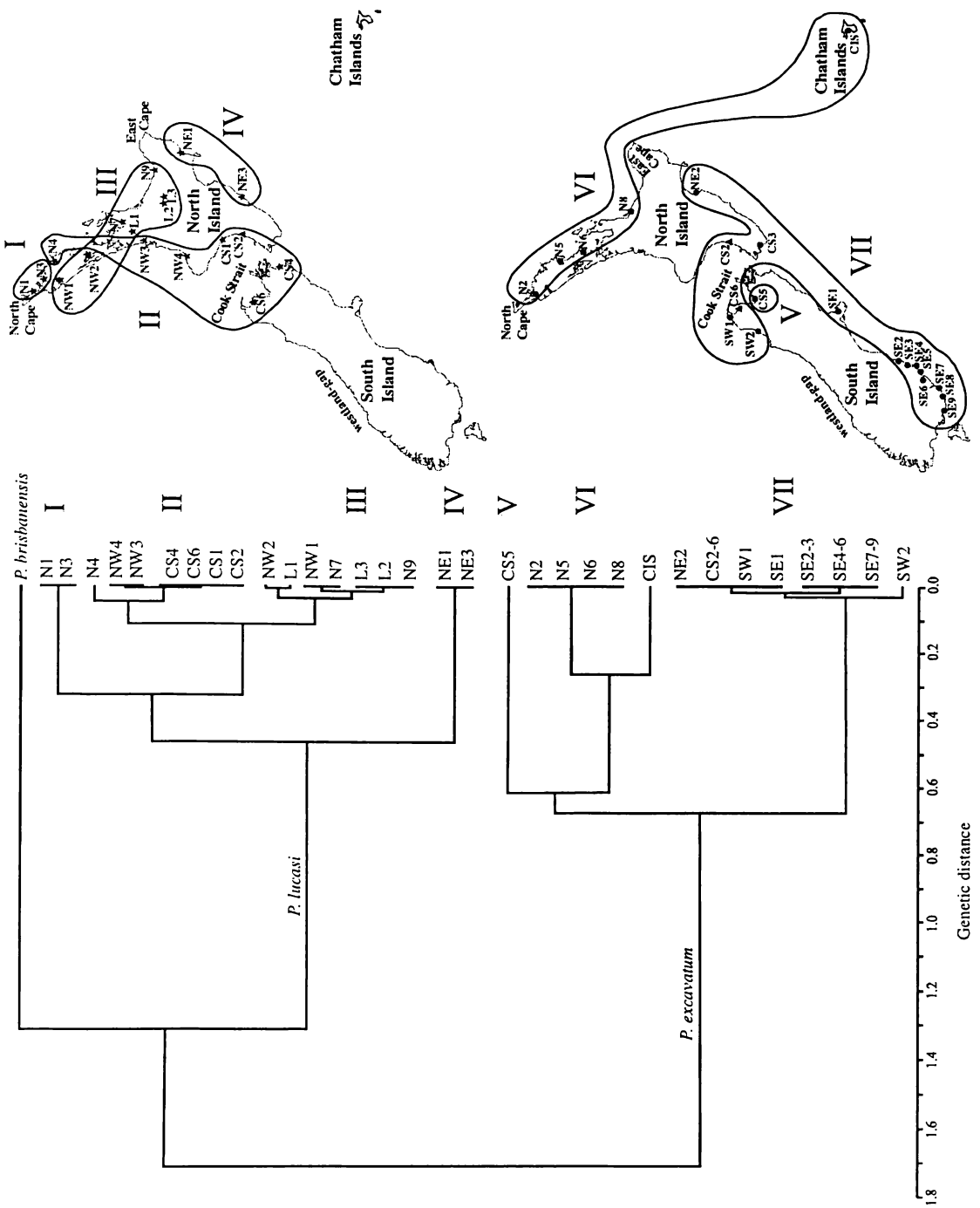

FIGURE 3. Phenetic analyses (UPGMA dendrogram) of the genetic distance (Nei 1978) between *Paracorophium brisbanensis* and populations of *P. lucasi* and *P. excavatum* (pooled populations correspond to Table 1).

Spatial genetic structure

We found fixed allelic differences corresponding to geographically isolated regions for both *P. lucasi* and *P. excavatum*, particularly from populations that were separated by the East Cape (Fig. 3). This geographic feature creates an isolating boundary between the ocean currents from the north and from the south (Chiswell 2000). Hence, such genetic isolation between Clusters III and IV (D = 0.47) for *P. lucasi* and between Clusters VI and VII (D = 0.67) for *P. excavatum* would suggest that the East Cape is an effective barrier to gene flow (Fig. 3). For *P. lucasi* an additional barrier appears further south. The two east coast *P. lucasi* populations (Cluster IV: NE1, NE2) were genetically distinct from the Cook Strait populations (D = 0.47; Fig. 3). By contrast, *P. excavatum* Cluster VII populations were genetically similar throughout this geographic region. A similar pattern was observed for the two most northern *P. lucasi* populations (N1, N3) of Cluster I which also showed moderate divergence to nearby populations (D = 0.32), but this pattern was not observed for *P. excavatum* populations (Cluster VI) for the same region (Fig. 3).

An isolation-by-distance pattern with high F_{ST} values for *P. lucasi* and *P. excavatum* (0.72 and 0.74, respectively; Table 2) suggests that populations are not in equilibrium (i.e., between gene flow and genetic drift, *sensu* Slatkin 1993) and a trend of geographic differentiation would be conducive to allopatric or parapatric speciational processes (Templeton 1980). For *P. lucasi* an island model can be used to describe the populations that were connected by an oceanic route, such as

the populations in Clusters I, II (without N4) and IV. However, when populations were separated by a land-bridge (Clusters II, III) they were better described by a stepping stone model (Table 2; Fig. 3), and these patterns were found whether direct geographic distance or aquatic distance was used. For *P. excavatum*, populations within all clusters corresponded to oceanic routes (Fig. 3). We found that an island model described Cluster VII (Table 2)—populations that span over a large geographic range (see Fig. 3). This pattern was also found for Cluster VI if Chatham Island (CIS) was not included in the analysis. By including CIS, which is approximately 950 km from any of the northern North Island *P. excavatum* populations, the analysis is biased towards a stepping stone model (Table 2). Generally, the overall genetic divergence and isolation-by-distance analyses suggest that *P. lucasi* is more divergent than *P. excavatum* over similar geographic distances (Fig. 3). Interestingly, *P. excavatum* is only known from brackish water habitats but *P. lucasi* is found to occupy habitats further upstream, including freshwater habitats (Chapman et al. 2002) and may reflect differing interhabitat dispersal distances for the two species.

Colonisation via oceanic surface currents is possible, and has been implicated for a number of Chatham Islands' taxa with affinities to both northern and southern New Zealand (Knox 1954; Craw 1988; Emberson 1995; Trewick 2000b). For *P. lucasi*, the relationship of N4 in Cluster II may infer overland dispersal (Fig. 3). Overland dispersal can also be suggested within Cluster III by the *LDH-2* locus with common alleles to NW3 (Cluster II). NW3 could represent either a hybrid zone or source population for both clusters (II and III). However, it may also represent rare dispersal for the Cluster III genotype from either NW1 or NW2 (Fig. 3). Overland transport by waterfowl has been implicated for many small

aquatic organisms (e.g., Rosine 1956; Maguire 1959, 1963; Proctor 1964; Schlichting 1960), but translocation by way of shipping activities may also provide an effective means of dispersal for many aquatic taxa (Carlton and Geller 1993; Stevens et al. 2002). Recently, Schnabel et al. (2000) suggested that the patterns of ocean circulation around New Zealand were the most likely barrier to gene flow for *Paracorophium*. Because ocean currents are generally described as a mean (averaged over time and space) they indicate the dominant surface circulation, but surface and coastal currents are highly variable and often affected by prevailing wind patterns (Roemmich and Sutton 1998; Chiswell 2000). Dispersal via ocean currents, as a vector for migration has not been explicitly tested. However, recent studies (Ford et al. 1999; M.I. Stevens unpubl. data) have shown that *Paracorphium* juveniles were abundant in the water column and prone to being flushed out of bays and estuaries during tidal flows allowing possible transport to neighbouring populations. Dispersal among populations may only be successful during times of rare or periodical climatic events, such as an ENSO (El Niño and southern oscillation), which are well known to bring intense rainfall and increased sea surface temperatures (Tomczak and Godfrey 1994). However, the present study suggests that dispersal at greater distances is less likely where significant geographic barriers exist (e.g., North Cape and East Cape).

Divergence through allopatric isolation

We found no evidence to indicate that recent hybrids can occur between *P. lucasi* and *P. excavatum* from two sympatric occurances in the present study (CS2, CS6) and no hybrids have been found among the three *Paracorophium* species at

Tauranga Harbour (N8) (Stevens et al. 2002). The single population from Cluster V (CS5) was found to have similarities to all three *Paracorophium* species and may represent an old hybrid zone or possibly an ancestral population. In addition, a few *P. lucasi* and *P. excavatum* individuals were found sympatrically within the Nelson (CS5) population suggesting the presence of reproductively isolated species. The degree of reproductive isolation among other clusters is uncertain in the absence of sympatry. However, the levels of genetic divergence reported here are likely to correspond to sibling species for both *P. lucasi* and *P. excavatum*, which have diverged through allopatric isolation. For example, similar levels of divergence were found among morphologically distinct species of the talidrid amphipod *Orchestia* (D = 0.51-0.59) (Conceição et al. 1998; De Matthaeis et al. 2000) and are similar to reported divergence among congeneric species of other Crustacea (Hedgecock et al. 1982; Stewart 1993; Thorpe and Solé-Cava 1994). Such patterns may also explain, to a lesser extent, Chatham Island's (CIS) genetic similarity to northern *P. excavatum* from Cluster VI, but common alleles with the southern Cluster VII may reveal common ancestry or recent hybridisation. The *P. lucasi* populations in Cluster I (N1, N3) are particularly interesting since they contained a null allele (data not shown) and were distinct from other populations based on *PEP*, *LDH-2* and *ME* loci. The presence of a null allele in three other *P. lucasi* populations (NE1, L2 and CS6) may suggest that N1 and N3 populations resulted from a founding event. Accordingly, such rare dispersal events may have resulted in genetic founder and/or bottleneck effects promoting rapid divergence among these populations.

The degree of divergence among the sibling species for *Paracorophium* would suggest that these groups were isolated well before the Pleistocene glaciations, and are more likely to have origins in the Pliocene (2-6 MY). The level of divergence of Chatham Islands (CIS) to northern *P. excavatum* from Cluster VI (Fig. 3) agrees remarkably well with Campbell's (1998) hypothesis that the Chatham Islands were totally submerged less than 4 MYA. The divergence of the Nelson (CS5) population would also suggest an older coalescence, possibly in the Miocene prior to the separation of North and South Island (Fig. 1). An increased speciation rate followed by a high rate of extinction has been hypothesised for New Zealand terrestrial biota during the Oligocene (Cooper and Cooper 1995). For aquatic taxa, we suggest that an increase in land surface during the Miocene isolated east and west coastal regions leading to many populations becoming extinct or locally restricted. Subsequent range expansion/overlap may have occurred during the Pliocene when more frequent east-west exchange among populations would have been possible (Fig. 1). During the Pliocene, tectonic changes altered the New Zealand archipelago from a collection of islands to the two main islands present today (Fleming 1979; Craw 1988; Stevens et al. 1995; Campbell 1998; Cooper and Cooper 1995) (Fig. 1). It is apparent that the Pleistocene glaciations and Pliocene landmass alterations have proved to be agents for divergence for several New Zealand terrestrial taxa (Craw 1988; Trewick and Wallis 2001, and references therein). For *Paracorophium*, we suggest that the development of ephemeral islands throughout the Pliocene for the New Zealand landmass (Fig. 1) may have allowed for more frequent aquatic dispersal, and is the

most likely explanation accounting for the barriers to dispersal that are now present.

The advance and retreat of glaciers has had a considerable influence on the distribution, abundance and diversity of biota in New Zealand (e.g., Craw 1988; Fleming 1979; Wardle 1991). Much of the west coast was covered by sea-ice during the last glacial maximum (ca. 17 kya) (Stevens et al. 1995) (Fig. 1), and the ability of poorly dispersing organisms to recolonise such denuded regions is especially evident. The westland-gap constitutes an area of the west coast of South Island that lacks beech forest between 42°30' and 43°30'S latitude (Wardle 1991) (Fig. 1). This region is now known to lack other biota such as non-diadromous galaxids (Main 1989; McDowall 1997) and upland bully (*Gobiomorphus breviceps*) (Main 1989), which have no migration to and from the sea. This contrasts to diadromous species, such as the shortjawed kokopu (*Galaxias postvectis*), that have been able to reinvade rivers located in the westland-gap (McDowall 1997). *Paracorophium* was absent from the westland-gap (sites SW1 and SW2 are north of the gap) (see Figs. 2 and 3) and may provide support for the effects of Pleistocene climate conditions. Re-colonisation via oceanic dispersal to the south appears unlikely during the present inter-glacial with the prevailing ocean current flowing northward along this coastline (Fig. 1). However, there is no evidence to suggest that divergence was promoted over the last 2 MY as a consequence of the Pleistocene climatic fluctuations.

We suggest that *P. lucasi* and *P. excavatum* species complexes consist of sibling species that are genetically distinct despite having no clear morphological differences. Such genetic patterns may reflect speciation processes throughout the genus since the Miocene. In addition, we found that the climatic shifts throughout the Pleistocene may have restricted the distribution of *Paracorophium*, particularly in the westland-gap (see Fig. 1). With no larval stage, isolation among amphipod populations may greatly reduce dispersal opportunities where geographical barriers are concerned, in particular historic geologic formations and the patterns of present-day ocean currents may be sufficient to isolate populations. Such barriers to gene flow among these fragmented island-like habitats suggests that these mechanisms have been conducive to speciational processes, in particular allopatric isolation. The deep genetic divergences within *Paracorophium* are marked by fixed allelic differences and allele frequency shifts consistent with physiographic barriers. With limited gene flow among biogeographic regions throughout New Zealand the apparent fragmentation of an ancestral regional gene pool suggests that allopatric speciation has played an important role in the origin of taxon diversity for New Zealand corophiid amphipods.

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APPENDIX 1. Allele frequencies at 13 loci for all populations of *Paracorophium lucasi*, *P. excavatum* (* = pooled populations corresponding to Fig. 3) and *P. brisbanensis*. Locations refer to those used in the text, figures, and tables. Alleles are labelled according to their relative mobility (see text).

	Paracorophium lucasi																		*Paracorophium excavatum*													
	N1	N3	N4	N7	N9	NW1	NW2	NW3	NW4	L1	L2	L3	CS1	CS2	CS4	CS6	NE1	NE2	N2	N5	N6	N8	NE2	CS*	CS5	SW1	SW2	SE1	SE2-3*	SE4-6*	SE7-9*	CIS	PB
n	26	29	26	26	27	23	17	41	27	37	31	26	23	11	26	24	28	18	26	24	26	32	30	57	25	28	27	27	34	35	51	80	38
AO-1																																	
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																	
B																	1.00	1.00															
C																							1.00	1.00		1.00	1.00	1.00	1.00	1.00	1.00		
D																			1.00	1.00	1.00	1.00										1.00	
E																																	1.00
F																									1.00								
AO-2																																	
A	1.00	1.00	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																	
B			0.04																														
C																	1.00	1.00															
D																													0.03		0.01		
E																							1.00	1.00		1.00	1.00	1.00	0.97	1.00	0.98		
F																			1.00	1.00	1.00	1.00									0.01	1.00	
G																																	1.00
H																									1.00								
PEP																																	
A	1.00	1.00									0.13					0.04	0.07																1.00
B																									0.02								
C			1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.87	1.00	1.00	1.00	1.00	0.96	0.93	1.00							0.98								
D																			1.00	1.00	1.00	1.00										1.00	
E																							1.00	1.00		0.98	1.00	1.00	0.98	1.00	1.00		
F																										0.02			0.02				

continued,

APPENDIX, *continued,*

	Paracorophium lucasi																		*Paracorophium excavatum*													
	N1	N3	N4	N7	N9	NW1	NW2	NW3	NW4	L1	L2	L3	CS1	CS2	CS4	CS6	NE1	NE2	N2	N5	N6	N8	NE2	CS*	CS5	SW1	SW2	SE1	SE2-3*	SE4-6*	SE7-9*	CIS	PB
IDH-1																																	
A																									0.35							0.04	
B					0.02														1.00	1.00	1.00	1.00	1.00	1.00	0.52	1.00	1.00	1.00	1.00	1.00	1.00	0.96	
C	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00							0.13								
D																																	1.00
IDH-2																																	
A																								0.01	0.08	0.07	0.61	0.02			0.01	0.01	
B								0.04	0.02						0.06	0.02					0.04		1.00	0.98	0.92	0.93	0.39	0.98	1.00	1.00	0.98	0.99	
C	1.00	1.00	1.00	0.98	1.00	1.00	1.00	0.92	0.96	1.00	1.00	1.00	0.94	0.96	0.87	0.94	0.70	0.64	0.98	0.94	0.96	0.98		0.01							0.01		
D				0.02				0.04	0.02				0.04		0.08	0.04	0.30	0.36	0.02	0.06		0.02											1.00
E													0.02	0.04																			
LDH-1																																	
A																			1.00	1.00	1.00	1.00						0.07	0.05	0.03	0.05	0.07	
B	1.00	1.00	1.00	1.00	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00						0.01	1.00							0.02	1.00
C					0.04																							0.05	0.03				
D																							0.75	0.92		0.75	0.59	0.52	0.69	0.77	0.89	0.91	
E																							0.23	0.07		0.25	0.41	0.36	0.23	0.20	0.06		
F																							0.02										
LDH-2																																	
A				1.00	1.00	1.00	1.00	0.01		1.00	1.00	1.00							1.00	1.00	1.00	1.00			1.00			0.02				0.03	
B	1.00	1.00	1.00					0.98	1.00				1.00	1.00	1.00	1.00	1.00	1.00														0.04	1.00
C								0.01															1.00	1.00		1.00	1.00	0.98	1.00	1.00	1.00	0.93	
G3PDH																																	
A																																	1.00
B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
C																				0.04													
D																			0.02														
6PGDH																																	
A				0.02																													
B	1.00	1.00	1.00	0.98	0.87	1.00	1.00	1.00	0.94	1.00	1.00	1.00	0.91	0.91	1.00	0.98								0.06	0.05						0.04	
C																0.02	0.21	0.50	1.00	1.00	1.00	1.00	0.88	0.81	0.95	0.98	0.98	1.00	1.00	0.97	0.89	0.90	
D					0.13				0.06				0.09	0.09			0.79	0.50					0.12	0.12		0.02	0.02			0.03	0.11	0.03	1.00
E																								0.01								0.03	

continued,

APPENDIX, *continued.*

	Paracorophium lucasi																		*Paracorophium excavatum*												
	N1	N3	N4	N7	N9	NW1	NW2	NW3	NW4	L1	L2	L3	CS1	CS2	CS4	CS6	NE1	NE2	N2	N5	N6	N8	NE2	CS*	CS5	SW1	SW2	SE1	SE2-3*	SE4-6*	SE7-9*	CIS	PB
ARK																																	
A																						0.05											
B					0.19								0.05						1.00	1.00	1.00	0.74	1.00	1.00		1.00	0.98	0.83	0.56	0.39	0.77	1.00	
C	1.00	1.00	1.00	1.00	0.81	1.00	0.97	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00	0.98	0.98	1.00				0.21			1.00		0.02	0.17	0.41	0.61	0.23		1.00
D							0.03									0.02	0.02																
E																													0.03				
MPI																																	
A																				0.04													
B			0.07		0.05			0.03					0.08	0.05	0.02	0.04	1.00	1.00		0.04					0.07					0.03	0.01		0.04
C				0.02												0.02																	
D	0.21	0.15	0.76	0.65	0.85	0.42	0.50	0.87	0.70	0.42	0.95	0.96	0.69	0.95	0.91	0.72			0.30		0.26		0.87	0.86	0.10	0.98	0.71	0.85	0.32	0.67	0.39	0.44	0.96
E	0.74	0.85	0.17	0.33	0.10	0.54	0.50	0.10	0.30	0.58	0.05	0.04	0.23		0.02	0.20			0.63	0.81	0.74	0.98	0.13	0.14	0.76	0.02	0.29	0.15	0.66	0.30	0.59	0.48	
F	0.05					0.04									0.05	0.02			0.07	0.11		0.02			0.07				0.02		0.01	0.08	
PGM																																	
A				0.02	0.52			0.24	0.02		0.47	0.56	0.11	0.09	0.04	0.09														0.02			
B															0.02																		
C	0.25	0.10	0.08	0.98	0.48	1.00	1.00	0.73	0.98	1.00	0.53	0.44	0.89	0.86	0.94	0.91	1.00	1.00	0.02	0.02	0.02	0.13	0.08	0.07		0.02		0.04	0.02	0.02	0.02		
D																						0.02											
E	0.67	0.69	0.88					0.03						0.05					0.98	0.96	0.98	0.85	0.92	0.92	1.00	0.71	0.93	0.96	0.94	0.95	0.97	0.97	
F	0.04	0.17	0.04																	0.02				0.01		0.25	0.07		0.02	0.02	0.01	0.03	1.00
G	0.04	0.04																								0.02			0.03				
ME																																	
A			1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00															
B																																	1.00
C	1.00	1.00																	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	

CHAPTER V

JUVENILE FEMALES DOMINATE DISPERSAL OF COROPHIID AMPHIPODS IN A NEW ZEALAND ESTUARY

Key-words: Corophium, Inbreeding avoidance, *Paracorophium*, Sex-bias, Tidal cycles

ABSTRACT

We examined the dispersal dynamics of the corophiid amphipods, *Paracorophium* spp. from a temperate estuarine habitat. During ten sampling occasions spanning 13 months, we measured the number, size, and determined the sex of individuals in the sediment and those transported in bed-load and suspended-load during two tidal cycles. Significantly greater numbers of juvenile females were caught in the bed-load and in suspension during the ebb tide relative to the flood tide, but this was not found for adults or juvenile males. Smaller individuals (<3 mm) were more often caught in the bed-load and in suspension, and adults had a significantly smaller body size compared to those in the sediment. Comparisons of adult sex-ratios suggest that adult males were significantly over-represented in the bed-load and in suspension compared to the sediment. However, the juvenile sex-ratios were significantly more female-biased in the sediment and in suspension compared to those in the bed-load. We suggest that the greater proportion of adult males in the bed-load and in suspension is likely due to reproductive behaviour (e.g. searching for mates), whereas the juvenile females in suspension are likely to be dispersing. We conclude that female-biased juvenile dispersal is an effective strategy to avoid inbreeding, reduce inter- and intraspecific competition, and maintain equilibrium among estuarine *Paracorophium* populations.

Dispersal among meta-populations is a key component of an organism's life-history affecting populations, the persistence of species, local adaptation, speciation, and other life history traits. For example, dispersal can be advantageous for avoiding predation (Hawkins 1985), competition among kin (Hamilton and May 1977; Taylor 1988) and for preventing inbreeding (Pusey 1987; Waser et al. 1994; Perrin and Mazalov 1999). Hence, assessing populations and communities necessitates an understanding of the nature and extent of dispersal linking them (Palmer et al. 1996) and has important consequences for the recovery of benthic assemblages from natural and anthropogenic disturbance.

Studies focussing on the dispersal of aquatic organisms have generally targeted taxa that have a pelagic larval stage (e.g. Söderström 1987; Boehlert and Mundy 1988; Abelson and Denny 1997; Garrison 1999). For those taxa lacking dispersive larvae (e.g. amphipods) the life-stage involved in dispersal is not clear (Hughes 1988; Lawrie and Raffaelli 1998a, 1998b; Ford and Paterson 2001). However, dispersal early in the life-cycle is often associated with reducing overcrowding that would otherwise lead to competition, and would be favoured by natural selection in preventing high density aggregations of related individuals (Pusey 1987; Hughes 1988; Jenson and Kristensen 1990). High rates of inbreeding are a direct consequence of philopatry of both sexes, and the dispersal of one sex should help prevent close inbreeding (Pusey 1987; Waser et al. 1994; Perrin and Mazalov 1999). Sex-biased dispersal has previously been documented in insects (Waldbauer and Sternburg 1979), fish, amphibians, and reptiles (Waldman and McKinnon 1993), and in general tends to be female-biased in birds, while male-

biased in mammals (Greenwood 1980; Pusey 1987). However, no studies have yet determined any potential sex-bias for juvenile amphipods, although Rajagopal et al. (1999) suggested that the female bias observed in adult *Corophium* may be due to high swimming activity of males in search of females.

For corophiid amphipods dispersal was initially thought to be accomplished by adults (Hart 1930; Meadows and Reid 1966) until Hughes (1988) showed that *Corophium volutator* juveniles were more frequently found in the plankton than any other size class, and that selective adult transport over long distances was unlikely. Furthermore, Lawrie and Raffaelli (1998a) showed that juveniles constituted the greatest proportion of swimming individuals and concluded that this was actual dispersal, with adult movement restricted mostly to males and likely to be related to reproductive behaviour. The behaviour of *C. volutator* in still versus flowing water was assessed by Ford and Paterson (2001) and suggests that juveniles could disperse far greater distances than adults. Hence, at least for *C. volutator*, natal dispersal appears to be most likely.

Here, we targeted the southern hemisphere corophiid amphipod *Paracorophium*—the most common benthic amphipod of muddy sandflats in New Zealand (Chapman et al. 2002; Stevens et al. 2002), and an important component of estuarine food webs (Segestråle 1959; Peer et al. 1986; Wilson 1989). Limited data on *Paracorophium* have shown that juveniles dominated recolonisation of sediment, and that adult sex-ratios are also female-biased (Mischewski 1994; Ford et al. 1999; Wong 1999). However, little is known about potential changes in sex ratios relative to life history stages and the implications this will have for determining population structure. Accordingly, we assessed whether the sex-bias for *Paracorophium* adults originates from differences in gender-specific aspects of

adult behaviour as suggested by Rajagopal et al. (1999). Furthermore, we hypothesised that if natal dispersal occurs in *Paracorophium*, then this should be sex-biased to avoid inbreeding and reduce competition (*sensu* Greenwood 1980).

MATERIALS AND METHODS

Study area

The Waimapu Estuary is a semi-exposed intertidal sandflat with a single river channel (Fig. 1). An initial survey was carried out of the entire Waimapu Estuary and nearby estuaries, indicating that amphipods were mostly restricted to the inter-tidal region surrounding the mouth of the river and none were found in the outer sub-tidal region (Fig. 1). We chose two sites to assess corophiid amphipods—Site 1 was located near the river mouth (at low tide) where we identified three corophiid amphipod species in the benthos: *Paracorophium lucasi* (Hurley 1954) Chapman 2002, *P. excavatum* (Thomson 1884) Stebbing 1899, and *P. brisbanensis* Chapman 2002—Site 2 was located 300 m downstream in the channel running through the estuary where no benthic amphipods were found (Fig. 1). We reasoned that Site 1 would capture both localised movement and larger scale dispersal due to the resident population, but at Site 2 individuals caught in the traps would be emigrating (on an ebb tide) or immigrating (flood tide).

The hydrological characteristics of the sites were assessed from October 1999 to October 2000. The estuary had approximately equal ebb and flood tides (semi-diurnal) with a tidal range from 2.6 m to 3.2 m (mean=2.9±0.2 m). During the ebb tide the water column was well mixed with a mean velocity of 0.27±0.01 m s^{-1}

(range 0.08–0.44 m s^{-1}) and was entirely freshwater for the last two hours of the tide (mean salinity=1.3±0.3 ‰). The flooding tide was detected by an intrusion of salt-water at Site 2 beneath the freshwater surface water. Within an hour the saltwater reached Site 1, and within 2 hours both sites were well-mixed (mean salinity=13.1±0.99 ‰) with a mean velocity of 0.11±0.01 m s^{-1} (range 0.01–0.23 m s^{-1}), although Site 1 consistently had a thin freshwater (20–30 cm) surface layer. At high tide, the depth at Site 1 ranged between 1.4–1.7 m and had a mean salinity of 17.2±0.1 ‰, and at Site 2 the depth ranged between 1.2–1.4 m with a mean salinity of 17.7±0.03 ‰. The mean riverine output during the sampling period (11 October 1999–12 October 2000) was 2.4 m^3 s^{-1}. On the ten sampling dates, flow varied from 0.8 m^3 s^{-1} in March 2000 to 5.3 m^3 s^{-1} in July 2000.

Sampling of amphipods

We collected samples between October 1999 and October 2000. Based on previous studies (Mischewski 1994; Hewitt et al. 1997; Wong 1999), we used three sediment cores to a depth of 5 cm to sample the resident amphipods at Site 1 on each sample date using a hand-held 11 cm diameter sediment corer at low tide (95 cm^2 cross-sectional area, 475 cm^3 volume).

Bed-load and suspended-load traps were used to assess movement of *Paracorophium* within the estuary. The bed-load traps were a 3 cm diameter core of 60 cm depth with an aspect ratio of 20 (Emerson and Grant 1991). The suspended-load traps were based on previous designs that have been used to capture macrofauna in the plankton (Butman 1986; Hewitt et al. 1997; Lawrie and

Raffaelli 1998b; Ford et al. 1999). We used a cylindrical container (10×15 cm, aspect ratio=1.5) with 2×2 cm baffling-tubes inserted in the opening to reduce vortices, and therefore minimise re-suspension of material within the trap. For traps with an aspect ratio less than 2, baffling has been shown to increase trapping efficiency (Butman 1986). These traps were suspended on a pole 30-40 cm above the substrate such that they remained submerged during low tide and had minimum exposure to the 1-2 hour period of stratification in the water column during the flood tide. At both sites, ten traps of each type were positioned across the main flow of the estuary. Traps were set at the end of an ebb tide and sampling proceeded over two tidal cycles (24 hours). The first sample was obtained at the end of the flood tide (after 6 hours) and the second sample was taken at the end of the following ebb tide (after 12 hours). This procedure was repeated for the following tidal cycle. This sampling regime resulted in suspended-load and bed-load samples for the ebb and flood tide with ten replicates at each site.

All samples were sieved through 10 mm and 2 mm mesh in the field, and the resulting sample was subjected to further sieving (using 250 μm mesh) in the laboratory. All biological material retained by the sieves was preserved in 70% ethanol for further measurement and classification. To identify mature *Paracorophium* we used the diagnostic characters suggested by Chapman (2002) and Chapman et al. (2002). However, using maxillipede and gnathapod 2 characters, specific identification of *Paracorophium* was not possible for individuals below 2.2 mm, and often identification was difficult for female individuals below 3.0 mm. Accordingly, we refer to the 3-species complex as *Paracorophium* spp.

Sex determination of *Paracorophium* was based on the sexually dimorphic gnathapod 2. We separated all individuals into the following demographic categories: adult males and females (>2.2 mm in body length); juvenile males and females (<2.2 mm); and gravid females. Gravid females were further classified according to the stage of the embryos in the marsupium. These stages are separated into five categories—stage I: the presence of first cleavage, holoblastic eggs; stage II: a ventral cleft appears and extends into a horseshoe-shaped furrow; stage III: cephalothorax turns orange-red; stage IV: cephalothorax large, abdomen small, limbs biramous, and red pigmented eyespots appear; and stage V: hatched embryo in the marsupium (Rajagopal et al. 1999).

Initially, 447 individuals were measured by head lengths as well as body lengths to the nearest 50 μm along the anterior margin of the rostrum to the posterior margin of the telson using a stereo-microscope with an eyepiece micrometer. These measurements were compared using a linear regression model that found a highly significant relationship between head and body lengths ($y=0.073+0.115x$, $R^2=0.95$, $P<0.001$). Accordingly, for the remaining 3423 individuals, we used head length to estimate overall body size.

Statistical analyses

Total numbers of individuals between Site 1 and Site 2 were compared among dates and between the tidal cycles (comparisons were done separately for the bed-load and suspended-load traps) and analysed using a repeated measures ANOVA. We used a linear regression to determine if body size changed over time, and multiple ANOVA's (type III) were used to compare body sizes among site, trap

type, and tide. Multiple comparisons were made using a Tukey test when significant ($P<0.05$) differences were found. To examine sex-ratios we combined sites (preliminary analyses indicated that sex-ratios were similar between the two sites and over time) and used 2×2 contingency tables to compare among the sediment, bed-load and suspended-load samples. In all cases, assumptions of parametric analyses were examined using the predicted versus observed standardised residuals. Where departures from homogeneity were detected, these data were log (×+1) transformed. Prior to running regression analyses, we further tested data to see if the model was appropriate (Zar 1984). Data were analysed using the statistical package SPSS for Windows version 10.7 (SPSS Inc.), and STATISTIX 7 for Windows (Analytical Software).

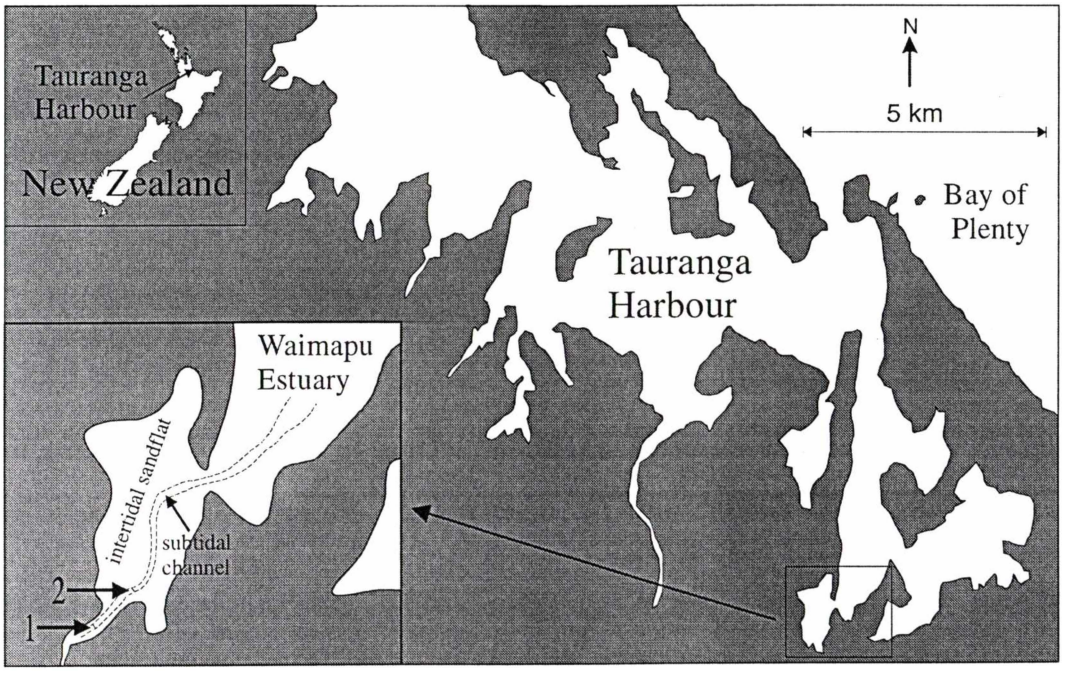

FIGURE 1. Location of the study site at Waimapu Estuary, Tauranga Harbour, New Zealand (37°40'S, 176°10'E). Inset: location of Site 1 at the mouth of the estuary and Site 2 in the sub-tidal channel running through the estuary.

Adult and juvenile transport

There were no significant differences between the number of individuals caught at Site 1 and Site 2 for the bed-load or suspended-load ($P>0.07$, in all cases; Table 1). Pooled data from Site 1 and Site 2 showed that juvenile females (<2.2 mm) were most frequently caught in the bed-load and suspended-load (mean=65.4%, range=52-75%), with adult females (11.7%, 7-23%), juvenile males (10.3%, 5-14%), adult males (10.0%, 4-19%), and gravid females (2.6%, 0.2-10%) making up the remainder (Fig. 2). We found a significantly greater number of juvenile females on the ebb tide for both the bed-load ($P<0.01$) and suspended-load ($P<0.001$; Table 1). The ebb tide was also found to have a significantly greater number of juvenile males in the suspended-load ($P<0.05$), and a significantly greater number of adult females in the bed-load ($P<0.01$; Table 1).

The mean body size of all individuals did not differ over time at either Site 1, Site 2 or in the sediment ($P>0.21$, in all cases). Table 2 shows the mean body sizes of adult males and females, gravid females, and juvenile males and females for each site, trap, and tide averaged over all sampling dates. Body sizes of juvenile males and females were significantly different between Site 1, Site 2, and the sediment ($P<0.01$, in both cases; Table 3). Post-hoc tests revealed that juvenile males were significantly larger at Site 1 compared to Site 2 (Tukey test, $P<0.001$), and juvenile females from Site 1 were significantly larger compared to those in the sediment and at Site 2 (Tukey test, $P<0.001$, in both cases). Body size differed

significantly for adult males and females among the sediment, Site 1, and Site 2 ($P<0.05$, in both cases; Table 3) and post-hoc tests revealed that adult male and female body size decreased from the sediment to Site 1, and further still at Site 2 (Tukey test, $P<0.05$, in all cases). The body size of gravid females was also found to decrease from the sediment to the bed-load, and further still in the suspended-load ($P<0.05$; Tables 2, 3).

Gravid females were present in the sediment and bed-load during October 1999 with brood stages I-IV, but were not caught in the suspended-load (Table 2). The sediment contained all brood stages, including stage V (mature) from November and December 1999 through February and March 2000 (Fig. 2). In the sediment from February to July 2000, juvenile females made up 14%, with 68% adult females (39% of adult females were gravid). In May 2000 a large proportion of females in the sediment were gravid (52%), but only two gravid individuals were caught in the bed-load and none in the suspended-load (Fig. 2). Over winter (June and July) only stage I brood was found. With the onset of spring (September 2000) we observed stage IV brood, and stage V in October 2000 (Fig. 2) when juvenile females again dominated the sediment (69%) with 24% adult females (59% gravid).

TABLE 1. Repeated measures ANOVA for each demography (e.g. adult male) for the bed-load and suspended-load traps (performed separately). Date=the ten sampling dates, Site=Site 1 and Site 2, Tide=ebb and flood tides, M=adult male, F=adult female, GF=gravid female, JM=juvenile male, JF=juvenile female.

			BED-LOAD			SUSPENDED-LOAD		
	Category	***df***	**MS**	***F ratio***	***P***	**MS**	***F ratio***	***P***
Corrected Model	M	11	1.81	3.24	0.000	1.16	5.03	0.000
	F	11	5.20	5.27	0.000	0.43	2.55	0.004
	GF	11	0.47	3.58	0.000	0.07	1.71	0.066
	JM	11	3.27	4.21	0.000	1.55	7.79	0.000
	JF	11	99.75	5.28	0.000	58.03	9.03	0.000
Intercept	M	1	46.08	82.16	0.000	11.76	50.80	0.000
	F	1	88.44	89.55	0.000	6.30	37.08	0.000
	GF	1	3.25	24.96	0.000	0.72	18.57	0.000
	JM	1	52.02	66.98	0.000	10.81	54.22	0.000
	JF	1	1740.50	92.19	0.000	619.52	96.44	0.000
DATE	M	9	1.93	3.45	0.000	1.41	6.07	0.000
	F	9	5.01	5.07	0.000	0.43	2.52	0.007
	GF	9	0.51	3.93	0.000	0.07	1.73	0.078
	JM	9	3.74	4.81	0.000	1.73	8.68	0.000
	JF	9	101.54	5.38	0.000	61.80	9.62	0.000
SITE	M	1	1.13	2.01	0.157	0.10	0.44	0.509
	F	1	1.13	1.14	0.286	0.55	3.24	0.072
	GF	1	0.06	0.47	0.493	0.05	1.16	0.282
	JM	1	0.72	0.93	0.336	0.45	2.26	0.133
	JF	1	2.88	0.15	0.696	0.25	0.04	0.845
TIDE	M	1	1.45	2.58	0.109	0.06	0.26	0.607
	F	1	11.05	11.18	0.001	0.36	2.13	0.145
	GF	1	0.45	3.46	0.063	0.08	2.06	0.151
	JM	1	1.62	2.09	0.149	1.05	5.27	0.022
	JF	1	180.50	9.56	0.002	81.92	12.75	0.000
Error	M	788	0.56			0.23		
	F	788	0.99			0.17		
	GF	788	0.13			0.04		
	JM	788	0.78			0.20		
	JF	788	18.88			6.42		
Corrected Total	M	799						
	F	799						
	GF	799						
	JM	799						
	JF	799						

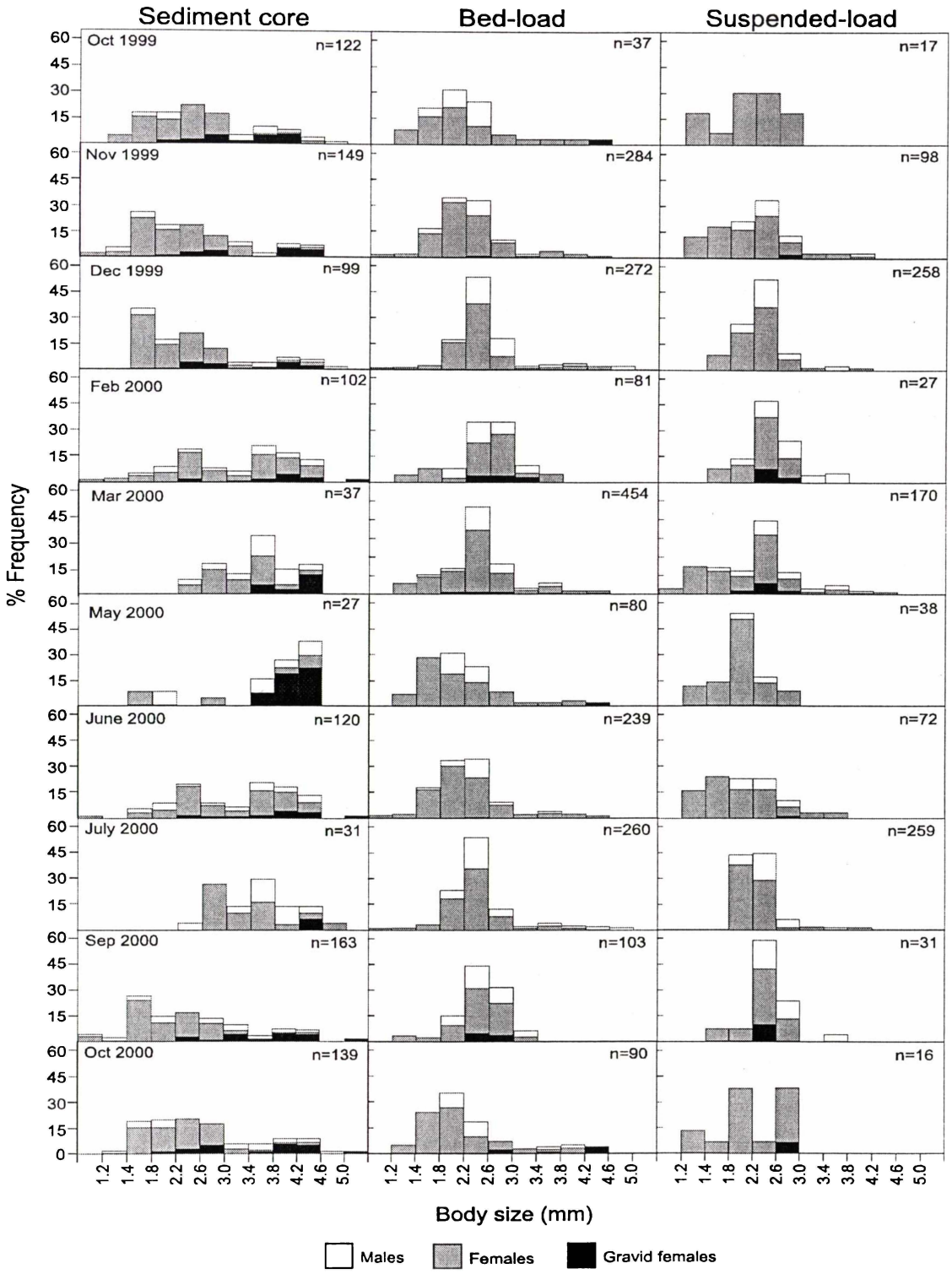

FIGURE 2. Length frequency histograms (body size) of the demographic categories (Males, Females, Gravids) for *Paracorophium* spp. from October 1999 to October 2000. All individuals from Site 1 and Site 2 were combined and grouped into 0.4 mm size classes (*n*=sample size).

TABLE 2. Mean body size (mm) and standard error (in parentheses) at Site 1 and Site 2 for each demography (e.g. Adult male) averaged over all sampling dates. *=when gravid females with stage IV brood were present, and †=when stage V brood was found.

			Adult male	**Adult female**	**Gravid female**	**Juvenile male**	**Juvenile female**
Site 1	Sediment		3.76 (0.06)	3.44 (0.05)	3.57 (0.06)	2.20 (0.03)	2.03 (0.02)
Site 1	Suspended	Ebb	2.62 (0.08)	3.16 (0.12)	2.34 (0.04)*	2.30 (0.04)	2.03 (0.03)
		Flood	3.08 (0.13)	3.11 (0.12)	2.68 (0.06)	2.23 (0.10)	2.15 (0.03)
	Bed-load	Ebb	2.72 (0.07)	3.18 (0.08)	3.01 (0.16)†	2.32 (0.03)	2.15 (0.02)
		Flood	2.85 (0.11)	3.29 (0.10)	2.69 (0.09)*	2.31 (0.03)	2.23 (0.03)
Site 2	Suspended	Ebb	2.48 (0.03)	2.91 (0.14)	2.50 (0.14)*	2.23 (0.03)	2.00 (0.02)
		Flood	2.48 (0.05)	3.00 (0.19)	nil	2.25 (0.03)	2.08 (0.03)
	Bed-load	Ebb	2.82 (0.09)	2.79 (0.05)	2.98 (0.24)†	2.21 (0.03)	2.02 (0.02)
		Flood	2.45 (0.06)	2.87 (0.08)	2.63 (0.35)†	2.24 (0.03)	2.12 (0.02)

TABLE 3. Three-way ANOVA's for each demography (e.g. Adult male) comparing the size distributions averaged over all sampling dates. Non-significant interactions were removed from each model. SITE=Site 1, Site 2; sediment core at site 1; TRAP=bed-load, suspended-load, sediment core at site 1; TIDE=ebb and flood tides.

Category	*df*	MS	*F ratio*	*P*
Adult male				
Corrected Model	4	21.96	77.05	0.000
Intercept	1	3179.12	11154.37	0.000
SITE	1	2.21	7.74	0.006
TRAP	1	0.29	1.01	0.315
TIDE	1	0.03	0.01	0.918
Error	374	0.29		
Corrected Total	378			
Adult female				
Corrected Model	4	8.77	25.20	0.000
Intercept	1	3415.99	9808.93	0.000
SITE	1	9.63	27.65	0.000
TRAP	1	0.00	0.01	0.918
TIDE	1	0.32	0.91	0.340
Error	503	0.35		
Corrected Total	507			
Gravid female				
Corrected Model	4	9.38	15.89	0.000
Intercept	1	739.35	1253.01	0.000
SITE	1	0.00	0.00	0.976
TRAP	1	3.66	6.20	0.013
TIDE	1	0.14	0.23	0.632
Error	244	0.59		
Corrected Total	248			
Juvenile male				
Corrected Model	4	0.13	3.07	0.017
Intercept	1	171.55	4150.51	0.000
SITE	1	0.47	11.36	0.001
TRAP	1	0.00	0.01	0.944
TIDE	1	0.00	0.00	0.964
Error	331	0.04		
Corrected Total	335			
Juvenile female				
Corrected Model	4	2.85	19.58	0.000
Intercept	1	8325.01	57107.26	0.000
SITE	1	4.11	28.16	0.000
TRAP	1	2.06	14.14	0.000
TIDE	1	3.80	26.09	0.000
Error	2348	0.15		
Corrected Total	2352			

The sex-ratios over all sampling dates showed that individuals in the sediment were female-biased for adults (3.6:1, n=467) and highly female-biased for juveniles (9.8:1, n=507). The bed-load was also female-biased for both adults (1.7:1, n=509) and juveniles (5.8:1, n=1384). The suspended-load had an approximately even ratio of adult females to males (0.98:1, n=192) but juveniles were highly female-biased (7.6:1, n=797).

Results of 2×2 contingency tables showed that adult and juvenile sex-ratios in the sediment were significantly more female-biased compared to the bed-load (adult: χ^2_1=30.0, P<0.001, n=976; juvenile: χ^2_1=9.6, P<0.01, n=1891). Likewise, adult and juvenile sex-ratios in the suspended-load were significantly more female-biased compared to the bed-load (adult: χ^2_1=9.4, P<0.01, n=701; juvenile: χ^2_1=4.1, P<0.05, n=2181). The adult sex-ratio in the sediment was significantly more female-biased compared to the suspended-load (χ^2_1=9.4, P<0.01, n=701). However, the juvenile female bias in the sediment was not significantly different to the suspended-load (χ^2_1=1.9, P=0.17, n=1304).

Dispersal is a key component of an organism's life-history determining patterns of distribution, recruitment of individuals and the colonisation of new habitat. In some situations selection may favour the overproduction of offspring of one gender (see Frank 1990 for review), creating a bias in the juvenile sex-ratio. However, this can also occur in the adult sex-ratio if females and males differ in various aspects of their life histories (Rajagopal et al. 1999), such as differing rates of mortality, dispersal, or the age at which they reach maturity.

We found that the adult sex-ratio was female-biased in the sediment (3.6:1), and this bias decreased in the bed-load samples (1.7:1), and further still in the suspended-load samples from the water column (0.98:1). The decrease in adult sex-ratios was due mostly to an increase in males (from n=101 to 192) and a reduction in gravid females (from n=173 to 51) (Fig. 2). This is most likely the result of reproductive behaviour for corophiid amphipods. For example, male *Corophium volutator* are known to search the sediment substrate for burrows that contain receptive females (Fish and Mills 1979; Hughes 1988; Lawrie and Raffaelli 1998a) and high swimming activity of *C. curvispinum* males in search of females has been suggested (Rajagopal et al. 1999). Furthermore, the adult female amphipod *Jassa marmorata* also spend much of their time in burrows while feeding, mating and brooding young, while adult males are more mobile (Clark and Caudill 2001).

For juveniles, we found that the female bias decreased from the sediment to the bed-load (9.8:1 to 5.8:1, respectively). However, we also found that the juvenile female bias increased from the bed-load to the suspended-load (7.6:1), suggesting

that more juvenile females were entering the water column. Furthermore, we found no difference in the juvenile female-biased sex-ratio between individuals in the sediment or in suspension. The variation in sex-ratios between the bed-load and suspended-load indicate that the water column was dominated by juvenile females (Fig. 2). The benefits of juvenile sex-biased dispersal are likely to be similar among taxa (see Greenwood 1980). For example, juvenile dispersal can have the effect of reducing overcrowding (Jenson and Kristensen 1990) resulting in a reduction of inter- and intraspecific competition, but sex-biased dispersal may also be necessary to avoid inbreeding (Pusey 1987; Frank 1990; Waser et al. 1994; Perrin and Mazalov 1999).

Previous studies have identified that the most abundant category of swimming *C. volutator* individuals were juveniles, and that this was most often during the ebb tide (Hughes 1988; Lawrie and Raffaelli 1998b; Ford and Paterson 2001). We also found that the total number of juveniles increased significantly during the ebb tide (Table 1). Smaller *Paracorophium* individuals, particularly juvenile females are more likely to be swept along by the stronger currents of the ebb tide (Sanders 1958; Ford and Paterson 2001) because they make up a large proportion of the smaller (<3.0 mm) size class (see Fig. 2). Similarly, a small size range was observed for surface-dwelling macrofaunal species caught in nets placed in the water column in Manukau Harbour, New Zealand (Hewitt et al. 1997), and is consistent with our study. However, in situations when populations are dense intraspecific competition may increase, and this has also been linked to an increased emigration of *Corophium* juveniles (Jenson and Kristensen 1990). The behaviour of *Paracorophium* in the water column, as well as why individuals leave the sediment is likely to be important for recruitment of individuals and for

the colonisation of new habitat patches (Palmer et al. 1996) and future studies would be useful to determine the gender-specific behaviour of *Paracorophium* juveniles.

In contrast to *Paracorophium* juveniles, adult movement was not significantly different between the tidal cycles (Table 1). Hughes (1988) found extensive swimming of *C. volutator* adults on the ebb tide, yet populations were maintained 15 km upstream. Our data suggest that populations are maintained (samples from month to month showed relatively small variability compared to the bed-load and suspended-load) and swimming by adults on the flood tide may partially compensate for downstream transport during the ebb tide. It is likely that *Paracorophium* male adults crawling and/or swimming near the substrate surface over short distances (centimetres to metres) may act as potential random dispersers, but the probability that they will become long-distance dispersers (10s–100s of metres) is dependent on the size of the individual. Adult dispersal, has been shown to be an actively, as opposed to passively controlled transport mechanism for *C. volutator*, but the distance travelled depends on the size of the individual and current velocity (Hughes 1988; Lawrie and Raffaelli 1998b; Ford and Paterson 2001). Furthermore, our data on *Paracorophium* support Ford and Paterson's (2001) results showing that the swimming behaviour of adult *C. volutator* did not change in still versus flowing water and flow velocity did not seem to be a cue to swim, but smaller individuals had less control and travelled further, particularly when exposed to current flows greater than 1.0 cm s^{-1}.

The small size of juveniles (see Table 2) suggests that they can be readily transported in the bed-load and in suspension. However, adult females were less likely to be found in the bed-load and in suspension compared to adult males, the

reverse of what we found for juveniles. Furthermore, body size of *Paracorophium* adults decreased with the distance away from the sediment at Site 1 (in both vertical and horizontal distance), such that individuals from Site 2 were significantly smaller than those from Site 1, and that individuals in suspension were significantly smaller than those in the bed-load at both sites (Tables 2, 3). Body sizes of individuals at both sites were also significantly smaller to individuals from the sediment taken at Site 1, but this pattern was not observed for juveniles (Tables 2, 3). Size-selective transport was particularly evident for *Paracorophium* gravid females with stage V (mature) brood in the sediment, which were found to be significantly larger ($P<0.001$) compared to those in the bed-load samples at both sites, but absent entirely from the suspended-load (Fig. 2).

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THESIS CONCLUSION

Dispersal is a means of colonising and re-colonising regions, as well as an agent of individual exchange between geographically separated populations. Phylogeographic studies have added new rigour to the field by enabling a critical evaluation of the gene flow connecting populations, histories of range alteration and of refugial origins (e.g. Avise 1998; Bermingham and Moritz 1998). Such investigations have provided new insights into the interactions of ecology and life history in shaping the evolution and distribution of extant populations. My research incorporated population genetic and biogeographical data for arthropods, and compared this to the geological record. Isolation and divergence among fragmented habitats have been promoted throughout the late Cenozoic and infer past histories of glacial extirpation in addition to possible isolating effects of geological alterations. These patterns are consistent with colonisation and limited dispersal pathways for these endemic Antarctic and New Zealand arthropods.

Despite a shifting thermal regime during the Pleistocene, endemic terrestrial arthropods in Antarctica are likely to have persisted in ice-free areas. Their prevalence in regions that were both ice-covered during the Pleistocene and distant from glacial refugia also indicates that many extant populations owe their origin to recent range expansion. Distributional records from Lakes Chad and Bonney in Taylor Valley, and from Cape Geology and Mt. England at Granite Harbour, indicate local dispersal within the last 40 years for Antarctic terrestrial arthropods (Chapter I). Furthermore, the present-day distribution of arthropods was restricted in comparison to previous records and implies anthropogenic impact in the vicinity of the McMurdo Station and former North Base. It is only

with accurate distributional records that species ranges can be examined, particularly with an increasing human presence in Antarctica, as well as the possibility of new habitats becoming available as a result of climatic shifts.

Passive dispersal has been suggested for introductions of collembolan species in Eastern Antarctica (Greenslade and Wise 1984), and the effects of an increase in human activity in Antarctica has been recognized (e.g. Strandtmann and George 1973; Broadbent 1994). However, considerably more is known about biological introductions of aquatic taxa world-wide (e.g. Mills et al. 1993), with crustaceans identified as the most abundant taxa in ballast water (Carlton and Geller 1993). By enabling movement of organisms by these means, bays, estuaries and inland waters have become some of the most threatened ecosystems in the world (Carlton and Geller 1993). The introduction of *Paracorophium brisbanensis* (Chapter III) may have consequences for the native fauna and flora, particularly if this species spreads to other New Zealand ports. Accordingly, studies are urgently required in order to assess the biological impact of this species introduction.

The behaviour of *Paracorophium* in the water column is likely to be important for creating patterns of distribution, for recruitment of individuals, and for colonisation of new habitat patches (Palmer et al. 1996). My present research (Chapter V) suggests that juvenile females dominate the potential dispersal of *Paracorophium* in Tauranga Harbour. Dispersal early in life has often been associated with reducing intraspecific competition, and natural selection is believed to favour dispersal by juveniles, preventing both high density aggregations of related individuals and the associated increased mortality risk (Hughes 1988). For *Paracorophium* juveniles it is possible that entering the water

column may be either passive entrainment or a behavioural response to overcrowding. Such natal dispersal, however, may be particularly beneficial when temporal fluctuations occur in habitat suitability or in situations of crowding. High rates of inbreeding are a direct consequence of philopatry of both sexes, and dispersal of one sex should help prevent close inbreeding (Pusey 1987; Waser et al. 1994; Perrin and Mazalov 1999). Sex-biased dispersal may also be due to overcrowding leading to inter- or intraspecific competition (Pusey 1987; Hughes 1988; Jenson and Kristensen 1990). However, the processes that control dispersal can be quite separate from the physical and biological processes that determine local resources, habitat suitability, and population densities and there are a number of significant studies that have addressed some of these issues (Nowell and Jumars 1984; Ólafsson et al. 1994; Eckman 1996; Abelson and Denny 1997). These studies emphasise the necessity to examine the potential role of dispersal processes when studying the ecology of populations.

To further ascertain the degree of contact between fragmented habitats in southern Victoria Land, I carried out a comprehensive analysis of population genetic structure to evaluate origins and extent of gene flow among populations (Chapter II). Examination of the spatial genetic structure of *G. hodgsoni* has revealed extreme isolation through habitat fragmentation, founding/bottleneck events followed by range expansion over long-term glacial effects. Overall, dispersal appears limited and reflects historical patterns found for other terrestrial taxa in Antarctica (Courtright et al. 2000; Fanciulli et al. 2001; Frati et al. 2001). The sympatric populations throughout Taylor Valley are most likely the result of genetic drift with past geographic isolation and may reflect incipient speciational processes. These data illustrate the usefulness of using a phylogeographic

approach to infer various speciation and colonisation mechanisms, particularly when coupled with population genetic, geological, and biogeographical data. Comparative phylogeography using other endemic Antarctic taxa, particularly nematodes, tardigrades, and free-living mites from southern Victoria Land will enable further examination of colonisation routes, range expansion, and long-term persistence of taxa in this extremely fragmented habitat.

Isolation among habitats was also revealed for *P. lucasi* and *P. excavatum* species complexes from New Zealand (Chapter IV). However, these genetic patterns are much older than the climatic shifts during the Pleistocene, and reflect speciational processes throughout the Pliocene and Miocene. The detection of sibling species that are genetically distinct and geographically isolated is also consistent with patterns found for other amphipods (e.g. De Matthaeis et al. 2000). With no larval stage, isolation among populations may greatly reduce dispersal opportunities where geographical barriers are present. In particular, historic geological events and the patterns of present-day ocean currents may be sufficient to isolate *Paracorophium* populations. With limited gene flow among biogeographic regions the apparent fragmentation of an ancestral regional gene pool suggests that allopatric isolation has played an important role in the origin of taxon diversity for New Zealand corophiid amphipods.

Enhancing our knowledge on the rates and causes of species expansion and decline through examination of the processes responsible for the maintenance of both indigenous and exotic populations is important in efforts to conserve biodiversity. Within the southern Pacific region, particularly Antarctica, the sub-antarctic, New Zealand, and Australia it is possible to identify at least three research areas that would benefit from further research:

1. Small-scale dispersal experiments, examining vertical and horizontal movements of arthropods in response to different thermal regimes and humidity gradients would be useful in establishing potential behavioural responses to environmental changes in Antarctica.
2. Long-term monitoring of dispersal to determine frequency of translocation events and their rates of successful establishment. Such information would provide estimates on population expansion and decline and the consequences that rare translocation may have for isolated populations.
3. We know very little about why some taxa are morphologically conserved, yet others show remarkable levels of diversity. Studies investigating the genetic and habitat developmental constraints on morphology would be useful, particularly for taxa among diverse habitats.

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APPENDIX 1

SOIL FAUNA OF ANTARCTIC COASTAL LANDSCAPES[†]

[†]This appendix includes data from Chapter II published under the same title as: Hogg ID and Stevens MI (2002). In: Geoecology of Antarctic Ice-Free Coastal Landscapes, Ecological Studies Analysis and Synthesis Volume 154 (eds Beyer L, Bölter M), pp. 265-278. Springer-Verlag, Berlin.

Introduction

The endemic soil fauna of the maritime and continental Antarctic is taxonomically limited and consists almost entirely of arthropods, particularly the springtails (Collembola), and mites (Acari). Chironomid midges (Diptera) and beetles (Coleoptera) have also been recorded from the Antarctic Peninsula and the South Shetland, and Orkney Islands (Gressitt 1964, Balfour-Browne and Tilbrook 1966, Wirth and Gressitt 1967). Notable exceptions to this generalisation are the smaller protozoans, tardigrades, rotifers and nematodes, which are also commonly found in both continental and maritime areas (Gressitt 1965, Utsugi and Ohyama 1989, Bullini et al. 1994). Unfortunately, ecological work on these latter taxa is limited, and our focus here will be primarily on the larger, soil-dwelling arthropods.

Much of the information available on the soil fauna, has come from reports documenting the fauna and/or describing new species (e.g. Janetschek 1963, Weiner 1980, Miller et al. 1988), with comparatively fewer studies examining ecological and physiological aspects (e.g. Matsuda 1977, Block 1985). More recently, interest has been targeted towards predicting the responses of invertebrates to environmental change (e.g. Block and Harrison 1995, Kennedy 1995) as well as evaluating patterns of diversity and dispersal among locations through studies of population genetics (e.g. Valbonesi et al. 1994, Frati et al. 1996). As we will discuss, these latter issues may be inextricably linked.

In this chapter we will discuss the invertebrate soil faunas of the continental and maritime Antarctic. We use the criteria and terminology of Holdgate (1977) referring to the Antarctic Peninsula and South Shetland Islands as "maritime" and all other

regions on the Antarctic coast (and inland) as "continental". In keeping with the theme of this volume we pay particular attention to those taxa associated with the continental oases (Windmill Islands and Ongul Island) of East Antarctica and those of the South Shetland Islands. The remainder of the chapter will consist of 5 sections. Sections 15.2-15.3 will compare and contrast the faunal composition of soil invertebrates from the two regions, and discuss ecological and physiological factors affecting their distribution. Section 15.4 will consider the interhabitat dispersal of taxa as well as the role of population genetic studies in assessing levels of gene flow among habitats. For this purpose we will also draw on our own and other data from habitats in and around Victoria Land in the Ross Sea sector. Section 15.5 will discuss the potential responses of the soil fauna to environmental changes resulting from both global (e.g. enhanced greenhouse effect) and local disturbances (e.g. habitat destruction), and finally section 15.6 will provide a summary of further research needs.

Faunal Composition

From the earliest Antarctic expeditions, several reports have described, added to and revised the inventories of soil invertebrates (e.g. Willem 1902, Salmon 1962, Greenslade and Wise 1984). Perhaps the most comprehensive lists have been provided by Wise (1967, 1971) for the Collembola, and Hunter (1967), Strandtmann (1967) and Wallwork (1967) for the mites. To date, roughly 15 species of springtail and 25 species of mite have been recorded from the Antarctic continent. Of these, only two species of springtail and six species of mite have been recorded from Ongul

I. and Windmill Is. (Tables 15.1, 15.2, respectively). The nearby maritime regions (e.g. South Shetland Islands), have recorded higher levels of taxonomic richness with 16 species of springtail and 36 species of mite found on the South Shetland Islands alone (Tables 15.1, 15.2). These lists are undoubtedly incomplete and will continue to be modified, particularly with the advent of molecular taxonomy (e.g. Valbonesi et al. 1994). Such studies have been able to assess diversity to levels previously unattainable, and have frequently revealed the existence of previously unknown, morphologically cryptic species (e.g. Valbonesi et al. 1994, Carapelli et al. 1995a, 1995b).

The taxonomic richness seen throughout the maritime and continental Antarctica contrasts with the considerably greater species richness found on the subantarctic islands (e.g. Crozet, Kerguelen, Heard, Marion, Bird and Macquarie Islands) where between 50-150 species of land arthropods have been recorded. It is evident that subantarctic islands contain mostly cosmopolitan species (Greenslade 1987, Greenslade and Wise 1984, Greenslade and Wise 1986). The same is also true for maritime regions and Marshall and Pugh (1996) noted that of the total number of mite species found in the maritime Antarctic, 42.5% are endemic contrasting with 85.7% for the continental Antarctic. Within the maritime Antarctic, the South Shetland Islands may harbour among the greatest diversities of soil invertebrates. Block and Starý (1996) found that 19% of all oribatid mite species recorded in the maritime regions were found in the South Shetland Islands, the highest of any of the sites they surveyed. The status of the beetle taxa (endemic or introduced) remains uncertain (Balfour-Browne and Tilbrook 1966), and at least two other species – one dipteran

and one beetle have been confirmed as likely introductions to the Prince Charles Mountains, East Antarctica (Rounsevell 1978, 1979).

Protozoans, tardigrades, rotifers and nematode worms occur widely on the Antarctic continent and in maritime areas (Spain 1971, Jennings 1976a,b, Barker 1977, Dastych 1984, Shishida and Ohyama 1989, Miller et al. 1988, Valbonesi et al. 1994), although these groups appear to be restricted primarily to areas of very high moisture content (Pryor 1962, Tilbrook 1967). Several studies have documented the diversity of these groups, particularly tardigrades (e.g. Barker 1977, Miller et al. 1988, Dastych 1989, Utsugi and Ohyama 1989), and nematodes (Barker 1977, Shishida & Ohyama 1989). To date, over 30 species of tardigrade have been reported from the Antarctic continent, including five confirmed from the areas surrounding Syowa Station (Utsugi and Ohyama 1989), and four species from the Vestfold Hills (Miller et al. 1988). For nematodes, three species (*Plectus antarcticus, P. frigophilus and Scottnema lindsayi*) found by Shishida & Ohyama (1986), were the first records of nematodes from Syowa Station. Yeates (1979) had previously found two of these species (*Plectus antarcticus, P. frigophilus*) from the Bunger Oasis, as well as a species of *Helicotylenchus*.

Table 1. List of the collembolan (springtail) fauna found in the maritime Antarctic and continental locations.

Taxon	Site[a]	References
Cryptopygus antarcticus antarcticus Willem	S	Willem (1901), Wahlgren (1906), Tilbrook (1967), Wise (1967, 1971), Weiner (1980), Greenslade & Wise (1984), Ohyama & Shimada (1990), Greenslade (1995)
Cryptopygus caecus Wahlgren	S	Wahlgren (1906), Tilbrook (1967), Wise (1967, 1971, 1974)
Cryptopygus sverdrupi Lawrence	O	Sømme (1986), Lawrence (1978), Greenslade (1995)
Cryptopygus badasa n. sp.	S	Greenslade (1995), Convey et al. (1996)
Archisotoma brucei (Carpenter 1907)	S	Tilbrook (1967), Wise (1967, 1971), Greenslade (1995)
Isotoma (Folsomotoma) octooculata (Willem 1901)	S	Wahlgren (1906), Tilbrook (1967), Wise (1967, 1971), Greenslade (1995)
Isotoma (Folsomotoma) o. kerguelenensis (Enderlein 1903)	S	Gressitt & Weber (1959)
Folsomia candida Willem 1902	S	Greenslade & Wise (1984)
Friesea grisea (Schäeffer 1891)	SO	Wahlgren (1906), Salmon (1962), Tilbrook (1967), Wise (1967, 1971), Ohyama & Sugawara (1989), Greenslade (1995)
Friesea woyciechowskii Weiner	S	Weiner (1980), Greenslade (1995)
Friesea topo n. sp.	S	Greenslade (1995)
Tullbergia mixta Wahlgren	S	Wahlgren (1906), Tilbrook (1967), Wise (1967, 1971), Greenslade (1995)
Onychiurus sp. Strong	S	Greenslade & Wise (1984)
Protophorura sp.	S	Greenslade (1995)
Hypogastrura viatica (Tullberg 1872)	S	Hack (1949), Salmon (1962, 1964), Tilbrook (1967), Wise (1967, 1971), Greenslade & Wise (1984), Greenslade (1995)
Achorutoides antarcticus n. gen., n. sp.	S	Willem (1901)

[a] S, South Shetland Is.; O, Ongul I.

Table 2. List of the Acari (free-living mites) found in the maritime Antarctic and continental locations.

Taxon	Site[a]	References
Ceratozetes gracilis (Michael)	S	Niedbala (1986)
Liochthonius mollis (Hammer)	S	Tilbrook (1967), Wallwork (1967)
Liochthonius australis Covarrubias	S	Covarrubias (1968)
Oppia loxolineata Wallwork	S	Wallwork (1965, 1967), Tilbrook (1967)
Oppia loxolineata longipilosa Covarrubias	S	Covarrubias (1968), Niedbala (1986)
Oppia pepiptensis brevipectinata	S	Covarrubias (1968)
Austroppia crozetensis (Richters 1908)	S	Block & Starý (1996)
Globoppia loxolineata longipilosa Wallwork 1968	S	Block & Starý (1996)
Globoppia loxolineata (Wallwork)	S	Wallwork (1965), Block & Starý (1996)
Alaskozetes antarcticus antarcticus (Michael)	S	Wallwork (1965, 1967), Tilbrook (1967), Niedbala (1986), Ohyama & Shimada (1990), Block & Convey (1995)
Halozetes antarctica (Michael 1903)	S	Gressitt & Weber (1959)
Halozetes belgicae belgicae (Michael 1903)	S	Dalenius & Wilson (1958), Wallwork (1965, 1967), Niedbala (1986)
Halozetes belgicae longisetae Wallwork	S	Wallwork (1967), Niedbala (1986)
Halozetes impeditus sp. nov.	S	Niedbala (1986), Block & Starý (1996)
Antarcticola meyeri n. gen., n. sp.	O	Tilbrook (1967), Wallwork (1967), Rounsevell (1979), Ohyama & Sugawara (1989), Kanda et al. (1990)
Petrozetes oblongus nov. gen., nov. sp.	O	Sitnikova (1969)
Edwardzetes dentifer Hammer 1962	S	Block & Starý (1996)
Magellozetes antarcticus (Michael 1895)	S	Wallwork (1965), Block & Starý (1996)
Magellozetes processus Hammer 1962	S	Block & Starý (1996)
Maculobates nordenskjoeldi (Trägårdh)	S	Wallwork (1965)
Cryptolaelops (Gamasellus) racovitzai (Trouessart)	S	Tilbrook (1967), Jumeau & Usher (1987), Usher et al. (1989), Convey et al. (1996)
Parasitus sp.	S	Tilbrook (1967)
Rhombognathus gressitti Newell	S	Pugh (1993)
Nanorchestes antarcticus Strandtmann 1963	SOW	Tilbrook (1967), Ohyama & Matsuda (1977), Ohyama (1977, 1979, 1984), Rounsevell & Greenslade (1988), Heatwole et al. (1989)
Nanorchestes nivalis (Trouessart)	S	Strandtmann (1982), Convey et al. (2000)
Stereotydeus villosus (Trouessart)	S	Tilbrook (1967), Graham (1975)
Stereotydeus meyeri Strandtmann	O	Strandtmann (1967)
Rhagidia gigas gerlachei (Trouessart 1903)	S	Strandtmann (1967), Edwards & Usher (1987), Dalenius (1965)
Rhagidia leechi Strandtmann 1963	S	Tilbrook (1967)
Tydeus tilbrooki Strandtmann 1967	S	Tilbrook (1967)
Tydeus erebus Strandtmann 1967	OW	Johnstone et al. (1973), Ohyama (1977, 1979, 1984) Ohyama & Matsuda (1977), Rounsevell (1977),
Cocceupodes australis n. sp.	S	Strandtmann & Tilbrook (1968)
Eupodes minutus (Strandtmann 1967)	SO	Tilbrook (1967), Ohyama (1977, 1979), Ohyama & Matsuda (1977), Convey et al. (2000)

continued,

Table 15.2 continued,

Taxon	Site[a]	References
Eupodes parvus grahamensis n.ssp.	S	Booth et al. (1985)
Ereynetes macquariensis Fain	S	Strandtmann & Tilbrook (1968)
Bakerdania antarcticus (Mahunka)	S	Pugh (1993), Convey et al. (2000)

[a] S, South Shetland Is.; O, Ongul I.; W, Windmill Is.

Ecology and Physiology

Moisture has been described as the major factor affecting the distribution and abundance of antarctic soil invertebrates (Tilbrook 1967, Kennedy 1993). Temperature also plays a critical role in dictating the amount of available water as well as providing taxa with considerable physiological challenges at extreme levels (Strong 1967, Spain 1971, Block 1985, Kennedy 1993). Protozoans, tardigrades, rotifers and nematodes appear particularly dependent on a very high moisture content (Pryor 1962), and thus may have a more limited distribution relative to the arthropod taxa. Several studies examining the effects of moisture have shown that invertebrates are usually associated with areas where relative humidity exceeds 70%, although arthropods have been collected from areas where values are as low as 30% (Wise and Spain 1967, Rounsevell and Greenslade 1988). This dependence on higher levels of relative humidity is likely related to the cutaneous mode of respiration used by all antarctic taxa thus far studied – despite having temperate relatives with tracheal respiratory systems (Pryor 1962, Rounsevell and Greenslade 1988). However, cutaneous respiration may offer advantages in coping with the Antarctic environment. For example, Rounsevell and Greenslade (1988), found granulations in the cuticle of *Nanorchestes* spp. and suggest that these help retain a layer of air over the body which facilitates cuticular respiration in soils that are waterlogged (e.g., during the

spring thaw). These features may further enhance survival by reducing the chances of freezing when the animal is in direct contact with ice (Rounsevell and Greenslade 1988).

From a feeding perspective, the vast majority of the soil fauna are herbivores and detritivores, and thus contribute directly to the breakdown of organic material and the cycling of nutrients. Studies have reported feeding primarily on algae, fungi, and dead moss shoots (Strong 1967, Fitzsimons 1971a, Lippert 1971), although there is little direct evidence for feeding on live mosses (Fitzsimons 1971a). Instead, mosses appear to serve more as a habitat, as well as maintaining suitable moisture content (Strong 1967, Convey and Block 1996). The presence of arthropods in association with decaying animals and feather accumulations, suggests that scavenging is also possible among these taxa (Strong 1967).

The rate at which the soil fauna feed is strongly temperature dependent with highest feeding rates and faecal production occurring in the temperature range of 5-15°C (Burn 1981, 1986). Little or no feeding occurs at temperatures less than 0°C, or greater than 20°C, where mortality rates, for at least some taxa, can be as high as 80% (Burn 1981). One of the main adaptations of antarctic taxa to their environment is the ability to maintain metabolic rates equivalent to their temperate relatives at temperatures as low as 5°C (Burn 1981). The selection of appropriate microhabitats also appears to be important in order to exist within preferred temperature ranges (i.e. 5-15°C), and as a result most taxa are limited to the upper 3-5cm of moss and/or soil (Tilbrook 1967). Surprisingly, and despite 24 hour daylight and temperatures >0°C during the summer months, some taxa still maintain diurnal activity patterns. For example, Wise and Spain (1967) found that arthropod activity was limited to the

hours of 0600 and 2100 and usually between 0900 and 1600 hours. Seasonal activity periods range from mid November to mid February at continental sites (e.g. Wise and Spain 1967), although this period will be extended somewhat in the warmer maritime areas (Convey 1996). Owing to these limited periods of activity, life cycles are typically very long compared with temperate relatives where mature animals have been estimated to live between 3-7 years (Burn 1984), contrasting with life cycles of two months to a year in temperate habitats (Young 1980).

Species interactions such as competition and predation are limited for the soil fauna, and in particular, the role of predator is greatly under-represented in most antarctic habitats, although exceptions exist. For example, the largest mite in Victoria Land, *Coccorhagidia gressitti* preys on other, smaller mite species (Gless 1967). Even in the maritime antarctic few predacious species have been found, and these consist almost exclusively of mites (Edwards and Usher 1987), with collembolans serving as the primary prey items. In particular, Lister et al. (1988) found predation by the mite *Gamasellus* racovitza was highly selective for one species of collembolan (*Cryptopygus antarcticus*), and constituted 80% of all prey traces found during gut content analysis.

The ability of antarctic taxa to survive the extremely cold temperatures has been studied extensively by W. Block and colleagues (e.g. Block et al. 1978, Young and Block 1980, Sømme 1981, Block and Convey 1995). Sømme (1981) reports that none of the antarctic springtails and mites, thus far examined, are freezing tolerant (i.e. freezing of the body is fatal). Accordingly, their ability to survive low temperatures is dependent on being able to keep their body fluids from freezing (i.e. supercooling). Two main methods appear to be employed, that of avoidance of ice nucleators within

their bodies and the use of antifreeze proteins. Surprisingly, these adaptations are not unique to antarctic taxa and have also been found in temperate relatives (Block and Convey 1995). The production of antifreezes is energetically expensive and occurs only in late autumn and winter. However, the production of antifreezes is not enough to prevent ice nucleators from forming and suppression of feeding is also necessary to avoid "contaminants" (i.e. food materials) that might initiate ice formation (Young and Block 1980). Accordingly, gut clearing usually occurs when ground temperatures drop below 0°C and further feeding is restricted as a preparation for winter (e.g., Young and Block 1980). However, not all animals stop feeding simultaneously and the presence of animals with and without food in their guts explains the bimodal distribution of cold tolerance often reported in animals (Sømme 1981, Pickup 1990).

Interhabitat Dispersal and Population Genetic Structure

Much of what is known of potential dispersal methods for the invertebrate fauna is based on anecdotal evidence and/or casual observation. For example, air currents are thought to be one of the main agents of dispersal for most antarctic taxa based on collections of mites in aerial wind traps (Pryor 1962, Strong 1967). However, Marshall and Pugh (1996), suggest that this mode of transport may not be particularly effective for larger invertebrates (e.g. mites) due to the lack of an anhydrobiotic dispersal stage.

Other modes of dispersal are also possible and may include accidental carriage on birds or mammals. Birds are frequently implicated in the transfer of passively dispersed taxa from habitat to habitat, and roosting birds such as skuas (*Catharacta*

skua) may provide a suitable dispersal vehicle. Strong (1967) observed individuals of *Alaskozetes* (Acari) on the feathers of a freshly killed skua, and likewise Tilbrook (1967) collected several species from both bird colonies and nesting areas. At our own study sites in and around Victoria Land, we have noted a strong association between roosting skuas and the presence of both springtails and mites (Hogg and Stevens unpubl. data). However, Marshall and Pugh (1996) have cast doubt on this mode of transportation, suggesting that the association of arthropods with these areas is more the result of the rich organic food supply, rather than the product of dispersal events *per se*. We suggest that given the close proximity of soil invertebrates to nesting sites, accidental carriage on birds is at least possible for some antarctic taxa.

An alternative method of dispersal is that of "rafting" using sea currents to move among coastal areas. Potential "rafts" might include iceflows (Strong 1967) or other materials such as feathers from penguins or skuas. Considerable accumulations of moulted feathers from penguins and skuas are present along coastal areas particularly during January when invertebrates are at their most active (Wise and Spain 1967). Indeed, even in the absence of these materials, Strong (1967) has reported that individuals of *Alaskozetes* were able to survive immersion in salt water for up to 55 days at temperatures of 0°C. The presence of springtails on the surfaces of meltwater streams (Pryor 1962, Tilbrook 1967) suggests that access to the sea is even possible for those individuals/populations not directly on the shoreline. Accordingly, movement via sea currents may also provide a viable means of interhabitat dispersal.

A more recent and potentially undesirable method of dispersal may be provided by humans, and there have been several examples of possible species introductions (e.g. Burn 1984, Greenslade and Wise 1984, Niedbala 1986, Rounsevell and Horne 1986).

The equally likely, and equally undesirable, transfer of existing species within the antarctic region is also possible, although this would be difficult to assess on the basis of casual observation only.

Although these observations provide useful information on potential dispersal methods, they rarely provide information on the actual frequency or success of such events. One method to address this issue is to evaluate the population genetic structure of taxa in an effort to quantify rates of dispersal among habitats. By examining the frequencies of alleles at gene or allozyme loci among locations it is possible to evaluate genetic similarities among populations, and thus estimate levels of gene flow (and hence dispersal) among locations.

Despite the usefulness of such studies in determining rates of dispersal among habitats, very few studies have been undertaken to date. Exceptions are the work of Frati and colleagues (e.g. Frati et al. 1992, 1996) on *Isotoma* sp. (Collembola) in northern Victoria Land, and our own work on springtails, predominantly *Gomphiocephalus hodgsoni* (Collembola), in southern Victoria Land and Ross Island (Hogg and Stevens unpubl. data). Frati et al. (1997b) found fixed allelic differences (non-shared alleles) at sites within 100km suggesting that no gene flow was occurring among these locations. Our results too, have suggested limited gene flow particularly between sites on Ross Island and those of southern Victoria Land on the Antarctic continent. For example, in a survey of sites on Ross Island and in the Dry Valleys, we found fixed allelic differences, at two of the ten loci examined between sites on Ross Island and the continent. However, differences on Ross Island were considerably less. Accordingly, these data suggest that gene flow and hence dispersal for antarctic taxa may be limited to local events.

Potential Responses to Environmental Change

The ability of the antarctic soil fauna to respond to environmental changes (e.g. global warming, habitat destruction), will depend to a large extent on the scale and rate at which the change occurs. For rapid, localised changes (e.g. habitat destruction), survival of individuals may be limited due to the relatively low dispersal rates of taxa (see Section 5.2.4) and/or the absence of suitable, alternative habitat. In these instances, local extirpation may be inevitable. However, for slower, more gradual changes (e.g. global warming), dispersal to new areas of suitable habitat may be possible provided that the rate of change does not exceed their ability to find new, alternative habitat. On a larger scale (e.g. hundreds of kilometres), this may occur in conjunction with other changes (e.g. new soil formation/moss growth), although long distance dispersal among habitats for most taxa may be extremely limited (Balfour and Tilbrook 1966, Convey and Block 1996). However, changes are also likely on relatively smaller spatial scales (e.g. centimetres to metres). For example, considerable temperature differences are known to occur even on relatively small (e.g. 1-5cm) spatial scales (Wise and Spain 1967), and animals are known to select particular microhabitats that ameliorate ambient air temperatures (Pryor 1962, Tilbrook 1967). Accordingly, and in the case of rising global air temperatures, taxa such as mites and springtails may be able to modify their micro-distribution in the soil/plant profile through behavioural responses to the temperature shift. However, the resulting changes in soil moisture content as well as corresponding changes in feeding, growth rates, and survival may be difficult to predict.

For both large-scale and small-scale changes (although particularly when long distance dispersal is required) the rate of change must be less than the rate at which individuals are able to find suitable new habitat. Unfortunately, global warming scenarios have suggested that the rate of change may far exceed that previously experienced by the earth's biota (Ojima et al. 1991). Furthermore, several studies have suggested that time since the last glaciation has been insufficient for successful colonisation of some seemingly appropriate habitats by soil taxa (Balfour and Tilbrook 1966, Tilbrook 1967, Convey and Block 1996). Accordingly, the natural migration of animals other than in very localised circumstances appears unlikely to provide a suitable response to most immediate environmental changes.

An alternative is for species to adapt to the changing environmental conditions *in situ* (i.e. evolutionary response). The ability to evoke an evolutionary response will, in turn, be dictated to a large extent on the underlying genetic variability that is contained within and among populations of a species. Conventional evolutionary theory suggests that species with greater overall levels of genetic variability may be better able to respond to selective pressures such as environmental changes relative to species with comparatively lower levels of variability. For example, if the timing of production of antifreeze proteins was under genetic control, species with the greatest variability for this trait may be more likely to persist following changing environmental temperatures.

However, what is frequently not considered is how this genetic variability may be partitioned among respective populations of a given species (i.e. genetic differentiation among populations). For example, some species with high levels of overall genetic variability, may have limited levels of variability within any given

population (i.e. have highly differentiated populations, each of limited variability). For species with limited interhabitat dispersal (i.e. antarctic taxa), variability within populations would be expected to be extremely limited due to the lack of gene flow (and hence genetic exchange) among populations. Accordingly, the ability of these individual populations to respond to environmental change may be extremely limited.

Surprisingly, initial studies had suggested that allelic variability within populations of collembolids was surprisingly high (Frati et al. 1997b, Hogg and Stevens unpubl. data). Although there are a number of possible explanations, it appears likely that these levels of variability are an artefact of morphologically based classification schemes, which have failed to identify reproductively isolated species or populations. Specifically, currently recognised "species" may be complexes of morphologically similar, but reproductively isolated taxa. Accordingly, and as initially expected, the allelic variability within true (reproductively isolated) species is likely to be limited. This remains highly speculative, and clearly is an issue that will require further clarification in the immediate future.

In the absence of this information, the preservation and remediation of a range of habitats, remains our best option for preserving levels of variability within and among antarctic taxa.

Further Research

Although there are a wealth of research opportunities within the maritime and continental antarctic, it is possible to identify at least three research areas that would benefit from immediate attention and greatly enhance our understanding of these unique systems:

1. Small-scale dispersal experiments, examining vertical and horizontal movements of animals in response to different thermal regimes and humidity gradients would be useful in establishing potential behavioural responses to environmental changes. Furthermore, complementary data on their feeding habits as well as growth rates and survival in these different microhabitats will aid in predicting ecological responses to global atmospheric changes (e.g. global warming).
2. Studies examining the population genetic structure for a range of taxa and throughout their latitudinal distributions will be useful for several reasons. These data will enable accurate determination of true levels of (reproductively isolated) species diversity within and among habitats. They will also provide estimates of gene flow among populations, thus allowing assessment of interhabitat dispersal, as well as providing data on levels of genetic variability within and among populations. This in turn will be important in designating "evolutionary significant units" which are essential for informed management decisions. Multidisciplinary studies on bird behaviour (e.g. roosting, migration), and invertebrate behaviour (e.g. attachment to feathers, feet, etc), in both continental and maritime locations would be useful for assessing local dispersal dynamics.

3. Ecological work on smaller taxa (e.g. protozoans, nematodes), is woefully lacking and in particular their role in the breakdown of material and cycling of nutrients, as well as their interactions, if any, with the arthropods. This paucity of information may reflect, in part, the challenges of working with these taxa. However, it is essential that their contribution to the biological processes in soils be accurately assessed.

In this chapter, we have presented an overview of soil invertebrates from the South Shetland Is. (maritime) and from Ongul I. and Windmill Is. (continental). It is clear that knowledge of this important component of the antarctic soil fauna is incomplete, and in this final section we have tried to outline areas where we feel further research would enhance our understanding of these systems.

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APPENDIX 2

SYNONYMY OF THE NEW ZEALAND COROPHIID AMPHIPOD GENUS, *CHAETOCOROPHIUM* KARAMAN, 1979, WITH *PARACOROPHIUM* STEBBING, 1899: MORPHOLOGICAL AND GENETIC EVIDENCE[†]

[†]This appendix includes data from Chapter IV published under the same title as: Chapman MA, Hogg ID, Schnabel KE and Stevens MI (2002). *Journal of the Royal Society of New Zealand*. 32: 229-241.

Abstract

The original diagnosis of the New Zealand endemic corophiid genus, *Chaetocorophium*, by Karaman (1979) was based on a comparison of an apparently freshwater species, *Paracorophium lucasi* Hurley, 1954, with the type species of the estuarine genus *Paracorophium*, *P. excavatum* (Thomson, 1884), but as re-described by Hurley (1954). Hurley's paper was inadvertently erroneous, being based on a mixture of two species by an earlier worker. A new description of *P. excavatum* by Chapman (2002) has clarified the rather slight morphological differences between these endemic species, though genetic (allozyme) analyses confirm their separate identities. Given the error in erecting the genus *Chaetocorophium*, the genus should now correctly revert to *Paracorophium*. Comparisons of maxilliped, male gnathopod 2 and other morphological features also suggest that they both belong in the genus *Paracorophium*.

Keywords: Amphipoda; *Paracorophium*; *Chaetocorophium*; systematics; New Zealand; Australia, molecular taxonomy

INTRODUCTION

Until recently (Hurley 1954, 1975; Barnard 1972; Chapman & Lewis 1976; Barnard & Karaman 1991; Fenwick 2001) the corophiid fauna of New Zealand was thought to include a freshwater species in an endemic genus, *Chaetocorophium lucasi* (Hurley, 1954) known then from 3 central North Island lakes, but now also from coastal habitats (Schnabel et al. 1999), and an upper estuarine species, *Paracorophium excavatum* (Thomson, 1884). New studies have revealed a situation of considerable taxonomic and ecological complexity, with evidence of both morphological confusion between *P. excavatum*, the type species of the genus, and a related Australian species, *P. brisbanensis* (Chapman 2002), and also of possible cryptic speciation (as shown by allozyme analyses) within the New Zealand *Chaetocorophium-Paracorophium* complex (Schnabel 1999; Schnabel et al. 1999, 2000; Stevens & Hogg, unpubl. data).

The genus *Paracorophium* was established by Stebbing (1899) for a brackish water New Zealand species, described as *Corophium excavatum* by Thomson (1884) from Brighton, near Dunedin and later reported from Lakes Rotorua, Rotoiti and Waikare in the central North Island, as well as Napier and Nelson (Chilton 1906) and from the Brisbane River (Chilton 1920). When Hurley (1954) re-described *P. excavatum*, using slides labelled by Chilton as being from Brighton material, he did not realise that Chilton had mixed his Brighton and Brisbane specimens (Chapman 2002). Hurley used other slides of Chilton's to distinguish (correctly) the supposedly freshwater *P. lucasi* from *P. excavatum.* Karaman (1979) reviewed the genus *Paracorophium* when describing *P. chelatum* from Palau and erected the New

Zealand endemic genus, *Chaetocorophium*, for *Paracorophium lucasi*. Unfortunately, his comparisons were based on Hurley's (1954) re-description of *P. excavatum*.

Chapman (2002) used new collections from New Zealand and specimens from the Queensland Museum (including the original collection examined by Chilton 1920) to show that Hurley's (1954) re-description of *P. excavatum* was of a mixture of *P. excavatum* from Brighton and a previously unrecognised, but similar, Australian species, *P. brisbanensis* Chapman, 2002. Given the morphological confusion and error in erecting *Chaetocorophium*, the genus should now correctly revert to *Paracorophium*. Furthermore, we demonstrate that *C. lucasi* does not differ sufficiently from the Australasian species of *Paracorophium* to warrant generic separation. Here we summarise the evidence.

COMPARISONS BETWEEN *C. LUCASI* AND *P. EXCAVATUM*

Geographical Distribution

The two species are broadly allopatric in their distribution (Fig. 1, Table 1) with *C. lucasi* occurring on both sides of the North Island, but also in the Nelson area of the South Island. *P. excavatum* has been found mainly in east coast habitats of both the South and North Islands. A few sympatric populations occur near Nelson and Wellington, and in the Bay of Plenty. In the South Island searches of several localities both north of Kaikoura on the east coast and between Haast and Westport on the west coast were unsuccessful, probably because of unsuitable substrates of mainly stones and boulders.

Both species occupy similar coastal habitats, living in fine organic sediments in the mid to upper tidal regions of sand- or mud-flats of harbours and inlets or in slow-flowing brackish regions of streams and rivers. There are no reports of *P. excavatum* in truly freshwater inland habitats nor of freshwater populations of *C. lucasi* other than in three central North Island lakes. The possibility that either occurs in further inland habitats cannot be ruled out, but sufficient surveys of benthic fauna in New Zealand lakes have been done to show that if so, they are not widespread.

Despite our extensive collecting, we were unable to distinguish between the species in the field. No obvious habitat or behavioural (as observed during live sorting of samples) differences between them were apparent in our on-site observations. Detailed studies of habitat features, such as salinity, water flow regimes, and substrate organic content, may show habitat differentiation, though these must operate only on a micro-scale as both species have been found together within areas of only 25–50 m^2.

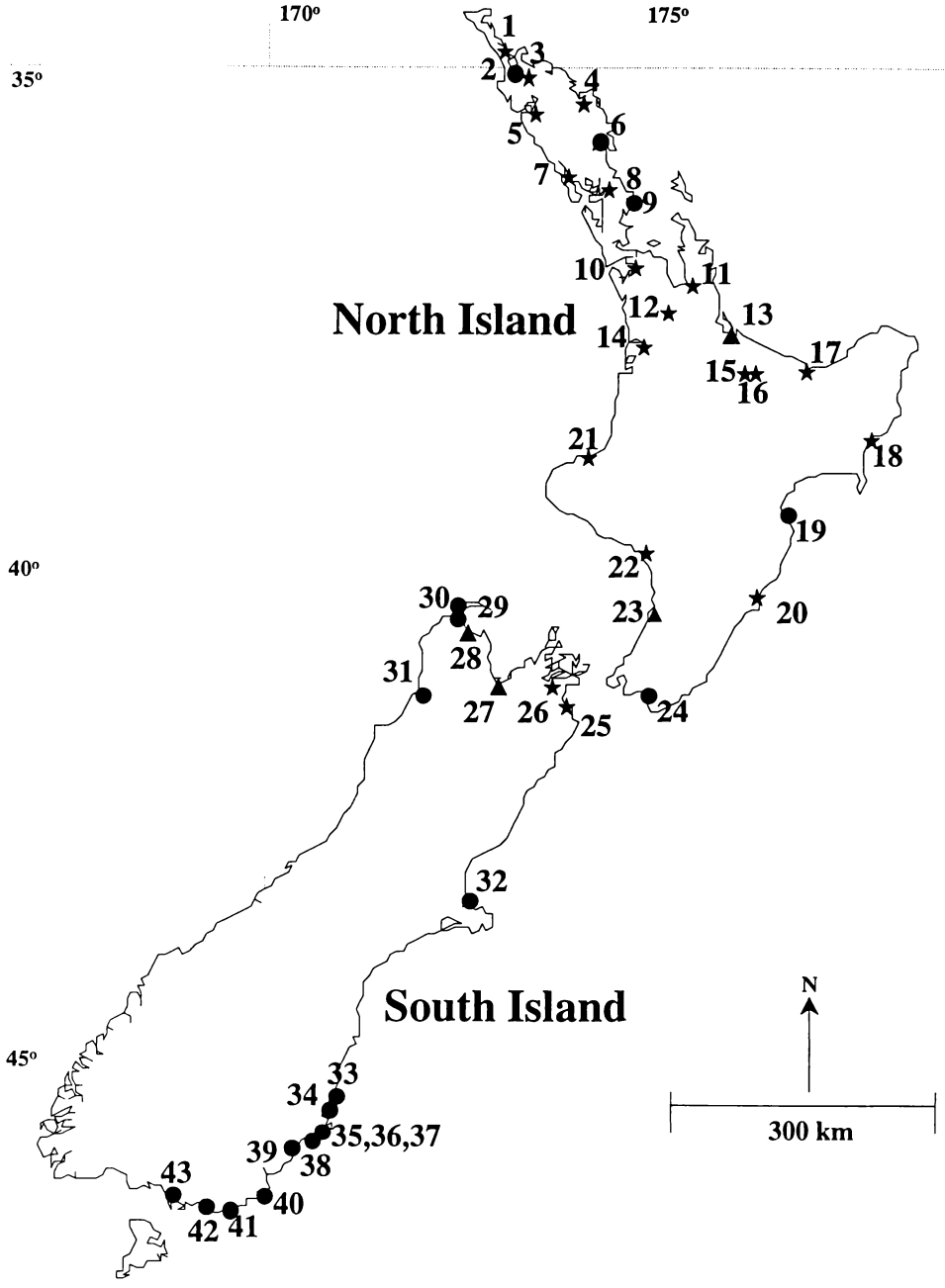

Fig. 1 Map of New Zealand showing the distribution of *C. lucasi* (stars) and *P. excavatum* (circles); triangles show sympatric occurrences. Numbers refer to those in Table 1.

Table 1. Collection sites for *Paracorophium excavatum* and *Chaetocorophium lucasi* throughout New Zealand. Morphological and molecular (allozyme) analyses were performed on all samples except those marked with an asterisk (*) which correspond to morphological analyses only. Site numbers correspond to those used in Figures 1 and 5.

North Island		
Location	**Latitude /Longitude**	**Species**
1 Houhora Harbour	34°48'/173°06'	*C. lucasi*
2 Rangauna Harbour	35°01'/173°15'	*P. excavatum*
3 Awanui River	35°01'/173°17'	*C. lucasi*
4 Taumarere	35°20'/174°06'	*C. lucasi*
5 Rawene	35°26'/173°31'	*C. lucasi*
6 Whangarei Harbour	35°43'/174°19'	*P. excavatum*
7 Dargavile	36°03'/173°58'	*C. lucasi**
8 Topuni River	36°13'/174°28'	*C. lucasi*
9 Omaha Bay	36°35'/174°76'	*P. excavatum*
10 Mangere	36°33'/174°31'	*C. lucasi**
11 Thames	37°05'/175°30'	*C. lucasi*
12 Lake Waikare	37°26'/175°13'	*C. lucasi*
13 Tauranga Harbour	37°40'/176°10'	*C. lucasi**
		P. excavatum
14 Raglan Harbour	37°48'/174°57'	*C. lucasi*
15 Lake Rotorua	38°02'/176°17'	*C. lucasi*
16 Lake Rotoiti	38°01'/176°21'	*C. lucasi*
17 Whakatane	38°00'/177°06'	*C. lucasi*
18 Gisborne	38°34'/177°56'	*C. lucasi*
19 Napier	39°30'/176°48'	*P. excavatum*
20 Porangahau River	40°38'/176°22'	*C. lucasi*
21 Waitara	39°04'/174°03'	*C. lucasi*
22 Whanganui River	39°55'/175°02'	*C. lucasi*
23 Foxton	40°18'/175°15'	*C. lucasi*
		P. excavatum
24 Lake Onoke	41°25'/175°09'	*P. excavatum*

continued,

Table 1. continued,

South Island		
Location	**Latitude /Longitude**	**Species**
25 Wairau River	41°29'/174°02'	*C. lucasi*
26 Havelock	41°16'/173°44'	*C. lucasi**
27 Nelson	41°17'/173°14'	*C. lucasi**
		P. excavatum
28 Collingwood	40°41'/172°40'	*C. lucasi*
		P excavatum
29 Waikato	40°37'/172°40'	*P. excavatum**
30 Whanganui Inlet	40°34'/172°38'	*P. excavatum*
31 Little Wanganui	41°23'/172°03'	*P. excavatum*
32 Christchurch	43°32'/172°43'	*P. excavatum*
33 Shag River	45°29'/170°47'	*P. excavatum*
34 Karitane	45°38'/170°38'	*P. excavatum*
35 Tomahawk Lagoon	45°51'/170°32'	*P. excavatum*
36 Papanui Inlet	45°54'/170°41'	*P. excavatum**
37 Waikouaiti River	45°37'/170°38'	*P. excavatum**
38 Brighton River	45°57'/170°20'	*P. excavatum*
39 Lake Waihola	46°01'/170°05'	*P. excavatum*
40 Catlins Lake	46°28'/169°38'	*P. excavatum*
41 Waikawa Harbour	46°38'/169°07'	*P. excavatum*
42 Fortrose	46°34'/168°47'	*P. excavatum*
43 Invercargill	45°25'/168°20'	*P. excavatum**

Morphology

The re-description of *P. excavatum* by Chapman (2002) enables clarification of the morphological distinctions between the New Zealand species: some of those noted by Hurley (1954) relate to the Australian species, *Paracorophium brisbanensis* (Chapman, 2002). Here we use the term 'spine' rather than the now generally customary 'robust seta' (Watling 1989) for convenience in comparisons with earlier descriptions. Similarly, 'inner' and 'outer' margins are used in describing maxilliped plates (=lobes) rather than 'medial' and 'lateral'. Specimens used in the descriptions are deposited in the Museum of New Zealand.

Without detailed examination of both maxilliped and male gnathopod 2 characteristics it is difficult to distinguish the New Zealand species. Unless large males are present, no features enabling identification without dissection or at the low magnifications used in routine sample sorting have been found. Such males have been poorly represented in our collections.

The antennae, mandibles and maxillae are all very similar in the two species, including the inner plate of maxilla 1 in *C. lucasi* (omitted in Hurley's (1954) Fig. 86). The armature of the maxillipeds is the major distinguishing character (Figs. 2–3). In both species the apico-distal margin of the inner plate has 3 spines interspersed between 6 plumose setae. The 2 most medial setae are on the inner margin of the plate in *P. excavatum*, but are less widely separated and more apical in position in *C. lucasi*. In *P. excavatum* the spines are approximately 2/3 the length of the apical setae which are 0.40–0.55x (n = 4) the length of the outer margin. In *C. lucasi* the spines are approximately 1/3 the length of the apical setae and the setae are long in relation to length of outer margin (0.69–1.00, n = 4). The inner margin of the outer plate in *P. excavatum* has thin spines along the distal half, with 3–4 longer ones apically, and submarginal rows of finer setae. In *C. lucasi* there are both marginal and submarginal setae but the marginal row includes some thicker, almost spine-like, setae. The submarginal setae extend proximally in both species.

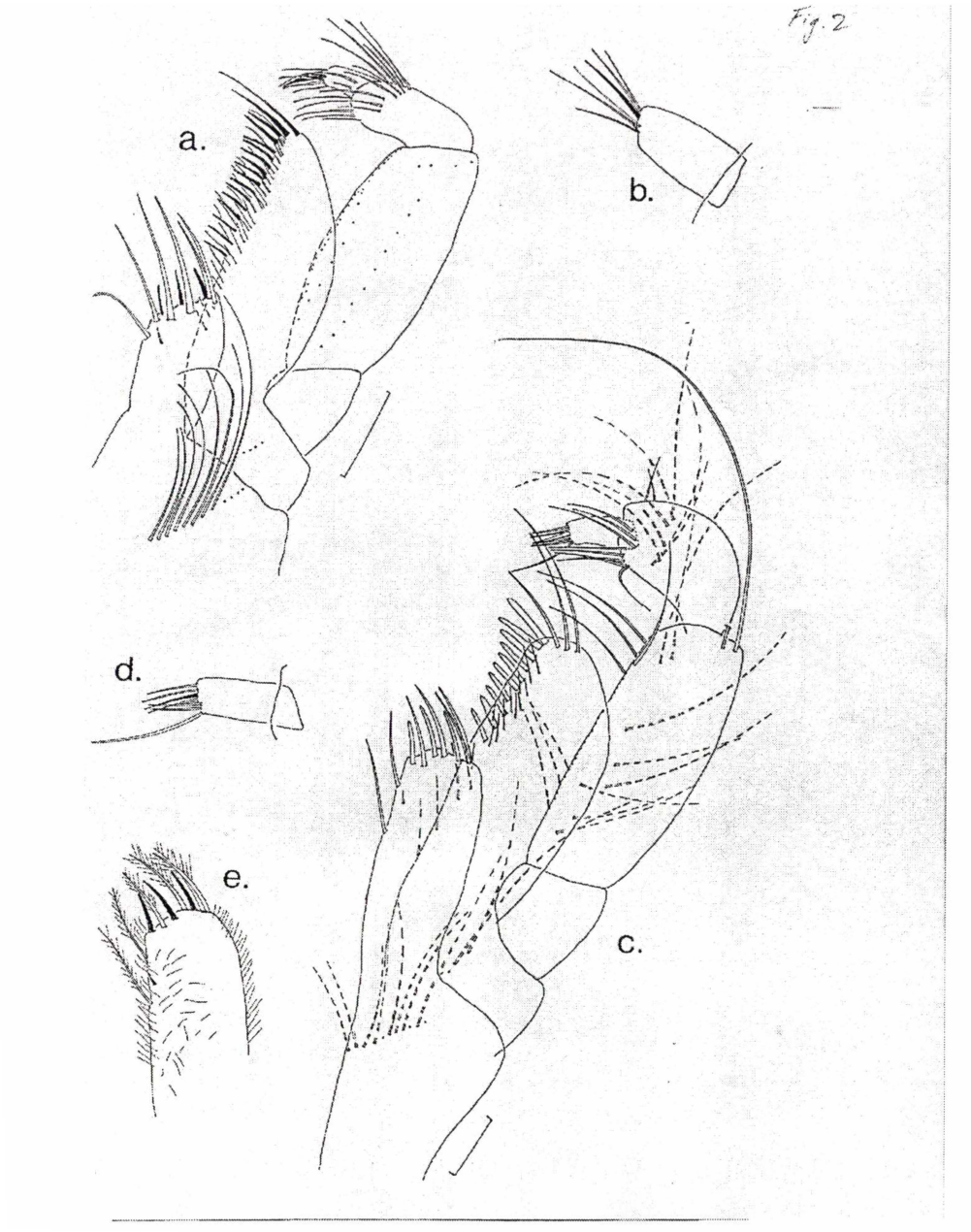

Fig. 2 Male maxillipeds. **a**. *C. lucasi*: right maxilliped (some basal and all palp article 2 setae omitted); **b**. palp article 4 of left maxilliped of same specimen; **c**. *P. excavatum*: right maxilliped; **d**. palp of same specimen; **e**. Inner plate of same specimen, showing setules (omitted in a and c). (Fig. 2a–b of Lake Rotorua specimen, MoNZ CR. 9823; Fig. 2c–e of Brighton, Otago specimen, MoNZ CR. 9815).

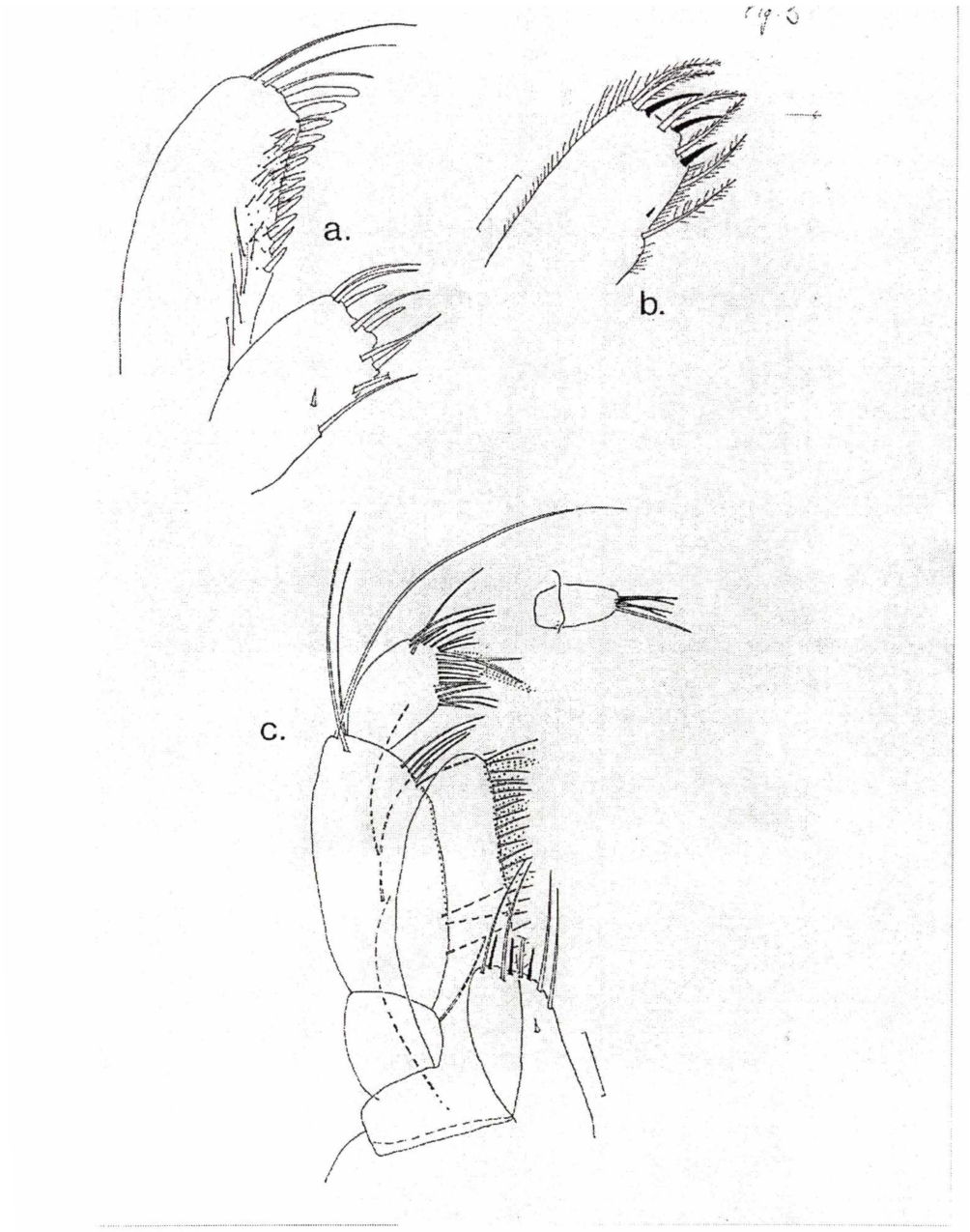

Fig. 3 Male maxillipeds. **a**. *P. excavatum*: inner and outer plates of left maxilliped; **b**. inner plate showing setules; **c**. *C. lucasi*: left maxilliped, with detail of article 4 on right. (Fig. 3a–b of same specimen as in Fig. 2c–e; Fig. 3c of Lake Rotorua specimen, MoNZ CR.9824).

Gnathopod 1 in both sexes and female gnathopod 2 are very similar in the two species, but the form of gnathopod 2 in older males is different. In *P. excavatum* (Fig. 4a-c), article 6 is elongated with ratios of length:breadth ranging from 1:0.31–1:0.39 in article lengths of 0.36–0.57 mm (n = 7), and the dactyl (claw) always greatly exceeds the palm. In *C. lucasi* (Fig. 4d–g) the ratio of length:breadth in article 6 increases with age, ranging from approximately 1:0.40 in smaller animals to 1:0.62 in the largest measured (articles 0.38–0.75 mm in length, n = 12). In large males the dactyl barely reaches the defining tooth, but in smaller males, where article 6 is narrower, it extends beyond the palm, as in *P. excavatum*. The mid-palm tooth in large males is more pronounced than in *P. excavatum* and there is a deep notch separating it from the defining tooth which does not project much beyond it. Both the dactyl and article 6 appear more heavily sclerotised and robust than in *P. excavatum*. In smaller *C. lucasi*, as Hurley (1954) noted, the dactyl can be up to twice as long as the palm (Fig. 3f–g). In such males the mid-palmar tooth and defining tooth are slightly developed with only a shallow notch between them, so that they are very similar to the gnathopods of *P. excavatum*. Thus, unless identifications are confirmed by maxilliped examination, the two species are easily confused.

We also noted differences in the numbers of spines on the posterior margins of coxae (sideplates) 1–2 (n = 24 *C. lucasi*, n = 20 *P. excavatum*; right and left gnathopods scored where posssible), though these may be in part age-related: gnathopod 1in *P. excavatum* typically had 2 spines (range 2–4, n = 31 gnathopods), and in *C. lucasi* had 4–5 spines (range 2–6, n = 39 gnathopods); modal numbers on gnathopod 2 were 2–3 (range 2–6, n = 37 gnathopods) in *P. excavatum*, and 4–5 (range 2–6, n = 42 gnathopods) in *C. lucasi*.

Differences in pereopods and uropods between the species are slight. In examining a range of 20–30 specimens of each species from the same and different localities we found great overlap in variations between populations, and detailed studies of morphological changes with age and sex in single populations will be needed before any further species-specific characteristics can be defined. As well there was a high frequency of asymmetry in setation between the left and right appendages in both species: the same article might have, for example, 2 spines on the left appendage but 1 or 3 on the right one.

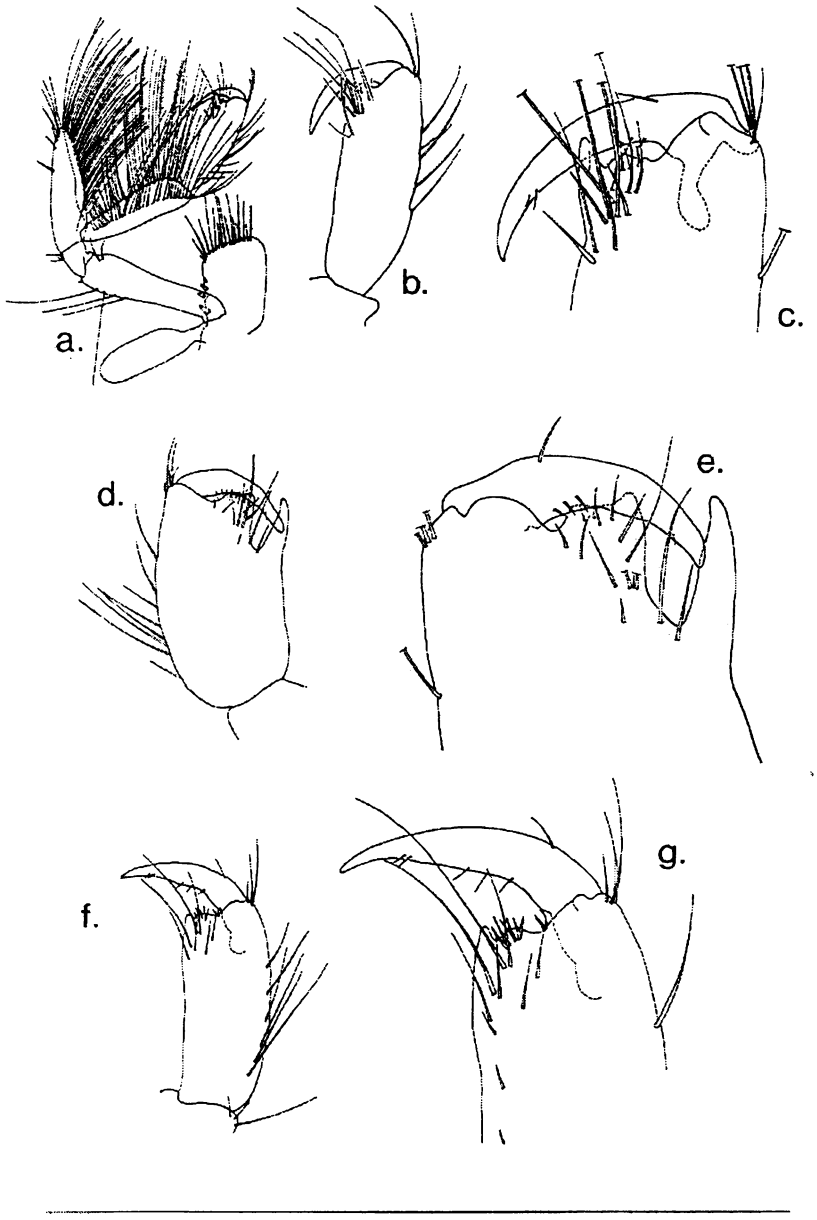

Fig. 4 Male gnathopod 2; medial views. *P. excavatum* (adult): **a**, gnathopod; **b–c**, article 6 and dactyl. *C. lucasi*:, **d–e**, article 6 and dactyl of adult male; **f–g**, article 6 and dactyl of juvenile male. Fig. 4a–c from Chapman (2002). (Fig. 4a-c of Brighton, Otago specimen, MoNZ CR. 9815; Fig. 4d–e of Waitara specimen, MoNZ CR. 9827; Fig. 4f–g of Lake Rotorua specimen, MoNZ CR. 9826).

Genetic Differences

Comparisons of the genetic similarity between populations of *C. lucasi* and *P. excavatum* have been made using cellulose acetate electrophoresis (*sensu* Hebert & Beaton 1993; Larose & Hogg 1998). Initial studies showed that the two species could be reliably separated (as confirmed by morphological examination) by the enzyme system AO (aldehyde oxidase, EC 1.2.3.1) (Schnabel 1999; Schnabel et al. 2000). Further analyses of 14 loci from 10 enzyme systems have now been made from 18 populations of *C. lucasi* and 20 populations of *P. excavatum* from 36 habitats around New Zealand (Stevens & Hogg unpub. data) to elucidate patterns of genetic differentiation.

When run in a Tris Glycine pH 7.0 buffer for 20 minutes at 200V, the staining bands of AO for *C. lucasi* (n = 177) were visible 20–24 mm above the application line, whereas electromorphs of *P. excavatum* (n = 140) had a lower mobility with bands 14.5–19.5 mm above the application line (Students *t*-test =46.23, d.f. = 315, $P < 0.001$).

No other alleles or allele combinations were unique to either species. However, lack of interbreeding between the species was indicated by the presence of fixed differences (non-shared alleles) in two sympatric populations at Collingwood and Foxton, at 9 and 11 loci, respectively, of the 14 loci examined. Furthermore cluster analysis (UPGMA dendrogram), using Nei's (1978) unbiased genetic identity values (I), showed considerable allelic differentiation between species (Fig. 5), with a mean I-value of 0.20. There were also considerable intraspecific differences with I-values as low as 0.46 between geographically disjunct populations. No morphological

criteria (as shown by qualitative examinations of maxilliped and gnathopod features) have yet been found to correlate with these allelic differences.

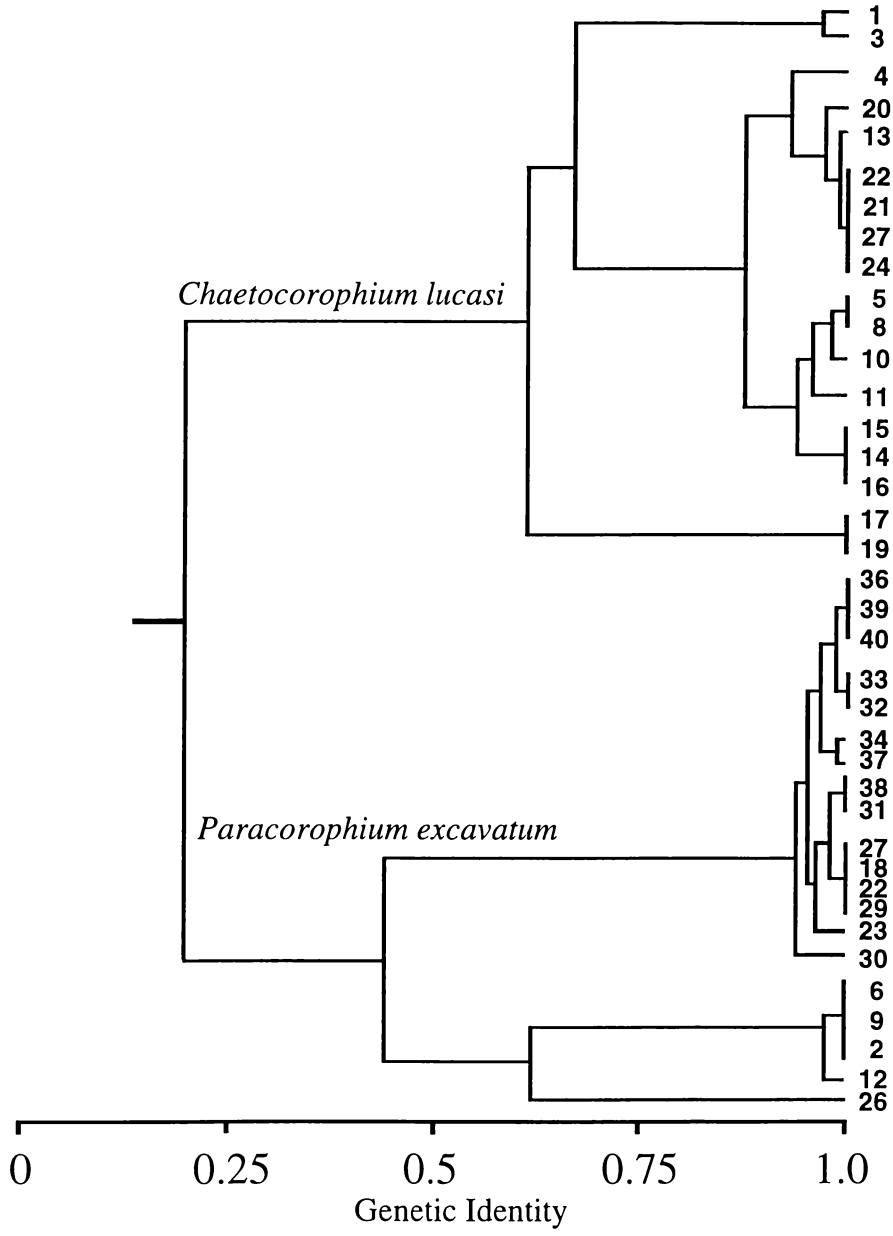

Fig. 5 A UPGMA clustering showing genetic identity (Nei 1978) between populations of *Chaetocorophium lucasi* and *Paracorophium excavatum* in the North and South Islands of New Zealand. Site names and locations are given in Table 1 and Fig. 1.

DISCUSSION

The original distinction between *Paracorophium* and *Chaetocophium* by Karaman (1979), and amplified by Barnard & Karaman (1991), was based on Hurley's (1954) descriptions of New Zealand species. All 3 papers are inadequate, largely as a result of Chilton's (1920) failure to recognise differences between what are now known to be 3 separate species, and his mis-labelling of slide material of *P. excavatum* used by Hurley (1954).

Hurley (1954) accurately described *P. lucasi* but his description of *P. excavatum* is actually of a mixture of *P. excavatum* and *P. brisbanensis,* as Chapman (2002) showed. It is also difficult to establish what criteria led Karaman (1979) to separate the genera since he mentions only characteristics of the maxilliped lobes, and neither his comments and descriptions, nor those of Barnard & Karaman (1999), correspond with those of Hurley (1954). Our examination of new collections of the New Zealand species, including some from their type localities, casts doubts on the validity of the generic distinction.

The two genera, as Karaman (1979) noted when erecting *Chaetocorophium*, are morphologically very similar. The distinction was perhaps biassed by an incomplete knowledge of the ecological distribution of *C. lucasi* (now known to occur in many coastal but only a few freshwater habitats). As well, Karaman (1979) evidently confused features of Hurley's supposed *P. excavatum* and what was then *P. lucasi.* He mentioned (p. 88) that '*P. lucasi* differs from all other three *Paracorophium* by very setiferous inner lobe of maxilla, and we separated it in a distinct new genus *Chaetocorophium.*' However Hurley (1954) correctly noted (p. 455) that in *P. lucasi*

'the inner plate had three small spines alternated with 6 long setae but said only that in *P. excavatum* there was an 'Inner plate margin with several long plumose setae', although his Figure 68 also showed 3 shorter setae or spines (differences are not distinguishable in his small figure) as well as the longer setae. In the generic diagnosis (p. 98) Karaman said ' Inner lobe of maxilliped with distal plumose setae; outer lobe long, provided with double row, a [sic] numerous setae' and remarked '*Chaetocorophium* Genus is very similar to *Paracorophium* Genus, but differs from later [sic] in presence of double row of numerous short setae along inner margin of outer lobe of maxilliped'. Hurley described the outer plate as having a '... double fringe of short setae down inner margin to end of inner plate, not with the 6 or so distinct small sharp teeth of *P. excavatum*.' (His description of *P. excavatum* (p. 451) says '8 or 9 increasingly longer teeth'). It is thus uncertain whether the (incorrect) features of the inner plates or of both maxilliped plates prompted Karaman's erection of a separate genus for *Chaetocorophium*. When Barnard & Karaman 1991 revised the generic descriptions, *Chaetocorophium* were said (p. 180) to have 'Inner plate... with distal spines' and *Paracorophium* to have 'Inner plate with distal setae' (p. 218). The contrast between Karaman's description of the setiferous inner margin of the outer lobe in *Chaetocorophium* (p. 98) and the 'several slender spines' in *Paracorophium* (p. 88) was changed to 'dense double row of short setae' in *Chaetocorophium* (p. 180) and to 'thin spines or setae in *Paracorophium'*.

Our comparison of the maxillipeds (Figs. 2–3) shows that the outer plate of the maxilliped in *C. lucasi* has some slender spines amongst the marginal setae, though such spines are more robust and obvious in *P. excavatum*. The difference in marginal armature is thus one of degree and less distinct than implied in Hurley's (1954)

description of *C. lucasi*. As well, he was (unknowingly) comparing it with the maxilliped of *P. brisbanensis*, which has fewer sub-marginal setae and fewer but similarly obvious spines than *P. excavatum* s.str on the outer plate (Chapman 2002).

Both species have spines as well as plumose setae on the apico-distal margin of the inner plate but show differences in the relative lengths of setae:spines and of setae:outer margin of lobe (Figs. 2a, 3b, c). Other species of *Paracorophium* also have spines and setae (Karaman 1979, Andres 1975, Varela 1983, Chapman 2002).

A further distinction between *P. excavatum* and *C. lucasi* is supposedly the length of the outer plate of the maxilliped. Hurley's (1954) descriptions said that in *P. lucasi* the outer plate almost reached the end of the palp carpus (= article 2) (p. 455), but in *P. excavatum* reached 2/3 along the carpus (p. 451). Karaman (1979) in his generic diagnosis noted only that the outer lobe was long in *Chaetocorophium*. Barnard & Karaman (1991) changed this to 'outer plate abnormal, long, almost reaching end of carpus...' in *Chaetocorophium* and said that in *Paracorophium* 'outer plate normal, not reaching apex of article 2 ...'. These distinctions are unsatisfactory as assessments of relative lengths are greatly dependant on appendage orientation and degree of flattening in slide preparations. Figures 2c and 3c show a longer plate in *C. lucasi* but we would hesitate to use any such differences as a major feature in species distinctions. As in the New Zealand species, the outer plates in other *Paracorophium* species are about 2/3–3/4 the length of palp article 2 (Karaman 1979, Andres 1975, Varela 1983, Chapman 2002).

A close relationship between the New Zealand species is also indicated by male gnathopod 2 features (Fig. 4). The narrow article 6, the length of the defining palmar tooth and the dactyl exceeding the palm of *P. excavatum* resemble those of juvenile

C. lucasi. Either condition could be derived from the other by retention of ancestral juvenile characteristics in *P. excavatum* or by alteration of relative growth patterns in *C. lucasi*. Another similarity is the presence of a large plumose seta near the base of the dactyls of pereopods 5–7 in both species, a character not present in the otherwise rather similar *P. brisbanensis* (Chapman, 2002). As well both New Zealand species have a very setiferous article 2 in pereopod 5 in larger males, especially in *P. excavatum*.

Karaman (1979) did not use male gnathopod 2 characteristics in distinguishing the genera, though he noted that *P. chelatum* differed from all other known species of *Paracorophium* in its chelate (propodochelate) gnathopod 2 (p. 97). His generic diagnosis of this genus said 'gnathopod 2 merochelate, distally chelate or subchelate'. However in his species diagnosis of *P. excavatum* (p. 97) he misquoted Hurley (1954) by reporting '2 strong fingers at distoposterior corner' of article 6'. Hurley correctly showed a single finger (and see our Fig. 4a–c). This error must be the origin of the statements in Barnard & Karaman (1991, p. 218) that article 6 has 'double chela' in their generic diagnosis and that the type [*P. excavatum*] is 'doubly parachelate'. Karaman's generic diagnosis of *Chaetocorophium* correctly said male gnathopod 2 was subchelate, which was altered to 'subchelate, almost parachelate. Article 6 with false chela' in Barnard & Karaman (1991). However, both Karaman (1979) and Barnard & Karaman (1991) confusingly refer to the chelate gnathopod 2 of *Chaetocorophium* in their comparisons with *Stenocorophium*.

The confusions and contradictions in the generic and species descriptions caused problems in identifications at the start of our study, which has shown the importance

of the re-examination of new collections of specimens from type localities and elsewhere in revising the work of earlier taxonomists.

We found that detailed morphological examination is required to distinguish the New Zealand species, with field observations of behaviour or habitat providing no reliable differences. Allozyme assays were helpful in confirming identifications and enabled clear separation of the two species. However they also showed considerable inter-population genetic differentiation within each species (Fig. 5). As Schnabel et al. (1999) noted, such inter-population differences may need to be taken into account in physiological (e.g. ecotoxicological) and ecological studies.

The low I-value of 0.20 between the populations of *C. lucasi* and *P. excavatum* suggests that, despite their close morphological similarities, these mainly allopatric taxa are evolving independently (sensu Stewart 1993), with fixed allelic differences indicating no present-day gene flow between them. There is no morphological or genetic evidence of hybridisation within the few known sympatric populations. This I-value fully justifies their separation as distinct species on morphological grounds: Thorpe (1982) suggested that I-values > 0.54 (SD ± 0.17) indicated conspecific status; Stewart (1993) more conservatively suggested I > 0.45.

No generally accepted criteria for the use of biochemical (allozyme) differences between populations for generic differentiations yet exist. The boundaries between genera are normally based on well-recognised morphological differences and hence the mainly northern hemisphere biochemical studies have concentrated on inter- and intra- species differentiation. In New Zealand both the formal taxonomy and the geographical distributions of freshwater and estuarine amphipods are still poorly known. There is a legacy of incomplete taxonomic descriptions, often based on single

specimens, and of uncritical assumptions relating such differences as were noted to degrees of maturity rather than to species distinction (e.g., Chilton 1920). Hurley's valuable reviews (1954, 1975) of the fauna suffered from the absence of geographically extensive collections and relied greatly on Chilton's papers and specimens. Chilton's descriptions and records amplified but sometimes confused the pioneer studies of the more reliable G.M. Thomson. Neither generic or specific boundaries are well defined morphologically for any New Zealand freshwater amphipod taxa (Fenwick 2001) and hence it is difficult to assess the importance of the genetic differences between *P. excavatum* and *C. lucasi.*

The genetic identity (I) value of 0.20 could be used to argue for or against the current generic separation. Thorpe (1982) found a mean of 0.27 (SD ± 0.11) between a range of genera. Thus the value for *Paracorophium-Chaetocorophium* lies in the grey area where additional evidence must be considered. Freshwater and estuarine amphipods in general have poor dispersal abilities, and occur in disjunct populations so that a high degree of genetic differentiation can be expected (Lopp & Oliver 1988; Stewart 1992, 1993; Conceição et al. 1998; Hogg et al. 1998; Thorpe & Solé-Cava 1994). As well, no data base yet exists on the degree of variation to be expected in large islands such as New Zealand with a history of long geographical isolation, coupled with the effects of marine transgressions, glaciations and periodic vulcanism (Stephens 1980).

We conclude that the morphological and ecological similarities between the New Zealand species indicate congeneric status despite their considerable genetic divergence. Accordingly, *C. lucasi* is now correctly restored to the genus *Paracorophium.*

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