Evolutionary genetics of southern stoneflies

Graham Alexander McCulloch

A thesis submitted for the degree of

Doctor of Philosophy

at the University of Otago, Dunedin,

New Zealand

July 2010

Abstract

Elucidating the historical processes that are responsible for the contemporary geographic distributions of evolutionary lineages is the major goal of phylogeography. Here I assess phylogenetic and biogeographic relationships within stoneflies (order: Plecoptera) using mitochondrial (COI) and nuclear (18S; H3) DNA. Sequence data were obtained from almost 500 southern hemisphere plecopteran specimens, with Bayesian phylogenetic analyses performed to elucidate the relationships among lineages. A variety of analyses were used to characterise the biogeographic forces responsible for the contemporary distribution of these lineages, and to examine how flight loss impacts stonefly evolution and biogeography.

Geological data are used to calibrate a multilocus phylogeny of stoneflies. The two stonefly sub-orders, Arctoperlaria (northern hemisphere) and Antarctoperlaria (southern hemisphere), are estimated to have diverged during the Jurassic, consistent with their vicariant formation driven by the breakup of Pangaea. Subsequently, a single Antarctoperlaria lineage apparently dispersed into the southern hemisphere during the Cretaceous. The disjunct distribution of southern hemisphere lineages is best explained by combination of both vicariance and dispersal, with most of the divergences between South American and Australian lineages consistent with vicariance, whereas dispersal best explains the origin of South African and New Zealand lineages.

Comparative phylogeographic analysis of six alpine stonefly genera was used to test a vicariant evolutionary hypothesis for the origin of the 'biotic gap' in the central South Island, New Zealand. The magnitude of north-south genetic differentiation was strikingly similar across all six genera (ranging from 0.074-0.091), with a test for simultaneous vicariance confirming that divergence is consistent with a single evolutionary event. The concordant cladogenesis detected across multiple taxa was consistent with vicariant isolation caused by the onset of glaciation in the late Pliocene. This indicated an important cladogenetic role for glaciation, an abiotic evolutionary process that is more typically associated with loss of biodiversity.

The genetic and biogeographic impact of wing reduction on New Zealand stoneflies was assessed via comparative phylogeographic analyses (COI; H3) of strong-flying *Zelandoperla decorata* versus the co-distributed but weak-flying *Zelandoperla fenestrata* species group. Consistent with its capacity for strong flight-mediated dispersal, *Z. decorata* exhibited no substantial phylogeographic differentiation across its broad South Island range, while conversely the weak-flying *fenestrata* species group exhibited substantial genetic structure across both fine and broad geographic scales. Intriguingly, the variable degrees of wing development observed within the *fenestrata* species group had no apparent impact on levels of phylogeographic structure, which were high regardless of morphotype, suggesting that even fully-winged specimens of this group do not fly. This finding implies that *Zelandoperla* flight-loss occurs independently of wing-loss, maybe reflecting underlying flight-muscle reduction.

This phylogeographic research sheds new light on the relative importance of dispersal and vicariant events in shaping the contemporary distribution of stonefly lineages in the southern hemisphere.

Acknowledgements

This thesis could not have been completed without the help of numerous people. Firstly I would like to thank my supervisors, Jon Waters and Graham Wallis, for all of the assistance they have given me. Both encouraged me to focus on publishing scientific papers, with three substantial papers produced during the course of my PhD research. In addition, Jon was always keen to go into the field collecting stoneflies, and always provided very helpful (and rapid) feedback on drafts. Graham provided valuable insight as to what was required to publish in particular journals, and taught me to focus on structuring my papers around key objectives.

When I began my PhD I knew next to nothing about stoneflies, and much of my knowledge about stoneflies was obtained by talking to Ian McLellan, who sadly passed away during the course of this study. Ian was widely regarded as New Zealand's stonefly 'guru', having described 72 new stonefly species (including 10 new genera) over the last 40 years of research. Ian taught me a huge amount about identifying the differences between species, provided me with a number of specimens from his personal collection, and always had a good supply of whitebait patties whenever I visited him.

Leigh Anderson, Chris Burridge, Steve Campbell, Diane Rowe, and Tom Young all assisted me in collecting specimens. In addition, a number of individual collectors and institutions provided specimens from their personal collections, including Ricardo Albarino, Pablo Pessacq, Derek Smith and Mike Winterbourn.

My family members - Mum, Dad, Andrew, Murray and Bronwyn - provided me with valuable support throughout my PhD. They were often roped into helping me collect specimens, and a few even attempted to read through my papers.

Initial funding for the research was provided by Marsden contract UOO-0404 (Royal Society of New Zealand), with subsequent funding provided by the Department of Zoology. A University of Otago PhD scholarship provided funding for my personal expenses, while the University of Otago also provided funding to attend several conferences in New Zealand, as well as the Southern Connections Congress in Argentina.

Finally, and most importantly, I have to thank my fiancée Sharlene. When we met I had just started my PhD, and she somehow managed to put up with me throughout my PhD. She was always keen to help me search for stonefly specimens, whether it be tramping through foot deep mud in the wilderness of Stewart Island, or driving up windy mountain roads in the hope of finding stoneflies at the top. Her support made completing this PhD possible

Table of contents

Appendices

List of figures

List of tables

Papers arising from this thesis

McCulloch GA, Wallis GP, Waters JM (2009) Do insects lose flight before they lose their wings? Population genetic structure in subalpine stoneflies. *Molecular Ecology,* **18,** 4073-4087.

McCulloch GA, Wallis GP, Waters JM (2010) Onset of glaciation drove simultaneous vicariant isolation of alpine insects in New Zealand. *Evolution*, **64,** 2033-2043.

McCulloch GA, Wallis GP, Waters JM. Molecular phylogenetics of Gondwanan stoneflies (Antarctoperlaria; Notonemouridae): vicariance or dispersal? (submitted)

Chapter 1: General introduction

1.1 Phylogeography

Phylogeography is a rapidly developing field that aims to determine which historical processes are responsible for the contemporary geographic distributions of lineages (Avise *et al.* 1987; Avise 2000; reviewed by Beheregaray 2008). Patterns of phylogeographic differentiation are frequently observed within species; these patterns can be attributed to fragmentation of widespread ancestors either by a vicariant event, or by dispersal over a preexisting barrier (Avise 1994). Due to the perceived 'untestability' of dispersal events (e.g. Croizat *et al*. 1974; Nelson & Platnick 1981), vicariant explanations have traditionally dominated biogeographic literature (Queiroz 2005; McGlone 2005). However, a recent upsurge of molecular phylogeographic studies has highlighted the role of dispersal in shaping the contemporary distributions of numerous lineages, and it is now clear that both biogeographic processes are common in nature (e.g. Sanmartin & Ronquist 2004; Queiroz 2005; Waters & Craw 2006; Wallis & Trewick 2009).

An extension of the phylogeographic analysis of single species is to compare patterns across multiple codistributed species (e.g. Bermingham & Avise 1986; Avise 1994). The main goal of this 'comparative phylogeography' is to search for concordant geographical distributions among lineages within different species, which would indicate the influence of common evolutionary and ecological processes. In particular, comparisons across multiple taxa can be used to clarify the histories of biogeographical regions, and this has emerged as an important tool for investigating such parallel evolutionary effects of geological or climatic processes (Avise *et al.* 1998; Bermingham & Moritz 1998; Schaal *et al.* 1998; Avise 2000; Arbogast & Kenagy 2001; Avise 2004).

In addition, these comparative studies can be used to contrast the dispersal ability or habitat preferences of the taxa involved (Eble *et al.* 2009).

1.2 New Zealand geography

New Zealand's (NZ's) isolation and dramatic geological and climatic history (Cooper & Millener 1993; Newnham *et al.* 1999) makes it an ideal region for studies of evolutionary biogeography. This southern landmass has been isolated from other major land masses since it detached from the supercontinent Gondwana 82 million years ago (Ma) (Cooper & Millener 1993), and it has been separated from the nearest major landmass (Australia) by more than 1500 km of ocean for the last 65 Ma (Kamp 1986; Campbell & Hutching 2007; Graham 2008). During the Oligocene (ca. 25–22 Ma), NZ's land area was dramatically reduced by marine inundation (Cooper 1989; Cooper & Millener 1993; Cooper & Cooper 1995; Campbell & Hutching 2007; Graham 2008), though the extent of NZ's submergence remains a controversial topic (Wallis & Trewick 2009).

NZ's re-emergence following the Oligocene drowning was a direct result of tectonic activity at the Alpine Fault — the boundary of the Pacific and Australian plates (Waters & Craw 2006; Landis *et al.* 2008). In addition to extensive uplift, Alpine Fault activity has generated 440–470 km of lateral displacement since 25–23 Ma (Sutherland 1994, 1999), while the Southern Alps were formed by compression along the fault line that intensified from the Pliocene onwards (6 Ma) (Gage 1980; Suggate 1982; Batt *et al.* 2000; Chamberlain & Poage 2000). Most recently, NZ has experienced several glacial periods since the late Pliocene (2.5 Ma), marked by the formation of a continuous complex of extensive glaciers and ice fields along the Southern Alps (Suggate 1990).

This turbulent geological and climatic history is thought to have strongly impacted the evolution of NZ's endemic biota (Wardle 1988; Wallis & Trewick 2009).

1.3 Loss of dispersal ability

The NZ fauna is dominated by sedentary endemic taxa, with many of these taxa apparently having descended from dispersive ancestors (Waters & Craw 2006; Wallis & Trewick 2009). This reduction of dispersal ability is generally a result of the loss of a particular life-history phase, for example the loss of a migratory phase in galaxiid fishes (Waters & Wallis 2001), or as a result of wing reduction in beetles (Emerson & Wallis 1995; Trewick 1997). In many cases, reduced dispersal ability is believed to be a major driver of speciation (Emerson & Wallis 1995; Waters & Wallis 2001; Bell *et al.* 2004). Similarly, loss of dispersal ability can often lead to increased phylogeographic structuring with species, and hence molecular data are often used to compare and contrast the relative dispersal ability of different lineages (Arbogast & Kenagy 2001 [North American boreal mammals]; Dawson *et al.* 2002 [California gobi's]; Eble *et al.* 2009 [Hawaiian surgeonfishes]).

Wing reduction is particularly common in the invertebrate and avifauna of NZ (Trewick 1997; Wallis & Trewick 2009; Phillips *et al.* 2010). This category of morphological change, leading to flight loss, is also common on oceanic islands, with the persistent loss of flighted emigrants from these islands thought to select locally against flight (Roff 1990; Wagner & Liebherr 1992; Denno *et al.* 2001). Similarly, flight loss is common in montane habitats, with a combination of small habitat size and high winds suggested to select against flighted individuals on these 'sky islands' (den Boer 1970; Harrison 1980; Roff 1990).

1.4 Plecoptera

Stoneflies (Plecoptera) are a small order of hemimetabolous insects, with more than 3497 described species worldwide (Fochetti & Figueroa 2008). These taxa represent an important part of many ecosystems, and are often used as bioindicators of water pollution levels (Krno 2007). In global terms, stoneflies have a widespread distribution, being found in all continents apart from Antarctica (Zwick 2000; Fochetti & Figueroa 2008). However, there is a strong geographic bias in taxonomic richness of stoneflies, with substantially more families and genera recorded in the northern hemisphere than in the southern hemisphere (Fig. 1.1). Specifically, 270 of the 340 recognised stonefly genera are restricted to the northern hemisphere (Fochetti & Figueroa 2008).

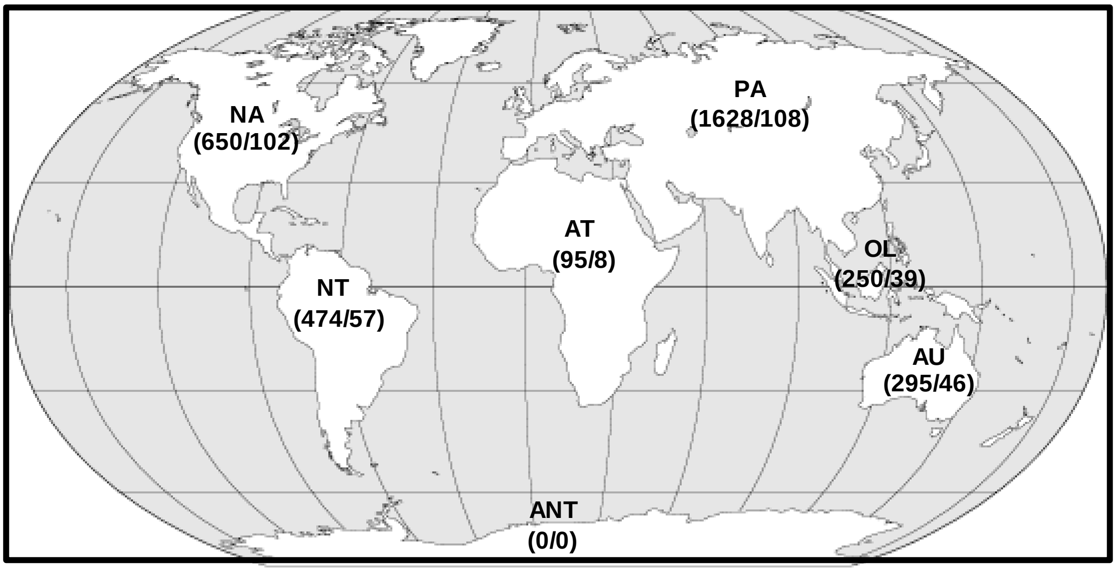


Fig. 1.1: Distribution of Plecoptera species and genera by zoogeographical regions (species number/genus number). PA – Palaearctic; NA – Nearctic; NT – Neotropical; AT – Afrotropical; OL – Oriental; AU – Australasian; ANT – Antarctic.

Stoneflies are considered to be an ancient insect lineage, with fossils dating back to the early Permian (Sinitshenkova 1987; Sinichenkova 1997; Zwick 2000). In taxonomic terms, stoneflies are traditionally placed within Polyneoptera, the largest and most diverse radiation of non-holometabolous insect orders (Terry & Whiting 2005). Despite a number of morphological and molecular phylogenetic studies, however, there is little consensus regarding the placement of Plecoptera within this large and diverse insect group (Wheeler *et al.* 2001; Grimaldi & Engel 2005; Zwick 2009).

Classification schemes within order Plecoptera have similarly varied substantially, even over recent decades. Klapalek (1909) originally divided Plecoptera into two sub-orders, Filipalpia and Setipalpia, with a third sub-order (Archiperlaria) later added by Illies (1965). In contrast, Zwick (1973, 2000) proposed a basal splitting of plecopteran lineages into two sub-orders: Arctoperlaria and Antarctoperlaria, with the names deriving from the nearly complete disjunction between northern hemisphere (Arctoperlaria) and southern hemisphere (Antarctoperlaria) lineages. The disjunction is not complete however, with two Arctoperlaria families (Perlidae and Notonemouridae) found in southern temperate regions. The two sub-orders are generally accepted in literature (Fochetti & Figueroa 2008), although a molecular analysis (based only on 18S rRNA) by Thomas *et al.* (2000) found both suborders to be polyphyletic.

Stoneflies have two distinct life phases, a long aquatic larval phase followed by a much shorter terrestrial adult stage, typically lasting between one and four weeks (Merritt & Cummins 1996). Stonefly larvae can disperse through streams by swimming or crawling, although passive larval drift is believed to be the primary dispersal mechanism for most plecopteran species (Waters 1965; Muller 1982; Dudgeon 1983; Palmer *et al.* 1996). By contrast, even fully-winged adults are generally considered to possess only limited flight ability (Brundin 1967; Brundin 1972; Zwick 2000; Schultheis *et al.* 2002; Fochetti & Figueroa 2008), which combined with a relatively short lifespan is thought to limit dispersal ability.

1.4.1 Southern hemisphere Plecoptera

Despite the relatively high diversity evident in the northern hemisphere, the southern hemisphere has a distinctive and highly endemic stonefly fauna. Specifically, five out of the seventeen plecopteran families (Eustheniidae, Diamphipnoidae, Austroperlidae, Gripopterygidae and Notonemouridae) are restricted to the southern hemisphere. Four of these five families are widely distributed throughout Australia, NZ, and South America, with the remaining family (Diamphipnoidae) restricted to South America. Gripopterygidae is the most diverse of these families, with 277 species from 49 genera, while in contrast Diamphipnoidae contains only three species housed in two genera. South Africa has a reduced stonefly fauna, with only 17 species from a single family (Notonemouridae). Due to the apparent ecological requirements of stoneflies — a broad preference for cool fast-flowing water — most Antarctoperlaria lineages are restricted to temperate regions, such as the southern parts of Australia and South America, though a few tropical lineages exist in northern regions of Australia and in southern Brazil (Fochetti & Figueroa 2008).

1.4.2 NZ Plecoptera

NZ has a well-characterised stonefly fauna, with 95 described species from 20 genera (McLellan 2006a). In addition, there are endemic species known from the subantarctic Auckland Islands (four species from one genus), Campbell Island (two species from one genus), and the Snares (one species). All NZ stonefly genera and species are unique to this country, with the sole exception of the genus *Notonemoura* which has a trans-Tasman distribution, though species are endemic to each country (McLellan 2006a). Despite the high endemicity within NZ, a few stonefly species are widely distributed throughout the country (e.g. *Austroperla cyrene*, *Megaleptoperla diminuata*), whereas most NZ stonefly species have relatively small ranges, with some apparently restricted to a single record/locality (e.g. *Nesoperla patricki*, *Apteryoperla lakiula*). Within NZ, the stonefly fauna of South Island is substantially more diverse than that of North Island (Fig. 1.2). This regional contrast in diversity has been attributed to increased rates of speciation occurring during the extensive geological activity in South Island since the Pliocene (McLellan 2003).

Certainly, NZ's unique landscape and climatic history have apparently resulted in a number of distinctive adaptations in its stoneflies. One of these adaptations, wing reduction, is particularly common, with 25 of the 104 endemic species having reduced wings (McLellan 2006a). In contrast, this adaptation is relatively rare in Australian, South American, and South African stoneflies, with only two of 194 Australian, five out of 474 South American, and zero out of 17 South African species with this condition (McLellan 1999). The extent of wing reduction can vary both among and within taxa, ranging from partial (microptery) to complete wing loss (aptery). Wing-reduction is broadly associated with high-altitudes (McLellan 1977, 1996), with a combination of small habitat size and high winds thought to select against flighted individuals (den Boer 1970; Harrison 1980). In another possible example of adaptation to the alpine environment, a number of NZ plecopteran taxa have apparently abandoned the plesiomorphic aquatic larval phase, and have adopted a terrestrial larval stage, with juveniles often found some distance from running water (McLellan 2003). These wholly terrestrial stoneflies generally live in moist habitats deep in alpine vegetation, or in damp depressions under stones. As with wing reduction, this evolutionary adaptation is generally associated with high altitudes, and is considered to be a response to the dramatic climatic conditions during the Pleistocene (McLellan 1977).

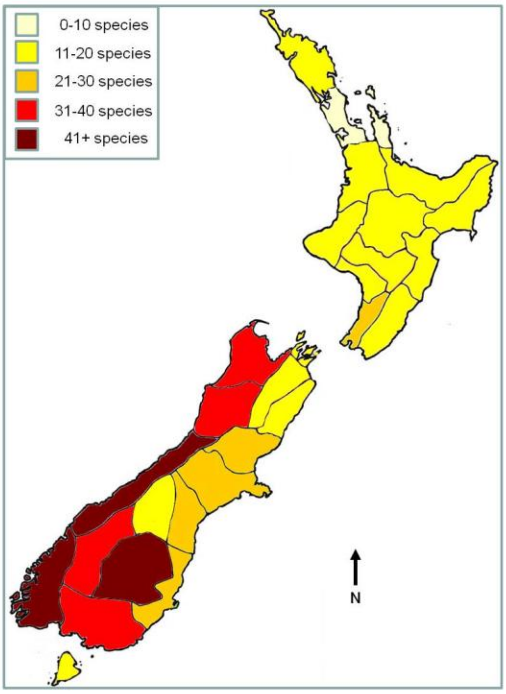


Fig 1.2: Number of species found in each NZ biogeographical region (see McLellan 2006a).

1.5 Thesis aims and structure

This thesis is in the form of three main chapters, plus an introduction and a general discussion section. Molecular data are used to reconstruct plecopteran phylogenies, with a variety of analyses used to infer the evolutionary history of NZ and southern hemisphere stoneflies.

Chapter Two examines the origin of southern hemisphere stoneflies, and determines whether the current distributions of lineages are best explained by dispersal or vicariance. Three main hypotheses are addressed in Chapter Two:

- 2a: The two stonefly sub-orders, Arctoperlaria and Antarctoperlaria, were formed by the breakup of Pangaea into Laurasia and Gondwana.
- 2b: The distribution of Antarctoperlaria lineages can be ascribed to vicariance caused by Gondwanan fragmentation.

- 2c: New Zealand stonefly lineages arrived by dispersal during or after the Oligocene.

Chapter Three uses comparative phylogeographic analysis of six alpine stonefly genera to test a vicariant evolutionary hypothesis for the origin of the ‘biotic gap’ in the central South Island. Two main hypotheses are addressed in Chapter Three:

- 3a: The six plecopteran genera will show evidence of simultaneous vicariance across the beech-gap.
- 3b: Cladogenesis will be more extensive, and any distributional gaps more prolonged in apterous species.

Chapter Four compares and contrasts the phylogeographic structure of two congenerics - the strong-flying *Zelandoperla decorata* and weak-flying *Z. fenestrata* species group. Two main hypotheses are addressed in Chapter Four:

- 4a: The weak flying *Z. fenestrata* species group will show a higher degree of genetic structuring than the strong-flying *Z. decorata*.
- 4b: The variable degrees of wing development within the *Z. fenestrata* species group will impact the level of phylogeographic structure observed.

The thesis is presented in the form of three inter-related papers intended for publication in peer-reviewed journals (2 published, 1 submitted for review). These papers are presented largely unaltered from accepted manuscripts; as a consequence some degree of repetition exists among chapters, particularly within the introduction sections. References, however, have been combined into a single list at the end of the thesis.

I collected the majority of NZ stonefly specimens with the help of field-assistants. Where I did not collect the specimens personally, the individuals or institutions that provided these specimens are acknowledged at the end of each chapter. All lab-work and phylogenetic analysis was conducted by myself, though supervisors provided assistance in interpreting results. All published and submitted papers are co-authored by my supervisors, who helped design the studies. Initial manuscripts were written by myself, though both supervisors read over manuscripts and suggested changes.

Chapter 2*: Molecular phylogenetics of Gondwanan stoneflies (Antarctoperlaria; Notonemouridae): vicariance or dispersal?

---

*The majority of this chapter has been submitted to *Journal of Biogeography* as: **McCulloch GA, Wallis GP, and Waters JM.** Molecular phylogenetics of Gondwanan stoneflies (Antarctoperlaria; Notonemouridae): vicariance or dispersal?

2.1 Abstract

The widespread but disjunct distributions of many southern hemisphere taxa have fascinated biogeographers for more than two centuries, with these distributions either attributed to vicariance associated with Gondwanan fragmentation or trans-oceanic dispersal. Stoneflies (order: Plecoptera) are a widespread order of freshwater insects whose poor dispersal ability and intolerance for salt water make them ideal candidates for Gondwanan relicts - taxa whose distribution can be explained by vicariant isolation driven by the breakup of Gondwana. Here we reconstruct the phylogenetic relationships among southern hemisphere stoneflies (5 families; 86 genera) using 2864bp of mitochondrial (COI) and nuclear (18S, H3) DNA, with a calibrated relaxed molecular clock used to estimate the chronology of diversification. Our analysis suggests that largely antitropical stonefly sub-orders, Arctoperlaria (northern hemisphere) and Antarctoperlaria (southern hemisphere), were formed 120 Ma (95% prior probability distribution 97–158 Ma), which is consistent with vicariant break up of the supercontinent Pangaea. Subsequently a single Arctoperlaria lineage has dispersed into southern hemisphere 76 Ma (95% range 63–97 Ma). The disjunct distribution of the five southern hemisphere plecopteran families is largely attributed to more recent long-distance dispersal rather than vicariance associated with Gondwanan fragmentation. Interestingly, we find no evidence for the continuous existence of stoneflies in New Zealand since its separation from Gondwana, with all New Zealand stoneflies estimated to have arrived during the Oligocene.

2.2 Introduction

The widespread but disjunct distributions of many southern hemisphere taxa have fascinated biogeographers for more than two centuries (Treviranus 1803; de Candolle 1820; Hooker 1853, 1860; Darwin 1859). Prior to the current understanding of continental movement, these biogeographic links were either explained by the existence of ancient land bridges (Matthew 1915; Simpson 1940; Darlington 1957), or in terms of trans-oceanic dispersal (Darwin 1859), with oceanic dispersal remaining the predominant explanation for over a century (Nelson 1978). After the widespread acceptance of plate tectonic theory in the late 1960s, however, these southern hemisphere links were typically explained in terms of vicariance: isolation of previously connected ancestral lineages as a result of the sequential fragmentation of the Gondwanan landmass (Croizat 1958; Brundin 1966; Raven & Axelrod 1972; Croizat *et al.* 1974; Rosen 1978; Humphries 2000; Nelson & Ladiges 2001; Ebach *et al.* 2003). Subsequently, the biogeographic importance of trans-oceanic dispersal was largely dismissed, until a recent upsurge in molecular studies suggested that vicariance alone could not explain many southern hemisphere distribution patterns (Cooper *et al.* 1992; Haddrath & Baker 2001; McDowall 2002; Briggs 2003; Givnish & Renner 2004; Sanmartin & Ronquist 2004; Knapp *et al.* 2005; McGlone 2005; Queiroz 2005; Phillips *et al.* 2010).

This paradigmatic shift back towards trans-oceanic dispersal has been particularly strong with respect to the New Zealand (NZ) biota. During the Oligocene (ca. 25–22 Ma), NZ's land area was dramatically reduced (Cooper 1989; Cooper & Millener 1993; Cooper & Cooper 1995; Campbell & Hutching 2007; Graham 2008), which would presumably have had a devastating effect on the NZ biota (Cooper & Cooper 1995).

The extent of NZ's submergence remains a controversial topic (Wallis & Trewick 2009): many geological studies have suggested that NZ was reduced to a few low-lying islands (Cooper 1989; Cooper & Millener 1993; Cooper & Cooper 1995), although more recent analyses have suggested that NZ may have been submerged completely (Landis *et al.* 2008). If the latter were the case, all of NZ's contemporary biota must have colonized since the Oligocene (Pole, 1994; Campbell & Landis, 2001; Pole 2001; Waters & Craw, 2006; Campbell & Hutching 2007; Trewick *et al.* 2007; Goldberg *et al.* 2008; Landis *et al.* 2008). Although there is evidence that some NZ lineages may have survived the Oligocene transgression (Knapp *et al.* 2007; Allwood *et al.* 2010), post-Oligocene arrival has been inferred for a large number of NZ lineages (Stöckler *et al.* 2002; Ericson & Johansson 2003; Zhang & Renner 2003; Donald *et al.* 2005; Knapp *et al.* 2005; Waters & Craw 2006; Wallis & Trewick 2009). Gibbs (2006) suggested that freshwater limited taxa, due to their intolerance for marine conditions, may provide the strongest evidence for a continuous presence of land in NZ.

Stoneflies (Plecoptera) are an ancient lineage of freshwater insects with fossils dating back to the early Permian (Sinitshenkova 1987; Sinichenkova 1997; Zwick 2000). They are a widely distributed group, with almost 4000 species described worldwide (Fochetti & Figueroa 2008). Plecoptera is divided into two geographically separated sub-orders: Antarctoperlaria in the southern hemisphere (Fig. 2.1), and Arctoperlaria in the northern hemisphere. These two sub-orders are believed to have been formed by breakup of the super-continent Pangaea at the end of the Triassic (Illies 1965; Banarescu 1990; Zwick 1990; Zwick 2000). The subsequent breakup of Gondwana has been invoked to explain the southern hemisphere distribution of the four Antarctoperlaria families throughout Australia, NZ, southern South America, and several subantartic islands (Fig. 2.2) (Illies

1965; Zwick 2000; Fochetti & Figueroa 2008), with their absence in southern Africa ascribed to climatic change (Banarescu 1990; Zwick 2003).

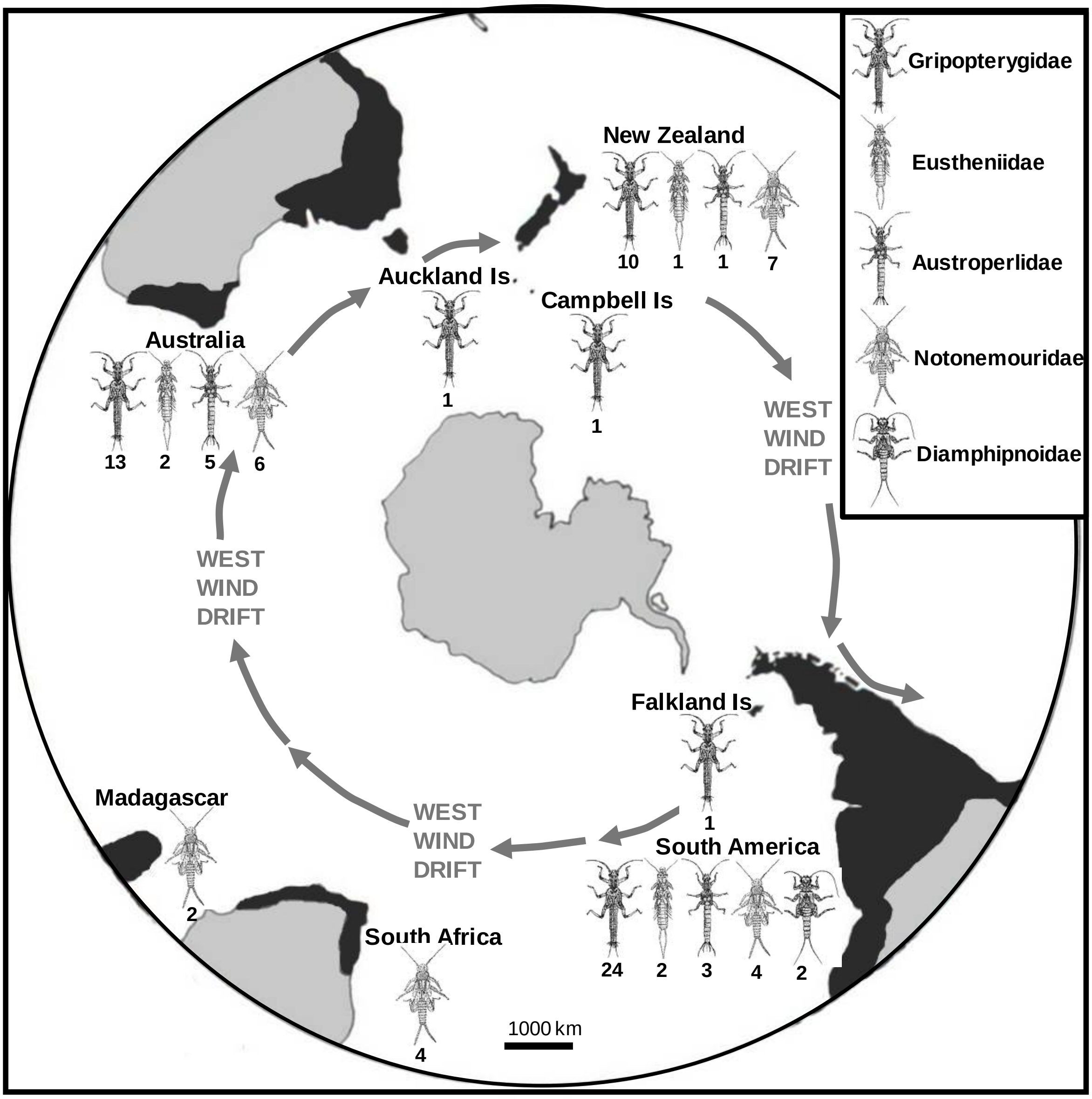


Fig. 2.1: Contemporary distribution of Plecoptera (black shading) in the southern hemisphere, with the number of genera from each family found in each region inset.

In addition to Antarctoperlaria, two families (Perlidae and Notonemouridae) of the otherwise northern hemisphere Arctoperlaria are found in southern temperate regions (Fig. 2.2). These southern hemisphere Arctoperlaria representatives are thought to reflect recent independent invasions from the north (Illies 1965; Stark & Gaufin 1976; Zwick 2000; Fochetti & Figueroa 2008). Perlidae is the only stonefly family found in both hemispheres, with genera in central South America, southern Africa, and throughout the northern hemisphere (Fochetti & Figueroa 2008). Unlike Perlidae, the family Notonemouridae is restricted to the southern hemisphere, and has a classical 'Gondwanan' distribution, resembling that of the Antarctoperlaria families. It has been suggested that Antarctica may have been an important dispersal route among the southern continents for this family (Illies 1965; Zwick 1981, 1990). The monophyly of Notonemouridae is, however, in question, with suggestions that it may be a polyphyletic assemblage, representing multiple southern invasions of northern lineages (Zwick 1981, 1990, 2000; McLellan 2000a; Fochetti & Figueroa 2008).

The level of endemicity of stoneflies in the southern hemisphere is high, with only one genus shared between distinct land masses (*Notonemoura*, NZ and Australia) (McLellan 2000a; McLellan 2006a). This high endemicity is perhaps unsurprising given that stoneflies are generally considered to be very poor dispersers due to their limited flight ability (Brundin 1967, 1972; Zwick 2000; Schultheis *et al.* 2002; Fochetti & Figueroa 2008; McCulloch *et al.* 2009). Indeed, the apparent dispersal limitations of stoneflies are further emphasized by their absence from the Chatham Islands, approximately one million years after the emergence of these islands, in contrast with several other arthropods (Goldberg *et al.* 2008). Stoneflies are thus ideal candidates as Gondwanan relicts- species whose distribution can be explained by vicariant isolation driven by the breakup of Gondwana.

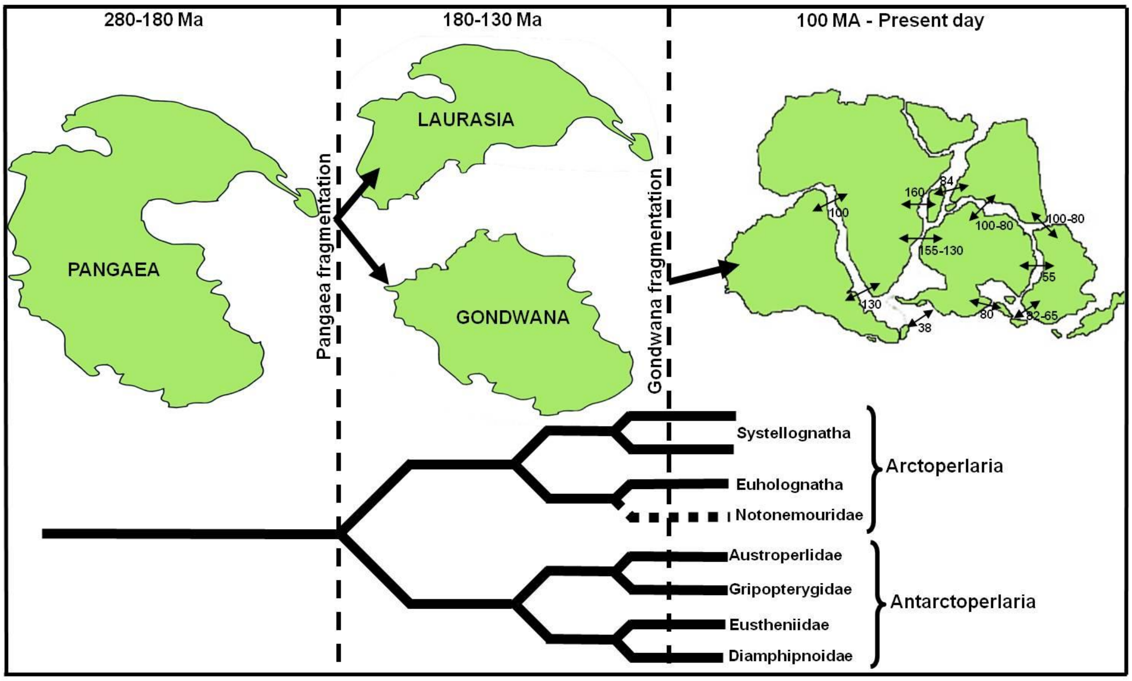


Fig. 2.2: Schematic explaining the disjunct distributions of southern hemisphere stoneflies. (a) Pangaea exists as a single supercontinent 280-180 Ma (b) The fragmentation of Pangaea results in the formation of the Arctoperlaria (Laurasia) and Antarctoperlaria (Gondwana) sub-orders. Antarctoperlaria families diverged during this time, while the progenitors of Notonemouridae dispersed into the southern hemisphere (c) Fragmentation of Gondwana (including approximate splitting times in millions of years; modified from Wallis & Trewick (2009)) results in the distribution of Notonemouridae and the Antarctoperlaria families throughout the southern hemisphere

In this paper we use DNA sequences (mitochondrial COI, nuclear 18S and H3) to reconstruct phylogenetic relationships among southern hemisphere stoneflies, and use a relaxed molecular clock to estimate the chronology of diversification. Evolutionary timescales are constrained using a variety of internal calibration points, primarily geological dates of island formation and mountain building. We test the hypothesis that the two sub-orders (Antarctoperlaria and Arctoperlaria) were formed by breakup of Pangaea into Laurasia and Gondwana. We determine whether the distribution of lineages can be ascribed to vicariance caused by Gondwanan fragmentation (Fig. 2.2), or whether dispersal has played an important role in the contemporary distributions of southern hemisphere stoneflies (Fig. 2.1). If Gondwanan vicariance is refuted for NZ taxa, we examine whether putative dispersal events to NZ predate Oligocene innundation. In addition we test for monophyly of the Arctoperlaria family Notonemouridae, and consider possible dispersal routes of this group across the southern hemisphere.

2.3 Materials and methods

2.3.1 Sampling

We obtained stonefly specimens from throughout South America, South Africa, Australia, NZ, Campbell Island, and Auckland Islands (see Appendix 1). Aquatic stonefly nymphs were collected by hand from underneath stones, or from vegetation suspended in riffle zones of streams. Recently-emerged adults of these species were similarly collected by hand from on and underneath stones at stream and river margins, or by sweeping streamside vegetation. Terrestrial larvae and their associated adults were collected by hand from damp depressions under stones, or from decaying vegetation deep in tussocks of alpine snowgrass, or other alpine vegetation. New Zealand specimens were identified using keys from Winterbourn *et al.* (2006) and McLellan (1991, 1993, 1996, 2000a, 2003). South American specimens were identified using keys from Zwick (1979), McLellan (2001), and McLellan & Zwick (2007). Australian specimens were identified using keys from Hynes (1978, 1981, 1989) and McLellan (1996), while South African specimens were identified using keys from Villet (2000).

2.3.2 *DNA extraction, amplification and sequencing*

DNA was extracted from femur tissue using 5% Chelex solution (BioRad; Walsh *et al.* 1991) containing 20 µg of proteinase K (Roche). A 644-bp portion of COI was amplified using the primers LCO1490 (5’-GGTCAACAATCATAAAGATATTGG) and HCO2198 (5’-TAAACTTCGGGTGACCAAAAAATCA) (Folmer *et al.* 1994). A 322-bp portion of the H3 gene was amplified using the primers H3AF (5’-ATGGCTCGTACCAAGCAGACVGC) and H3AR (5’-ATATCCTTRGGCATRATRGTGAC) (Colgan *et al.* 1998). A section of the 18S gene was amplified using primers 18S1F (5’ -TACCTGGTTGATCCTGCCAGTAG) and 18S9R (5’ -GATCCTTCCGCAGGTTCACCTAC) (Whiting *et al.* 1997), with the size of the amplified section ranging from 1766-bp to 1807-bp. PCRs (25 µl) contained Taq buffer (200mM Tris pH 8.4, 500mM KCl), 1.5 mM $MgCl_2$, 200 µM of dATP, dGTP, dTTP, and dCTP, 1 pmol of each primer, 0.5 units of Taq DNA polymerase (BioTaq; Bioline) and c. 100 ng of template DNA. PCR was performed in a Mastercycler ep Gradient S (Eppendorf), with the following profile: 94°C for 2 min; 40 cycles of: 94°C for 30 s, primer pair specific annealing temperature for 30 s (45°C for 18S and COI, 50°C for H3), 72°C for 30 s; a final extension for 4 min at 72°C. Sequencing reactions were performed using a capillary ABI3730 Genetic Analyzer (Applied Biosystems), using the forward primer (H3AF for H3, and LCO1490 for COI), with internal primers 18Sai (5’ -CCTGAGAAACGGCTACCACATC) and 18Sa2.0 (5’ -ATGGTTGCAAAGCTGAAAC) (Whiting 2002) used for 18S.

2.3.3 *Sequence analysis*

Both COI and H3 coding sequences obtained were aligned by eye as length variation was absent, and discrete haplotypes were identified using PAUP 4.0b10 (Swofford 2003). The 18S sequences contained substantial length variation, with alignment achieved using CLUSTALX (Larkin *et al.* 2007) followed by manual adjustment using secondary structure (Kjer 2004). Several regions of 18S could not be aligned with confidence and were excluded prior from phylogenetic analysis. Selecting the optimal portioned model for multiple genes is difficult due to the large number of potential ways of partitioning and pooling groups of sites (Nylander *et al.* 2004). As such, we opted for a conservative approach whereby we partitioned the protein coding genes (COI, H3) by gene and then by codon position, though we pooled H3 first and second codon positions due to the low number of varied sites. The 18S gene was not partitioned further because there is no biologically meaningful way of separating sets of sites (Simon *et al.* 1994; Pagel & Meade 2004). The most appropriate model of sequence evolution for each partition was selected using jModelTest 0.1.1 (Posada 2008) under the AIC selection criterion. Phylogenetic relationships for the concatenated dataset were estimated via Bayesian analysis using MRBAYES v3.12 (Huelsenbeck & Ronquist 2001). In addition, maximum likelihood topologies were recovered using Garli version 0.951 (Zwickl 2006), with nonparametric bootstrap support (Felsenstein 1985) for these topologies assessed based on 1000 pseudoreplicates. While the analysis of multiple data partitions in a concatenated form can mislead phylogenetic inference (Kubatko & Degnan 2007), this is most likely to occur during studies at shallow phylogenetic levels, where incomplete lineage sorting can produce discordant gene trees among individual data partitions. Four Markov chains (one cold and three heated; temp = 0.20) were run for a total of 20,000,000 generations, with chains sampled every 100 generations (the first 5,000 trees were discarded as burn-in). In order to root the phylogenies, we selected 10 outgroup taxa, composed of exemplars of each of the remaining polyneopteran orders (*Gromphadorhina portentosa*, *Celisoches annulatus*, *Notoligotoma* sp., *Galloisiana nipponensis*, *Mastermes darwinesis*, *Mantoida* sp., *Tyrannophasma gladiator*, *Ellipes minutus*, *Timema knulli*, *Zorotypus hubbardi*). To mimimize the effects of outgroup instability on assessment of ingroup relationships, a separate analysis was conducted with the outgroups removed. TRACER 1.4 (Rambaut & Drummond 2003) was used to check that the effective sample size was not too low, and to select appropriate burn-in cycles. Five final runs were completed, and concatenated to estimate posterior distributions.

2.3.4 Molecular clock analysis

Tests for clock-like evolution of DNA sequences among lineages were conducted in PAUP 4.0b10 (Swofford 2003) using a likelihood ratio test (Felsenstein 1988). Given the detection of significant rate heterogeneity across taxa, a exponential distributed relaxed molecular clock was used to estimate divergence times and phylogenetic relationships (Drummond *et al.* 2006). Bayesian analyses were performed to estimate divergence times using the program BEAST 1.4.8 (Drummond & Rambaut 2007). Data were partitioned by locus and codon position (see above), with a separate GTR + I + γ model of sequence evolution applied to each partition, with a speciation: Yule Process tree prior. The analysis was run ten times, with 20 million generations per run, and the ten separate runs were combined using LogCombiner. We used TRACER 1.4 (Rambaut & Drummond 2003) to monitor convergence of all parameters from the phylogenetic model to ensure that all effective sample sizes were > 200 and to select appropriate burn-in cycles. A maximum clade credibility tree was generated in TreeAnnotator and visualized in FigTree v1.1.2.

2.3.5 Molecular clock calibration

Molecular clock calibration points include several fossil calibration points and seven biogeographic calibrations within Plecoptera (Table 2.1). The oldest definitive plecopteran fossils are from the Early Permian (Sinitshenkova 2004), dated at 251 Ma.

A maximum age constraint for Plecoptera is taken at 295.4 Ma, as representatives of this lineage are absent from the rich insect fauna of Boskovice Furrow (dated at 294.6 Ma; Zajic 2000), or any older deposits. Therefore we constrained the root of Plecoptera between 251 Ma and 295 Ma by using a normal prior with a mean of 270 Ma and a standard deviation of 10 Ma, chosen such that 95% of the prior probability density was bounded by our constraints.

Fossil data are not used for calibrations within Plecoptera owing to the uncertain phylogenetic placement of individual fossils (Illies 1965; Zwick 2000). The stonefly sub-orders and contemporary families are defined on the basis of muscle morphology and behavioral characteristics, neither of which is preserved in fossils (Grimaldi & Engel 2005). There are, however, a number of stoneflies recorded from Baltic and Dominican amber from the Eocene (38–54 Ma) (Hagen 1963, Keilbach 1982; Stark & Lentz 1992). These complete stonefly specimens can be placed in contemporary Arctoperlaria families Nemouridae, Perlidae, Perlodidae, Taeniopterygidae and Leuctridae. As such the minimal age for these Arctoperlaria families is constrained to the Eocene (38 Ma).

A number of biogeographic calibration points are also included. These points include upper bounds based on island calibration events, and divergence of lineages coincident with the uplift of mountain ranges (Southern Alps in NZ, Andes in South America). Soft upper bounds were used, meaning much older ages are possible, but are associated with decreasing probabilities (see Sanders & Lee 2007). Extensive volcanic activity along the Campbell Plateau during the Miocene (19–12 Ma) formed the subantarctic Campbell and Auckland Islands (Hoernle *et al.* 2006), each of which have their own endemic stonefly genera. The upper bound for divergence among these subantarctic taxa and their sister taxa is therefore set at the formation of these Islands, 7 Ma and 17

Ma respectively (Hoernle *et al.* 2006). Banks Peninsula was similarly formed by volcanic activity during the late Miocene by the Akaroa (9.5–7.5 Ma) and Lyttleton (12–6 Ma) volcanoes (Graham 2008), and though now connected to the mainland, was originally an island off the east coast of South Island. Banks Peninsula contains a number of endemic flora and fauna, including two endemic stonefly lineages (McLellan 1993; McCulloch *et al.* 2009). The upper bound for divergence between the endemic Banks Peninsula taxa and their sister species was therefore set at 12 Ma.

Table 2.1: Summary of calibration points used to constrain molecular clock, including the age of the calibration event, the taxa involved, and geological and taxonomic references.

Calibration point	Age(Ma)	Taxa	References
Oldest fossil	250-300	*Palaeonemoura sp.*	Sinitshenkova (1997), Zwick (2000)
Amber fossils	>38	Perlodidae	Hagen (1863)
Amber fossils	>38	Perlidae	Hagen (1863)
Amber fossils	>38	Taeniopterygidae	Hagen (1863)
Amber fossils	>38	Leuctridae	Hagen (1863)
Amber fossils	>38	Nemouridae	Hagen (1863)
Banks Peninsula	<10.0	*Zelandobius wardi*	McLellan (1993), Hoernle *et al.* (2006)
		Z. fenestrata group	Hoernle *et al.* (2006), McCulloch (2009)
Auckland Island	<16	*Aucklandobius gressitti*	Hoernle *et al.* (2006)
Campbell Island	<7.5	*Rungaperla longicauda*	Hoernle *et al.* (2006)
Southern Alps	<5.0	*Taraperla johnsi*	Sutherland (1996), McLellan (2003)
Andes	<30	*Megandiperla kuscheli*	Veblen *et al.* (2007)
		Andiperla willinki	Veblen *et al.* (2007)

A number of South American and NZ taxa are wingless, with this wing-loss almost exclusively associated with high altitudes (McLellan 2006a). The uplift of NZ's Southern Alps accelerated 5 Ma, before this time little alpine habitat is thought to have existed (Wellman 1979; Sutherland 1996). This date is used to calibrate a soft maximum age of divergence between alpine adapted wingless taxa (*Taraperla johnsi)* and their fully-winged sister taxa (*Taraperla ancilis*). Similarly, the extensive uplift of the Andes in southern South America within the last 30 Ma (Veblen *et al.*2007) is used as a soft maximum age of divergence between the wingless genera (*Megandiperla, Andiperla*) and their fully-winged relatives.

To test whether our priors dominated the posterior distribution, all our BEAST analyses were also performed without data, with the results obtained from these runs compared with those obtained when the actual data were analysed.

2.4 Results

2.4.1 Data characteristics

All new sequences obtained in this study have been submitted to GenBank (accession numbers xxxxxxx-xxxxxx). COI sequences lacked any ambiguous sites or stop codons, consistent with true mitochondrial origin (Zhang & Hewitt 1996). Following concatenation of distinct gene regions, the edited DNA alignment comprised 2864 bp of sequence, from 115 individuals (105 ingroup, 10 outgroup). Sequence statistics and substitution model parameters are provided in Table 2.2.

2.4.2 DNA phylogeny

Maximum likelihood analysis (Appendix 2) and Bayesian inference (Fig. 2.3) of the combined data returned concordant and well-supported relationships across the depth of the phylogeny, although relationships at the shallowest levels tended to be unsupported. Removing the outgroups had no affect on the basic topology or branch support of either of these maximum likelihood or Bayesian phylogenies.

Table 2.2: Summary statistics for the three data partitions employed during phylogenetic analysis of Plecoptera. "AIC model" represents the inferred substitution model derived using ModelTest with the Akaike Inference Criterion, and "α" is the shape parameter of the gamma-distributed variation of rates among characters, while "I" is the proportion of invariant characters.

	COI	**H3**	**18S**
Number of sites	646	354	2030
Variable sites (%)			
All taxa	333 (51.5)	135 (38.4)	673 (33.2)
Ingroup only	281 (43.5)	125 (35.3)	509 (25.1)
Informative sites (%)			
All taxa	291 (45.0)	119 (33.6)	434 (21.3)
Ingroup only	270 (41.8)	107 (30.2)	291 (14.3)
Substitution model			
AIC model	TVM + I + γ	GTR + I + γ	TIM + I + γ
α	0.72	0.98	0.26
I	0.47	0.61	0.40

A Bayesian phylogeny reveals two main clades, broadly corresponding to the sub-orders Antarctoperlaria and Arctoperlaria (Fig. 2.3). All of the four recognized Antarctoperlaria families (Eustheniidae, Austroperlidae, Gripopterygidae and Diamphipnoidae) are represented by well-supported, reciprocally monophyletic assemblages. Likewise the Antarctoperlaria family Notonemouridae forms a well-supported clade, sister to the remaining Euholognatha families.

Within both Austroperlidae and Eustheniidae, neither Australia nor South America is represented by a monophyletic species assemblage (Fig. 2.3). As a case in point, Eustheniidae is divided into two clades consistent with taxonomy rather than geography: our analysis supports the monophyly of Eustheniidae (in South America and Australia) and of Stenoperlinae (in South America, NZ and Australia) (McLellan 1996).

The sole NZ eustheniid genus, *Stenoperla*, is placed sister to the South American *Neuroperla*, while the sole Austroperlidae genus, *Austroperla*, is sister to a clade containing both Australian and South American genera. Australian, NZ and South American Gripopterygidae assemblages do not form reciprocally monophyletic lineages, although there are several geographically-restricted clades (Fig. 2.3). One such clade contains eight of 12 Australian Gripopterygidae genera, while another clade contains 11 of 12 NZ Gripopterygidae genera. The close affinity between these NZ Gripopterygidae lineages is broadly consistent with existing taxonomy, with ten of them included within the sub-family Zelandoperlinae (McLellan 1977). Placement of *Vesicaperla* within this group was surprising, as it had been previously placed within the sub-family Antarctoperlaria, sister to *Zelandobius* (McLellan 1977). Four major clades are identified within Notonemouridae (Fig. 2.3), three of these consistent with generic groupings proposed by (McLellan 1991): the Austrocercella group, the Notonemoura group, and the Spaniocercoides group. Each of these clades contains genera from NZ, South America and Australia, with NZ genera sister to South America genera in two of the three clades. The fourth Notonemouridae clade contains South African genera previously suggested to be part of the Spaniocercoides group (McLellan 1991).

2.4.3 Divergence dates

Analyses without data confirmed that the priors were not dominating the data. The evolutionary divergence between the two major stonefly sub-orders, Antarctoperlaria and Arctoperlaria, was dated at 120 Ma (95% range 97–158 Ma). The oldest split within Antarctoperlaria - the clade containing Eustheniidae and Diamphipnoidae diverging from Gripopterygidae and Austroperlidae - was dated at 72 Ma (95% range 53–86 Ma). Austroperlidae subsequently diverged from Gripopterygidae approximately

59 Ma (95% range 44–72 Ma). The initial divergences within the Antarctoperlaria families occurred approximately 50 Ma, with the majority of the genera apparently formed post Oligocene.

The divergence between Notonemouridae and the remaining Euholognatha families was dated at 76 Ma (95% range 63–97 Ma). The oldest divergence within Notonemouridae, between the South African notonemourid genera and the remaining Notonemouridae, occurred approximately 58 Ma (95% range 46–79 Ma), with the remaining three Notonemouridae clades diverging less than 50 Ma (Fig. 2.3).

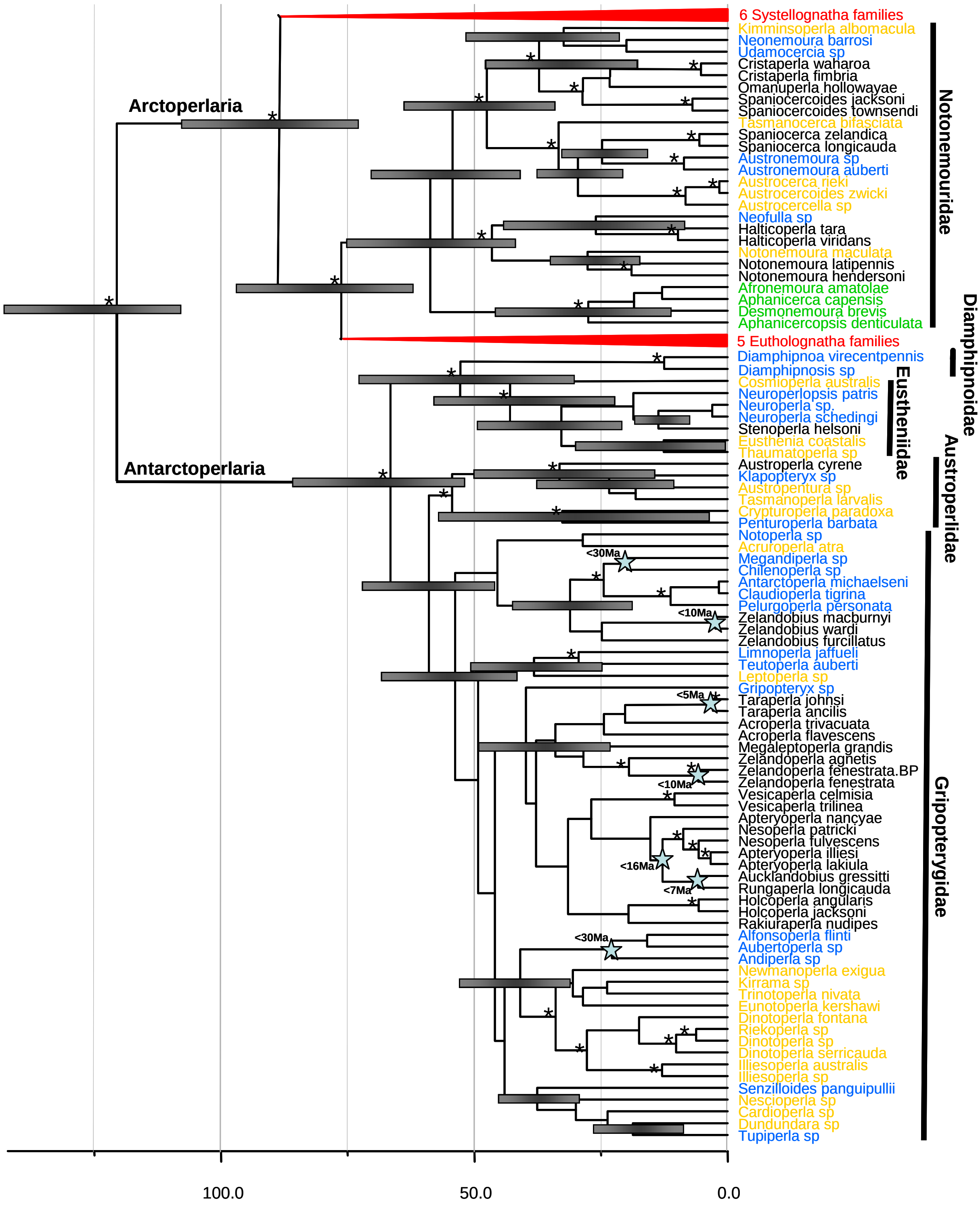


Fig. 2.3: Bayesian maximum clade consensus phylogeny of the relationships among Plecoptera, based on 3 regions: 18S, H3, COI. Posterior probabilities exceeding 95% are indicated by * above each node. Stars represent nodes that were bounded during age estimation (see Table 2.1). Horizontal bars at nodes of biogeographic significance indicate the 95% posterior probability of the age of the node. Scale bar across the bottom represents ages in million years. Outgroups are removed for diagrammatic purposes. Red = northern hemisphere, blue = South American, green = African, orange = Australian, black = NZ.

2.5 Discussion

2.5.1 Vicariant formation of sub-orders

Stoneflies are an ancient lineage, though their area of origin is unknown (Zwick 2000). Stoneflies have a largely anti-tropical distribution (Giller & Malmqvist 1998), with Plecoptera divided into two predominantly disjunct sub-orders: the austral Antarctoperlaria and Northern Arctoperlaria (Zwick 1990). Our analysis supports the monophyly of these sub-orders, with Antarctoperlaria and Arctoperlaria forming strongly supported clades (Fig. 2.3). Despite the clear distinction between sub-orders at a molecular level, their monophyly is not supported by any distinctive external features. Arctoperlaria is united by the single putative synapomorphy of drumming, an audible form of communication amongst adults involving tapping, rubbing or scraping of the abdomen against a rock or log, whereas Antarctoperlaria is united by the unique depressor of the fore trochanter (Baumann 1975; Beer-Stiller & Zwick 1995) and the presence of floriform chloride cells, which are thought to have a sensory function (Berthelemy 1968). This striking disjunction between the northern and southern hemisphere stonefly fauna is unusual among freshwater organisms, with such anti-tropical distribution patterns more typically observed in marine organisms (see Briggs 1987, 1995). Though similar anti-tropical patterns are evident within a few other archaic freshwater invertebrate groups (Korovchinsky 2006), these patterns are usually observed at lower taxonomic levels, such as between genera or species (Dumont 2002).

The origins of anti-tropical distribution patterns are typically attributed to either transequatorial movement during periods of cold climate (e.g. Pleistocene glaciations; Lindberg 1991), or as a result of the vicariant fragmentation of Pangaea during the Mesozoic (Croizat *et al.* 1974; Rosen 1978). A number of molecular studies have been conducted on anti-tropical Pacific fish taxa, with over half of these studies inferring transequatorial dispersal during the Pleistocene (Burridge 2002). Only one transequatorial divergence exceeding 20 Ma has been inferred for marine fishes: northern hemisphere and southern hemisphere atherinopsines are estimated to have diverged 40 Ma. A similar transequatorial dispersal event has been inferred for the freshwater fish superfamily Osmeroidea (Waters *et al.* 2002), with southern hemisphere family Retropinnidae estimated to have diverged from the northern hemisphere Osmeridae around 80 Ma (Burridge *et al.* in review).

In the current study, our analysis suggests that the two stonefly sub-orders diverged 120 Ma (97–158 Ma; 95% posterior probability), a date range that approaches the fragmentation of supercontinent Pangaea in the Jurassic (McLoughlin 2001), and therefore perhaps consistent with vicariant formation of these two sub-orders (Illies 1965; Banarescu 1990; Zwick 1990; Zwick 2000) (Fig. 2.2). Among insect taxa, the unusually low dispersal ability of many stoneflies (Brundin 1967; Marden & Kramer 1994; Thomas *et al.* 2000; Zwick 2000; Schultheis *et al.* 2002; McCulloch *et al.* 2009) may explain the inferred important role of vicariance for these weak-flying taxa. In addition, ecophysiological factors — the broad preference for cool, swift-flowing waters — may have helped to maintain this pattern of anti-tropical divergence.

2.5.2 Antarctoperlaria

The disjunct distribution of the stonefly sub-order Antarctoperlaria across the southern hemisphere has been suggested to be a result of vicariance associated with Gondwanan fragmentation (Illies 1965; Zwick 2000; Fochetti & Figueroa 2008). Our results do not support this hypothesis, however, with the majority of the cladogenesis within Antarctoperlaria postdating the initial fragmentation of Gondwana. The divergence between NZ lineages and their sister taxa all post-date 82 Ma, suggesting that they arrived in NZ via more recent dispersal (see below). Australia and South America, on the other hand, remained connected through Antarctica until the opening of the Drake Passage 41 Ma (Scher & Martin 2006). As the majority of divergences between South American and Australian stonefly lineages coincide with this fragmentation period, we suggest vicariant isolation of these landmasses may be responsible for these biogeographic disjunctions.

Antarctoperlaria are not present throughout all remnants of Gondwana, with their notable absence from both Africa and India. India and Madagascar split from Gondwana approximately 132 Ma, while Africa began rifting from the rest of Gondwana 135 Ma, and by 105 Ma all land connections are thought to have been severed (McLoughlin 2001; Torsvik *et al.* 2009). Our analysis suggests that the major diversification of Antarctoperlaria postdates the separation of Africa and India from Gondwana. This is supported by the fact that no plecopteran fossils have been identified in India, and though a few fossil plecopterans are recorded from Africa, they are from the late Permian (250 Ma). These late Permian fossil genera, *Euxenoperla* and *Euxenoperlella* (Riek 1973, 1976), were placed in Antarctoperlaria: Gripopterygidae, though this placement was considered to be largely arbitrary (Zwick 2000). Our analysis suggests that these fossils likely represent early plecopteran lineages, prior to the divergence of the Antarctoperlaria and Arctoperlaria sub-orders.

The absence of any antarctoperlarian lineages from South Africa and India is often attributed to extinction driven by climatic change (Banarescu 1990; Zwick 2003). After separating from Gondwana, South Africa and India drifted northwards, with the climate in these regions believed to have become warmer and drier (Banarescu 1990; Zwick

2003). The complete absence of antarctoperlarian lineages from the fossil records of these regions, however, suggests it is more likely that these lineages they were never there. The climate of Gondwana is not considered to have been uniform, with paleobotanists and biogeographers usually recognizing two climatically distinct biogeographic provinces: a northern tropical province (including northern South America, Africa, Madagascar and Northern Australia), and a southern temperate province (including southern South America, Australia, Antarctica, NZ, and New Caledonia) (Craw 1982; Karol *et al.* 2000). Given the ecological requirements of stoneflies, early antarctoperlarian lineages may have been restricted to the cooler southern province of Gondwana, which would explain their absence from both South Africa and India.

2.5.3 Arctoperlaria in the southern hemisphere

Notonemouridae is considered by many authors to represent a polyphyletic assemblage of Arctoperlaria lines that have independently dispersed into the southern hemisphere (Zwick 1990, 2000; McLellan 2000b; Fochetti & Figueroa 2008). Our analyses (which incorporate 19 of 25 recognized Notonemouridae genera) do not support this evolutionary biogeographic scenario, as they provide strong support for the combined monophyly of Notonemouridae (Fig. 2.3). We dated the divergence of Notonemouridae from remaining Euholognatha at 76 Ma (95% range 63–97 Ma), and infer that notonemourid ancestors probably spread to the southern hemisphere during the late Cretaceous.

It has, alternatively, been suggested that the distribution of Notonemouridae throughout the southern hemisphere is best explained by Gondwanan vicariance (Illies 1965; McLellan 1993; Zwick 2000). In contrast, our results suggest that the arrival of notonemourids in the southern hemisphere postdates the separation of both Africa (McLoughlin 2001) and NZ (Kamp 1986; Cooper & Millener 1993; Campbell & Hutching 2007) from Gondwana (Fig. 2.3), though Australia and South America were still connected via Antarctica (McLoughlin 2001). This finding suggests that the disjunct distribution of the southern hemisphere Notonemouridae is broadly attributable to long-distance dispersal, though the divergences between South American and Australian lineages can potentially be attributed to vicariance, as within Antarctoperlaria (see above).

The route of dispersal of Notonemouridae into the southern hemisphere is unknown, though Zwick (2000) suggested that this lineage may have dispersed into the southern hemisphere through Africa. The African lineages form a unique clade restricted to South Africa, with the unfavorable (warmer) northern conditions likely restricting the range of these stoneflies. This African clade is sister to the remaining Notonemouridae, and is estimated to have diverged from its sisters 58 Ma (95% range 46–79 Ma). This timing implies an early dispersal of Notonemouridae to Africa from another southern hemisphere landmass, or from Africa to the rest of the southern hemisphere. Given there is only a single South African clade, a single dispersal seems likely, with Africa’s mid-latitude and warm climate perhaps preventing the more frequent dispersal inferred among cool-temperate southern regions (Australia, NZ, and South America).

2.5.4 The origin of NZ stoneflies

NZ boasts a phylogenetically diverse stonefly assemblage that is characterized by recent cladogenesis (Fig. 2.3). While NZ Plecoptera are considered traditionally to have an ancient vicariant origin (Illies 1965; Brundin 1967; Zwick 2000), our molecular estimates for the ages of the NZ lineages completely postdate fragmentation of NZ from

Gondwana ca. 82 Ma (Fig. 2.3). The majority of NZ lineages appear to have arrived during the Oligocene (Fig. 2.3), consistent with the colonization of an emerging NZ following the end of marine inundation around 25 Ma (Cooper & Millener 1993; Landis *et al.* 2008). Though the extent of marine transgression in the Oligocene remains controversial (Wallis & Trewick 2009) it is clear that extensive land reduction took place (Landis *et al.* 2008). This land reduction is believed to have had a major influence on the composition of NZ's biota with the subsequent re-emergence of land providing new habitats for colonization (Cooper & Cooper 1995; Wallis & Trewick 2009).

Our molecular phylogenetic analysis suggests independent colonization events involving notonemourid and gripopterygid ancestors, with additional single colonization events inferred for Austroperlidae and Eustheniidae. NZ's gripopterygid taxa are paraphyletic, though 11/12 NZ Gripopterygidae genera (including the two subantarctic genera) form a monophyletic group that appears to have dispersed from South America 36 Ma (22–46 Ma). Wing reduction and loss is particularly common within this group, with five of the genera completely wingless, while each of the other six genera possess wingless species (McLellan 2006a,b; McCulloch *et al.* 2009, 2010) The second NZ gripopterygid clade consists of a single highly speciose genus (*Zelandobius*), containing 30 described species as well as a number of undescribed species (McLellan 2003, 2006a). *Zelandobius* is included within a clade containing a number of South American genera, consistent with the sub-family Antarctoperlinae (McLellan 1977). Antarctoperlinae were traditionally considered part of Gripopterygidae, though Zwick (1973) placed them between Gripopterygidae and Austroperlidae – our analysis supports their placement within Gripopterygidae. The estimated divergence between the *Zelandobius* group and the South American lineages again dates to the Oligocene, estimated at 30 Ma (20–43 Ma). The divergence could possibly be more recent, as the

South American species *Neopentura* (not sampled) resembles *Zelandobius* more closely than any other South American gripopterygid taxa, suggesting it may be the sister species (Vera & Camousseight 2006; McLellan & Zwick 2007). The effect of missing taxa in causing overestimation of divergence times is well known (Douzery *et al.* 2004; Crisp & Cook 2005; Waters & Craw 2006; Goldberg *et al.* 2008; McCormick *et al.* 2011).

NZ's sole austroperlid and eustheniid genera are generally considered to have a vicariant history (Illies 1965; Zwick 2000), though McLellan (1975) suggested that these lineages may have been more recent wind-drift invaders. Our results are consistent with McLellan (1975), with evidence that these families arrived during the Oligocene from Australia and Chile respectively (Fig. 2.3). Likewise, NZ's notonemourid genera appear to have arrived more recently by dispersal, with all divergences dating to the Oligocene. This includes the divergence between Australia and NZ *Notonemoura*, with this deep divergence between congeneric taxa suggesting that the taxonomy of this genus may need to be readdressed.

This analysis therefore rejects a direct Gondwanan derivation of contemporary NZ lineages, and is broadly consistent with an Oligocene colonization of the landmass. This conclusion is becoming commonplace, indicating that most NZ lineages dispersed from elsewhere, and thus lack a direct Gondwanan ancestry (Stöckler *et al.* 2002; Winkworth *et al.* 2002; Ericson & Johansson 2003; Zhang & Renner 2003; Arensburger *et al.* 2004; Donald *et al.* 2005; Knapp *et al.* 2005; Waters & Craw 2006; Wallis & Trewick 2009).

2.5.5 Dispersal rates

Given the preponderance of dispersal between NZ and other southern landmasses, it is intriguing that our data provide no evidence of any particularly recent (<1 Ma) dispersal events. This result is further reinforced by the fact that no species, and only a single genus, is shared between these regions (*Notonemoura*: NZ and Australia). Colonization rates are affected not only by dispersal ability, but also by habitat availability and ecological competition (Whittaker & Fernández-Palacios 2007). Current inhabitants may competitively exclude more recent dispersers, potentially explaining the apparent lack of ongoing dispersal between other isolated southern landmasses (South America, Australia). The re-emergence of NZ during the Oligocene may explain the inferred extensive dispersal to NZ during this time, and likewise volcanic islands are likely to have been colonized relatively rapidly after their emergence. As a case in point, colonization of the subantarctic Auckland Islands (320 km south of NZ) by gripopterygids is inferred to have taken place 14 Ma (10–17 Ma) (Fig. 2.3), soon after their volcanic formation ca. 17 Ma (Hoernle *et al.* 2006). Interestingly, the Auckland Island and Campbell Island (600 km south of NZ) endemic stonefly genera are sister taxa (Fig. 2.3), consistent with a common origin for these subantarctic lineages. It seems likely that the common ancestor of these taxa dispersed to Auckland Islands 13 Ma, and then subsequently to Campbell Island 6 Ma (4–7 Ma), not long after the volcanic emergence of the latter. Both Campbell Island and the Auckland Islands were moderately affected by glaciations during the Pleistocene (McGlone *et al.* 1997, 2000; McGlone 2002), though it seems clear that these divergent lineages survived these periods. Most species from these regions are flightless and possess terrestrial or semi terrestrial-larvae, adaptations that have been suggested to promote recruitment and aid survival during glacial periods (McLellan 1977; McCulloch *et al.* 2010). Along similar lines, Banks Peninsula was apparently colonized by a *Zelandoperla* lineage not long after its formation 12 Ma, whereas an endemic Banks Peninsula *Zelandobius* lineage appears to have colonized more recently. Similarly, it appears that *Nesoperla patricki* colonized the Dunedin volcano 9 Ma (7–12 Ma) soon after its formation 10 Ma.

Despite successful colonization of NZ's subantarctic islands, stoneflies have failed to colonize either the Chatham or Antipodes Islands (McLellan 2006a). These islands, however, have a not been available for colonization for long, with the Antipodes Islands formed by volcanic activity within the last 500,000 years (Adams 1981), while Chatham Islands, although geologically old (formed by volcanic activity 70–80 Ma) are thought to have been submerged under water for a long period prior to their re-emergence in the Pleistocene (Campbell *et al.* 1993; Campbell 1998; Trewick *et al.* 2007).

2.5.6 Methods of dispersal

Stoneflies are typically considered to be weak flyers with very limited dispersal ability (Brundin 1967; Brundin 1972; Zwick 2000; Schultheis *et al.* 2002; Fochetti & Figueroa 2008; McCulloch *et al.* 2009). Our results, however, suggest a number of dispersal events between continents, and to oceanic islands, suggesting that – over geological time - these taxa are more capable of long distance dispersal than previously suggested. Although it is possible that such dispersal is active, it is more likely a passive process achieved in the form of wind-dispersal via aerial plankton. Though the majority of aerial plankton consists of microscopic invertebrates, it has been shown to also include large, weak-flying insects such as stoneflies (Bilton *et al.* 2001; Kelly *et al.* 2001). Dispersal around the southern hemisphere is likely driven by the predominantly westerly winds that circulate around Antarctica, know as the west wind drift (WWD). This circumpolar dispersal mechanism (Sanmartin & Ronquist 2004; Waters 2008) was initiated followed the separation of Antarctica from Australia, and Antarctica from

South America (Scher & Martin 2006). While it might be expected that long-distance dispersal is typically in the direction of the prevailing wind (e.g. from Australia to NZ), as suggested by our data, there also appear to be possible instances of dispersal against this prevailing wind (e.g. from South America to NZ), though these nodes are not strongly supported. This is also consistent with the findings of (Sanmartin & Ronquist 2004), whose meta-analysis identified that the majority of animal dispersal is in the direction of these prevailing winds, but with some notable exceptions (see also Winkworth *et al.* 2002).

Like many recent studies, our results point to a dominant role of long-distance dispersal in explaining the presently disjunct distribution of lineages across ocean basins. Though the slow drift of tectonic plates likely explains the biogeographical distribution of some deep lineages (e.g. the Antarctoperlaria/Arctoperlaria divergence, and a number of South America/Australia divergences), it appears that these vicariant events are largely overlain and obscured by a number of more recent dispersal events.

2.6 Acknowledgements

R. Albarino, L. Anderson, C. Burridge, S. Campbell, B. McCulloch, J. McCulloch, I. McLellan, T. Ormandy, P. Pessacq, T. Reed, D. Rowe, D. Smith (Australian Museum), S. Waddingham, B. Wilson, M. Winterbourn and F. Wright assisted in collecting specimens or providing tissue samples. This research was supported by a University of Otago PhD Scholarship to GAM, and Marsden contract UOO-0404 (Royal Society of New Zealand).

Chapter 3*: Onset of glaciation drove simultaneous vicariant isolation of alpine insects in New Zealand

---

*The majority of this chapter has been published as: **McCulloch GA, Wallis GP, Waters JM** (2010) Onset of glaciation drove simultaneous vicariant isolation of alpine insects in New Zealand. *Evolution*, **64**, 2033-2043.

3.1 Abstract

The origin of the New Zealand ‘beech gap’, a low-diversity zone in the central South Island corresponding with a disjunction in the distribution of many taxa, has been the focus of biogeographical debate for many decades. Here we use comparative phylogeographic analysis (COI; H3) of six alpine stonefly genera (116 individuals, 102 localities) to test a vicariant evolutionary hypothesis for the origin of this ‘biotic gap’. We find strikingly similar phylogeographic patterns in all six genera, with the deepest genetic divergences always found between samples north and south of the ‘beech gap’. The magnitude of north-south genetic differentiation for COI is similar across all six genera (ranging from 0.074-0.091), with a test for simultaneous vicariance confirming that divergence is consistent with a single evolutionary event. The concordant cladogenesis detected across multiple taxa is consistent with vicariant isolation caused by the onset of glaciation in the late Pliocene. This study thus indicates an important cladogenetic role for glaciation, an abiotic evolutionary process that is more typically associated with loss of biodiversity.

3.2 Introduction

Understanding the evolutionary forces that structure genetic diversity over space and time is a central goal of phylogeography (Avise 2000). It has long been hypothesized, for instance, that co-distributed taxa are likely to be subject to similar abiotic evolutionary forces, yielding similar phylogeographic signatures across multiple species (Darwin 1859; Avise *et al.* 1998; Johns & Avise 1998; Avise 2000; Riddle *et al.* 2000; Johnson & Cicero 2004; Hickerson *et al.* 2006). In recent times, comparative phylogeography has emerged as an important tool for investigating such parallel evolutionary effects of geological or climatic processes (Avise *et al.* 1998; Bermingham & Moritz 1998; Schaal *et al.* 1998; Avise 2000; Arbogast & Kenagy 2001; Avise 2004). Most recently, new analytical tools have paved the way for rigorous statistical phylogeographic tests of simultaneous vicariance effecting cladogenesis across multiple taxa (Hickerson *et al.* 2006).

New Zealand (NZ) is an ideal region for studies of evolutionary biogeography due to its isolation and dramatic geological and climatic history (Cooper & Millener 1993; Newnham *et al.* 1999). This "small continent" (Wallis & Trewick 2009) has been isolated from other major land masses since it separated from the supercontinent Gondwana 82 million years ago (Ma) (Cooper & Millener 1993), and has been separated from the nearest major land mass (Australia) by more than 1500 km of ocean for the last 65 Ma (Kamp 1986; Campbell & Hutching 2007; Graham 2008). Following Oligocene innundation (Pole 1994; Cooper & Cooper 1995; Waters & Craw 2006; Trewick *et al.* 2007; Landis *et al.* 2008), NZ's re-emergence is a direct result of tectonic activity at the Alpine Fault — the boundary of the Pacific and Australian plates (Waters & Craw 2006; Landis *et al.* 2008). In addition to extensive uplift, Alpine Fault activity has generated 440–470 km of lateral displacement since 25–23 Ma (Sutherland 1994, 1999), with the majority of this movement occurring within the last 11–16 million years (myr) (Cutten 1979; King 2000). The Southern Alps were formed by compression along the fault line that intensified during the Pliocene and early Pleistocene (6–5 Ma) (Gage 1980; Suggate 1982; Batt *et al.* 2000; Chamberlain & Poage 2000). Most recently, NZ has experienced several glacial periods since the late Pliocene (2.5 Ma), marked by the formation of a continuous complex of extensive glaciers and ice fields along the Southern Alps (Suggate 1990). Ice coverage was most extensive in central South Island, with glaciers extending to sea level in the west (Newnham *et al.* 1999). In contrast, glacial cover remained relatively patchy in South Island's northern region (Fig. 3.1; Newnham *et al.* 1999).

This turbulent geological and climatic history is thought to have strongly impacted the evolution of NZ's endemic biota (Wardle 1988; Wallis & Trewick 2009). Many recent studies have discussed the biological impacts of mountain building in South Island (McGlone 1985; Trewick *et al.* 2000; Buckley *et al.* 2001; Arensburger *et al.* 2004; Chinn & Gemmell 2004; Trewick & Morgan-Richards 2005; Buckley & Simon 2007; Greaves *et al.* 2007; O'Neill *et al.* 2008). In addition, it has long been recognized that South Island is characterised by two regions of high endemicity (Otago-Southland, and north-west Nelson) separated by a central region of low endemicity (Burrows 1965; McGlone 1985; Craw 1989; Rogers 1995; Wallis & Trewick 2001; Gibbs 2006). This 'low-diversity' central zone is also the site of biogeographic disjunction for many taxa (Burrows 1965; Heads 1998), and is often termed the 'beech-gap', reflecting the conspicuous absence of the southern beech (*Nothofagus*) forest from this region (Cockayne 1926).

The central South Island biotic 'gap' has been variously explained in terms of tectonic activity (lateral displacement, mountain building) or, alternatively, as an effect of climate change (Pleistocene glaciations) (McGlone 1985; Wardle 1988; McGlone *et al.* 2001; Trewick & Wallis 2001; Wallis & Trewick 2001; Greaves *et al.* 2007). Cockayne (1926), for instance, proposed that taxa from the central region were eliminated through glacial advance and unstable glacial outwashes during ice ages, whereas northern and southern populations likely persisted in ice-free refugia (Fig. 3.1). This glacial refugial hypothesis has remained the predominant explanation for this biotic gap for many decades (Willett 1950; Burrows 1965; McGlone 1985; McGlone *et al.* 2001; Leschen *et al.* 2008). In contrast, (Heads 1998) proposed that slow but persistent lateral displacement along the fault line over the last 25 million years may have gradually separated once adjacent populations of Nelson (now north western South Island) and Otago (southern South Island), leaving the central area uninhabited.

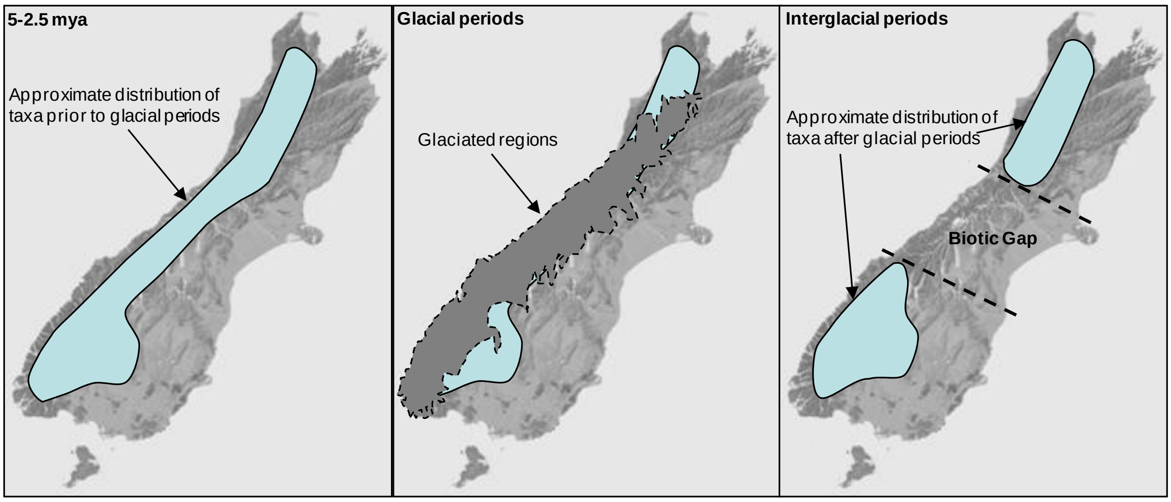


Fig 3.1: Hypothetical model for glacially-mediated divergence of alpine biota in the South Island, New Zealand. This schematic scenario explains the origin of the 'biotic gap', and the associated genetic divergence of northern and southern alpine lineages.

Using mtDNA from a collection of 45 invertebrate species, Trewick & Wallis (2001) identified several distinct phylogeographic patterns in flightless South Island taxa.

Specifically, these authors described three broadly repeated patterns (termed ‘regional’, ‘gap’, and ‘colonization’), with alpine taxa showing distinctive ‘gap’ phylogeographies (Trewick & Wallis 2001). The ‘gap’ pattern refers to taxa with disjunct northern and southern populations, whereas ‘regional’ pattern refers to taxa that lack such broad north-south structure, and ‘colonization’ patterns indicate relatively shallow phylogeographic histories (recent dispersal) (Trewick & Wallis 2001). The approximate timeframe of north-south genetic divergence was inferred to be less than 5.5 Ma in all taxa (Trewick & Wallis 2001), consistent with either alpine uplift or glaciations. Subsequently, NZ phylogeographic studies have identified similar “gap” patterns in a range of other invertebrate taxa, ascribing them to the effects of mountain-building (Trewick *et al.* 2000; Chinn & Gemmell 2004; Boyer *et al.* 2007; Leschen *et al.* 2008), or glaciations (Brown *et al.* 1999; Trewick 2001; Neiman & Lively 2004; Marshall *et al.* 2008; Hill *et al.* 2009; Marshall *et al.* 2009).

NZ’s distinctive alpine biota has presumably evolved since the formation of the Southern Alps 5 Ma (Fleming 1975; Gibbs 2006). Many of the regions insect taxa exhibit convergent adaptations to the alpine habitat, often involving increased size and wing reduction or even complete wing-loss (Mark & Adams 1995; Patrick 2003; Gibbs 2006). Wing-loss is particularly common in stoneflies (Plecoptera), with five of the 11 endemic genera in the Gripopterygidae being completely wingless (apterous) (McLellan 1977, 1983, 2003, 2006a). In addition to wing-reduction, the nymphs of a number of alpine stoneflies have apparently abandoned their traditional aquatic lifestyle, instead inhabiting damp terrestrial habitats beneath stones, in caves, and in alpine vegetation (McLellan 1967, 1977, 1983, 1993, 2003, 2006b).

In this paper we use both the mitochondrial COI region and the nuclear H3 region — both of which are commonly-used markers for invertebrate phylogeographic studies (e.g. Trewick & Wallis 2001; McCulloch *et al.* 2009) — to test for simultaneous vicariance in six co-distributed sub-alpine stonefly genera. These genera (*Apteryoperla*, *Holcoperla*, *Vesicaperla*, *Cristaperla*, *Halticoperla*, *Spaniocerca*) have broad distributions throughout South Island's sub-alpine zone, including distinct species at either end of the 'beech-gap' biogeographic break. We hypothesized that these six genera would show evidence of simultaneous vicariance across the beech-gap, as they are predicted to have been similarly affected by a shared climatic and geological history. In addition, we contrast the phylogeographic structure of three apterous genera (*Apteryoperl*a, *Holcoperla*, *Vesicaperla*) with that of three fully-winged (macropterous) genera (*Cristaperla*, *Halticoperla*, *Spaniocerca*). It was hypothesized that cladogenesis will be more extensive, and any distributional gaps more prolonged, in apterous species reflecting lower dispersal ability of these taxa.

3.3 Materials and Methods

3.3.1 Sampling

Specimens of the six stonefly genera were collected from 102 localities throughout South Island and lower North Island, NZ (Fig. 3.2; Appendix 3). Aquatic nymphs (*Cristaperla*, *Halticoperla*, *Spaniocerca*, and a single *Apteryoperla* species) were collected by hand from underneath stones or in suspended vegetation in riffle zones of streams. Recently-emerged adults of these species were similarly collected by hand from on and underneath stones at stream and river margins, or by sweeping streamside vegetation. Terrestrial larvae and their associated adults (*Holcoperla*, *Vesicaperla* and four *Apteryoperla* species) were collected by hand from damp depressions under stones or from decaying vegetation deep in tussocks of alpine snowgrass, or other alpine vegetation. Specimens were identified using keys from McLellan (1977, 1991, 1993, 2000b, 2003). All specimens were preserved in 95% ethanol.

3.3.2 DNA extraction, amplification and sequencing

DNA was extracted from femur tissue using 5% Chelex solution (BioRad; Walsh *et al.* 1991) containing 20 μg of proteinase K (Roche). A 644-bp portion of COI was amplified using the primers LCO1490 (5'-GGTCAACAATCATAAAGATATTGG) and HCO2198 (5'-TAAACTTCGGGTGACCAAAAAATCA) (Folmer *et al.* 1994). Additionally a 322-bp portion of the H3 gene was amplified using the primers H3AF (5'-ATGGCTCGTACCAAGCAGACVGC) and H3AR (5'-ATATCCTTRGGCATRATRGTGAC) (Colgan *et al.* 1998). PCRs (25 μl) contained Taq buffer (200mM Tris pH 8.4, 500mM KCl), 1.5 mM $MgCl_2$, 200 μM of dATP, dGTP, dTTP, and dCTP, 1 pmol of each primer, 0.5 units of Taq DNA polymerase (BioTaq; Bioline) and c. 100 ng of template DNA. PCR was performed in a Mastercycler ep Gradient S (Eppendorf), with the following profile: 94°C for 2 min; 40 cycles of 94°C for 30 s, 45°C for 30 s, 72°C for 30 s; a final extension for 4 min at 72°C. Sequencing reactions were performed using a capillary ABI3730 Genetic Analyzer (Applied Biosystems), using the forward primer (H3AF for H3, and LCO1490 for COI). Typically, we sequenced one individual per species per site.

3.3.3 Sequence analysis

Both COI and H3 coding sequences obtained were aligned by eye as length variation was absent, and discrete haplotypes were identified using PAUP 4.0b10 (Swofford 2003). MODELTEST 3.7 (Posada & Crandall 1998) was used to select the most appropriate model of sequence evolution for the data under the AIC selection criterion.

Pairwise sequence divergences were calculated using the selected model implemented in PAUP4.0b10 (Swofford 2003). Unrooted phylogenetic relationships within genera were estimated via Bayesian analysis using MRBAYES v3.12 (Huelsenbeck & Ronquist 2001). Four Markov chains (one cold and three heated; temp = 0.20) were run for a total of 20,000,000 generations, with chains sampled every 100 generations (the first 5,000 trees were discarded as burn-in). TRACER 1.1.1 (Rambaut & Drummond 2003) was used to check that the effective sample size was not too low, indicating that the MCMC mixed well and that the samples were independent. Bayesian analyses were repeated to ensure convergence of split frequencies across independent runs, as assessed using AWTY (Wilgenbusch *et al.* 2004).

We used the program MSBayes (Hickerson *et al.* 2007) to test explicitly for simultaneous divergence of the deepest South Island lineages within each of the six genera studied. We used this hierarchical ABC model to estimate the number of possible divergence times for the six taxon pairs (Ψ). The hyper-posteriors were obtained from 1,000 accepted draws from 1,000,000 simulated draws from the hyper-priori using acceptance/rejection with local regression algorithm (Beaumont *et al.* 2002). Reruns with different prior distributions of parameters were conducted to see whether these affected posterior distribution of the parameters estimates, but no effects were detected.

3.4 Results

Sequencing of 116 specimens yielded 97 discrete COI haplotypes (GenBank accession numbers GQ471345-GQ471442). These sequences lacked any ambiguous sites or stop codons, consistent with true mitochondrial origin (see Zhang & Hewitt 1996). A COI phylogeny of NZ stoneflies supported monophyly of each genus (data not shown). Central South Island populations previously described as *Holcoperla magna* (samples 12, 13) were phylogenetically distinct from southern South Island samples of this species (samples 7–11), and instead placed sister to the northern South Island taxon *H. jacksoni*. These central South Island *Holcoperla* samples (12, 13) are subsequently referred to as a distinct taxon, *H.* sp. nov.

In addition to strongly-supported clades associated with described taxa, several species also exhibited substantial phylogeographic differentiation. For instance, regional phylogenetic structuring of COI was detected within both the apterous *A. illiesi* and *H. magna*, and the macropterous *C. fimbria* and *S. zelandica* (Fig. 3.2). Haplotype sharing across sample sites was common for macropterous genera, with 15 of 58 (26%) macropterous haplotypes detected in multiple localities (Table 3.1). Substantially stronger population structuring was observed within the apterous species, with only four of 39 (10%) haplotypes shared between localities (Table 3.1). Moreover, these shared haplotypes always involved sites less than 10 km apart (Fig. 3.2). Similarly, mean levels of intraspecific divergence within apterous taxa (0.007–0.013) were higher than in macropterous species (0.006–0.007) (Table 3.1), despite the relatively small geographic ranges of the former taxa.

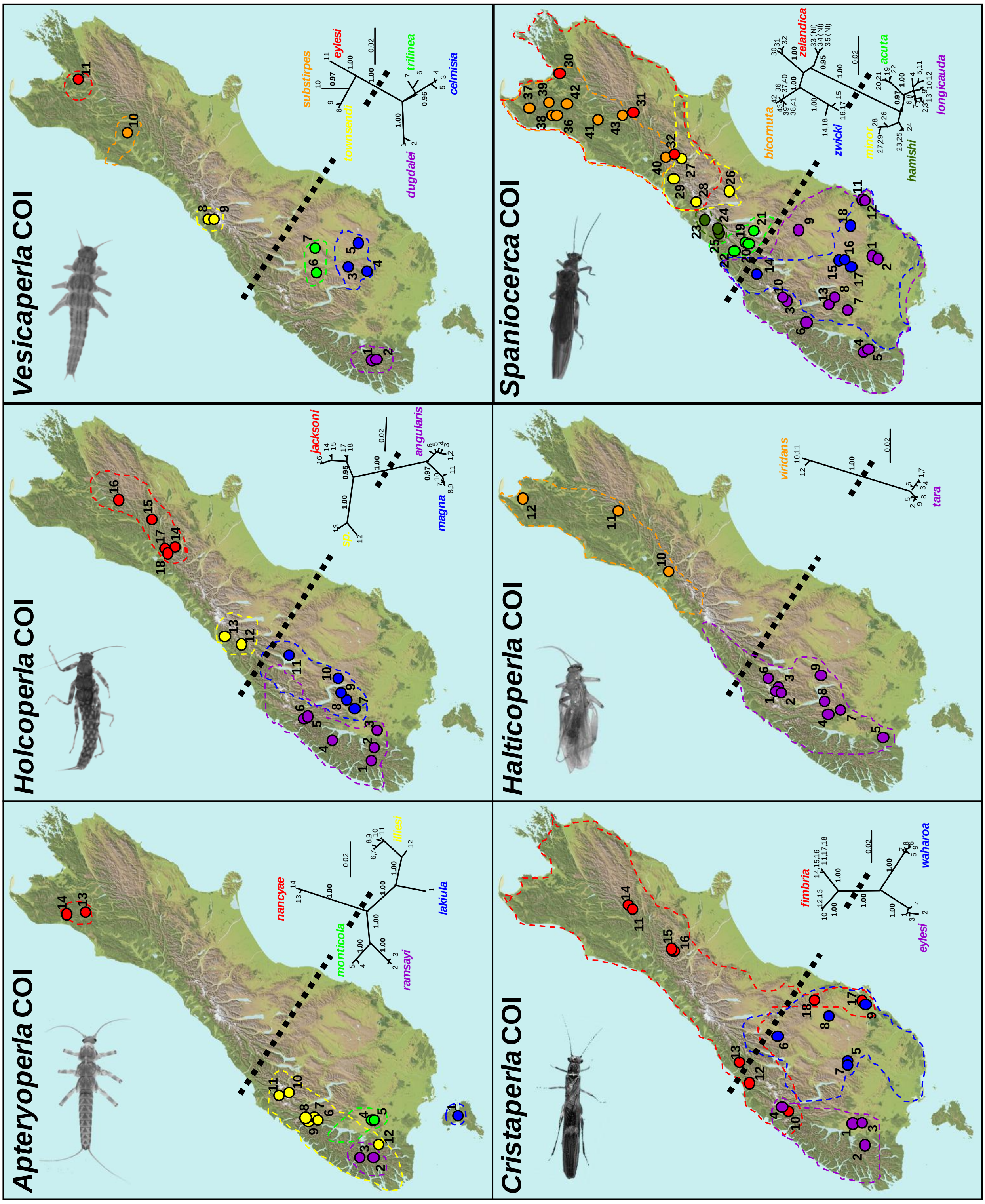


Fig 3.2: Sample sites and known species ranges (dashed colored lines) for three apterous (*Apteryoperla, Holcoperla, Vesicaperla*) and three macropterous (*Cristaperla, Halticoperla, Spaniocerca*) stonefly genera, with associated COI Bayesian phylogenies inset. Posterior probabilities (when exceeding 0.95) are indicated on internal edges of the Bayesian phylogenies. Dashed black lines represent the deepest genetic divergence within each genus, with the corresponding geographical location of this phylogeographic break indicated on the map.

Sequencing of 67 specimens for H3 yielded 37 discrete sequences (GenBank accession numbers GQ471443-GQ471479). H3 phylogenies were generally congruent with COI phylogenies for each genus (Fig. 3.3). *S. longicauda* and southern South Island *H. magna* (7–11) were, however, paraphyletic for H3 (i.e. had yet to evolve monophyly; Fig. 3.3).

Table 3.1: Model selected by ModelTest, number of variable sites, and number of informative sites for each genus.

Genus	Gene	Model selected	Variable sites	Informative sites
Apteryoperla	COI	TVM + γ	165	132
	H3	HKY	*18*	*18*
Holcoperla	COI	TVM + γ	169	156
	H3	TrN + γ	11	11
Vesicaperla	COI	HKY	147	129
	H3	GTR + I	*17*	*17*
Cristaperla	COI	TVM + γ	116	106
	H3	HKY + γ	14	13
Halticoperla	COI	HKY + γ	109	99
	H3	GTR + γ	6	6
Spaniocerca	COI	TrN + γ	143	127
	H3	GTR + γ	25	23

In all genera examined here, the deepest COI genetic divergence detected with South Island involved comparisons between samples north and south of the “biotic gap” (Fig 3.2 and 3.3). In all cases, with the exception of *Halticoperla,* this well-supported north-south divergence also represented the deepest split detected within each genus (i.e. including any North Island samples; data not shown). The magnitude of the deepest

COI divergence detected among congeneric species distributed north and south of the ‘biotic gap’ was very similar across all genera, ranging from an average of 0.074 (within *Halticoperla*) to 0.091 (within *Cristaperla*) (Table 3.1). A test for simultaneous vicariance using MSBayes showed that this divergence is consistent with a single vicariant event (Fig. 3.4), with the number of divergence events estimated at 1.37 (95% confidence interval 1.00–1.98).

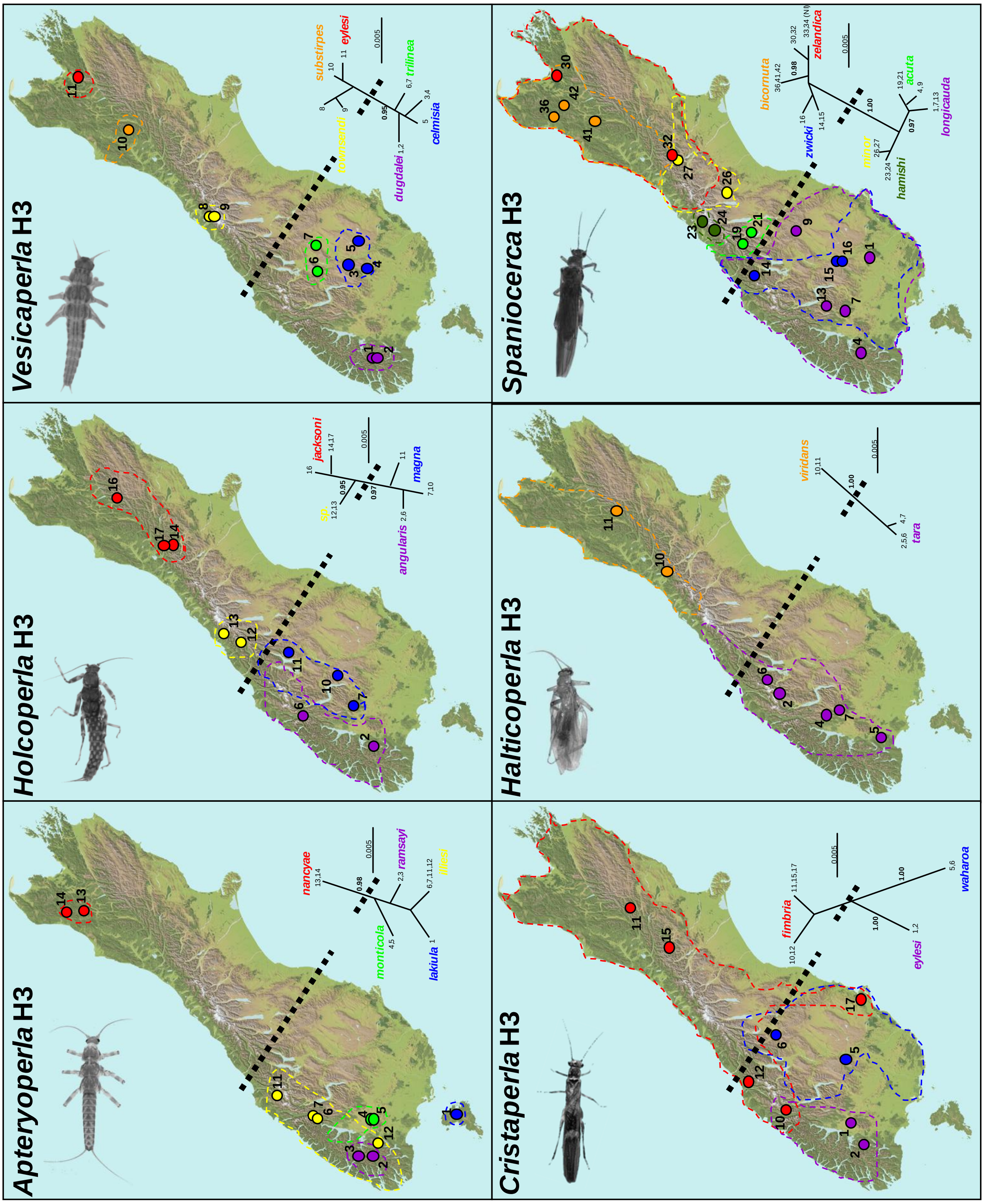


Fig 3.3: Sample sites and known species ranges (dashed colored lines) for three apterous (*Apteryoperla*, *Holcoperla*, *Vesicaperla*) and three macropterous (*Cristaperla*, *Halticoperla*, *Spaniocerca*) stonefly genera, with associated H3 Bayesian phylogenies inset. Posterior probabilities (when exceeding 0.95) are indicated on internal edges of the Bayesian phylogenies. Dashed black lines represent the deepest genetic divergence within each genus, with the corresponding geographical location of this phylogeographic break indicated on the map.

The average numbers of species per genus was similar for macropterous (4.0) versus apterous (4.5) genera (Table 3.2). In biogeographic terms, the apterous genera (*Apteryoperla*, *Holcoperla*, *Vesicaperla*) were particularly poorly represented in the South Island 'biotic gap', with only two species found in this central region (*Vesicaperla townsendi* and *Holcoperla* sp. nov) (Fig. 3.2).

Table 3.2: Sample size, number of species, shared COI haplotypes between sampling localities, and corrected COI genetic divergences for three apterous and three macropterous alpine stonefly genera. "North" indicates samples found north of the southern limit of the beech gap, while "South" indicates samples found south of this line.

Genus	Sample size (North/South)	North/South divergence	No. of species	Intraspecific divergence	Haplotypes shared
Apterous					
Apteryoperla	14 (2/12)	0.089	5	0.013	2/12
Holcoperla	18 (7/11)	0.088	4	0.012	2/16
Vesicaperla	11 (4/7)	0.078	6	0.007	0/11
totals	*43 (13/30)*	*0.081*	*15*	*0.012*	*4/39*
Macropterous					
Cristaperla	18 (9/9)	0.091	3	0.006	3/13
Halticoperla	12 (3/9)	0.074	3	0.007	2/12
Spaniocerca	43 (25/18)	0.076	7	0.007	10/33
totals	*73 (37/36)*	*0.078*	*13*	*0.007*	*15/58*

In contrast, the distributional gap between northern and southern species of macropterous genera was either relatively small (*Halticoperla*) or completely absent (*Cristaperla, Spaniocerca*). In these latter macropterous taxa, it appears the gap has been 'closed' as a result of dispersal into and across this central region. Indeed, published distributional data for macropterous species indicate that their ranges are considerably greater than those of apterous taxa (Fig. 3.2). This pattern is further emphasized by the fact that several macropterous species (e.g. *C. fimbria*, *S. zelandica*, *H. gibbsi*) have ranges extending across Cook Strait to North Island.

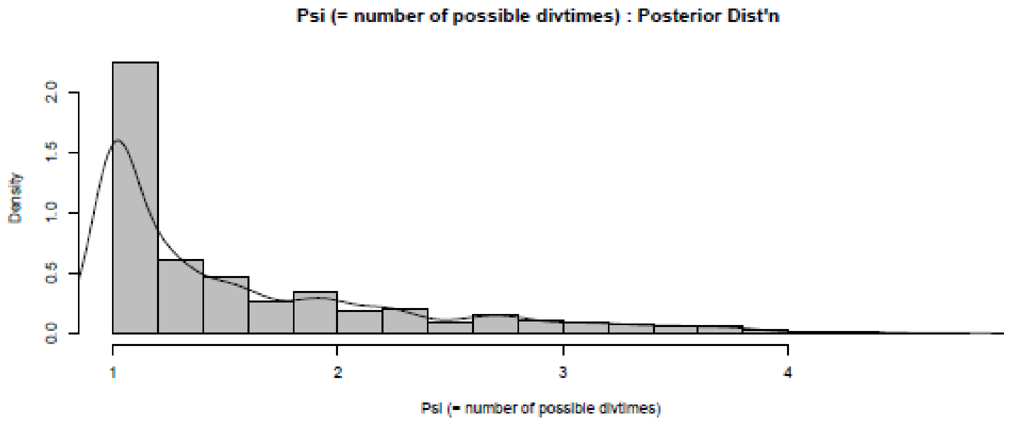


Fig 3.4: Posterior estimates of temporal concordance among six north-south lineage pairs (within *Apteryoperla, Holcoperla, Vesicaperla, Cristaperla, Halticoperla, Spaniocerca*) of NZ Plecoptera, for the COI gene. This analysis supports a simultaneous cladogenetic event across all six genera.

3.5 Discussion

3.5.1 Concordant vicariance

The phylogeographic analyses presented here support the hypothesis that a single vicariant event has driven simultaneous cladogenesis in alpine stoneflies across central NZ. In five of the six stonefly genera studied here, the deepest South Island divergence clearly corresponded to a north-south phylogeographic split across the central 'biotic gap' (Figs. 3.2 and 3.3). In addition to this spatial concordance, the magnitude of genetic divergence (0.074–0.091) was very similar across all six genera, consistent with a common evolutionary event (simultaneous vicariance). If standard insect COI calibrations are applicable here (e.g. 2–3% divergence per myr; (DeSalle *et al.* 1987; Brower 1994; Juan *et al.* 1995, 1996), this putative vicariant event likely occurred in the late Pliocene, substantially later than would be expected if the separation were a result of movement along the alpine fault, as previously proposed (Heads 1998). On the other hand, our inference of Pliocene cladogenesis is consistent with the findings of phylogeographic studies conducted on a range of NZ subalpine fauna, including skinks (O'Neill *et al.* 2008), cockroaches (Chinn & Gemmell 2004), cicadas (Buckley *et al.* 2001; Hill *et al.* 2009) and scree weta (Trewick *et al.* 2000). Future clock calibration studies should aim to further refine the estimated timing of these important divergence events.

The consistent north-south divergence pattern detected here is consistent with the glacial vicariant model (Fig. 3.1). Although it is difficult to disentangle the effects of late Pliocene uplift and glaciation, two features are instructive in this regard: there is a clear longest edge to the trees (as opposed to star phylogenies), and this edge spans a narrow heavily-glaciated region. These features indicate that the onset of glaciation best explains the origin of the 'beech gap'.

3.5.2 Postglacial range expansion

The ability of a species to re-colonize the 'biotic gap' following glacial extirpation is predicted to reflect its dispersal ability (Cockayne 1926; Trewick & Wallis 2001). Published biogeographic data (McLellan 1967, 1977, 1983, 1993, 2003; 2006a,b) provide clear evidence supporting this prediction, with a number of macropterous species (e.g. *C. fimbria*, *C. waharoa, H. viridans, S. longicauda*, *S. zelandica*; Fig. 3.2) having apparently re-colonized the 'biotic gap' since the last glacial maximum 14,000 years ago, whereas most apterous taxa have clearly failed to do so.

Given that there have been numerous glacial cycles in the last 2.5 myr (Graham 2008), it might be predicted that some taxa would show genetic evidence of multiple vicariant events. Specifically, taxa that successfully re-colonize the gap during interglacials may be subject to additional (more recent) vicariance driven by subsequent glacial cycles. We see possible evidence of this process within *C. fimbria*, where south-western samples (10, 12, 13) are phylogeographically distinct from their northern counterparts (11, 14-16) (Fig. 3.2). Incidentally, McLellan (1993) noted that Westland populations of *C. fimbria* are morphologically distinct from northern populations, the latter having larger ovipositors (females) and shorter hairs on cerci, keel and apical patch (males). We can infer similar postglacial dispersal and isolation within macropterous *Spaniocerca*: *S. zwicki* appears to have expanded south across the gap and has subsequently become isolated, whereas *S. minor*, *S. acuata*, and *S. hamishi* all appear to have expanded north into the 'biotic gap'.

3.5.3 Refugia within central South Island?

Interestingly, a single apterous species (*V. townsendi*), and *Holcoperla* sp. nov (Fig. 3.2) are apparently restricted to the gap region, suggesting the possibility of localized glacial refugia in this region. While glaciers are thought to have extended to sea-level in western South Island (Newnham *et al.* 1999), it has been speculated that deforested glacial outwash to the east of the biotic gap may have provided localized habitat for alpine fauna throughout the glacial periods (McGlone 1985; Smissen *et al.* 2003; Alloway *et al.* 2007). These central South Island endemics add to a growing list of taxa that appear to have survived recent glacial cycles within the gap region (Buckley *et al.* 2001; Lockhart *et al.* 2001; Trewick 2001; Heenan & Mitchell 2003; Smissen *et al.* 2003; Chinn & Gemmell 2004; Leschen *et. al.* 2008; Waters & Craw 2008; Buckley *et. al.* 2009; Hill *et al.* 2009; Marshall *et. al.* 2009).

3.5.4 Macropterous versus apterous taxa

Based on our study, it seems clear that macropterous stonefly species exhibit wide biogeographic ranges relative to apterous taxa, with the majority of the latter species restricted to single mountain ranges. These contrasting biogeographic patterns reflect the intrinsic correlation between dispersal ability and species range (Rundle *et al.* 2002; Beck & Kitching 2007). Moreover, macropterous taxa show evidence of gene flow among populations, with haplotypes often shared among geographically remote sites. Along similar lines, McCulloch *et al.* (2009) reported a clear correlation between flight-ability and gene flow in the widespread NZ stonefly genus *Zelandoperla*.

In addition to having increased adult dispersal through flight, all of the macropterous species possess aquatic nymphs that have the potential to disperse in streams through passive larval drift (Palmer *et al.* 1996; Bilton *et al.* 2001). Although the relative importance of adult versus nymph dispersal is not well known (Schultheis *et al.* 2002; MacNeale *et al.* 2005; McCulloch *et al.* 2009), it is clear that the only flightless species possessing an aquatic nymph (*A. illiesi*) has a geographic range substantially larger than any other member of its genus (Fig 3.2.). Therefore, we suggest that larval dispersal may play a major role in shaping stonefly biogeography.

Given that flight loss is thought to be a major driver of speciation (Roff 1994a; Emerson & Wallis 1995; Bell *et al.* 2004; Voje *et al.* 2009), it may seem surprising that the macropterous versus apterous genera studied here are equally speciose. Although apterous populations are clearly more easily isolated in small geographic regions, we speculate that the wider distributions of macropterous taxa make them more susceptible to multiple (repeated or parallel) isolation events (e.g. multiple north-south divergence events in *Spaniocerca*) (Darwin 1859).

3.5.5 Climate change and speciation

While historic climate change has been implicated in causing extinction and reducing genetic diversity across many parts of the globe (Sage & Wolff 1986; Crowley & North 1988; Hewitt 2000), it has also been suggested that climate change may sometimes increase genetic diversity by driving vicariant speciation (Hewitt 1996; Avise *et al.* 1998; Carstens & Knowles 2007; Voje *et al.* 2009). Glaciations, in particular, appear to have been important in driving cladogenesis in a variety of taxa, both globally (Knowles 2000, 2001; Weir & Schluter 2004; Shepard & Burbrink 2008, 2009) and in NZ (Trewick & Wallis 2001; Neiman & Lively 2004; Trewick & Morgan-Richards 2005; Leschen *et al.* 2008). Along similar lines, our study shows that severe glaciation of central South Island has played a major role in driving evolution in NZ's alpine ecosystems.

3.6 Acknowledgements

L. Anderson, C. Burridge, B. McCulloch, J. McCulloch, I. McLellan, D. Rowe, S. Waddingham, and M. Winterbourn assisted with sampling. This research was supported by a University of Otago PhD Scholarship to GAM, and Marsden contract UOO-0404 (Royal Society of New Zealand).

Chapter 4*: Do insects lose flight before they lose their wings? Population genetic structure in subalpine stoneflies.

---

*The majority of this chapter has been published as **McCulloch GA, Wallis GP, Waters JM** (2009). Do insects lose flight before they lose their wings? Population genetic structure in subalpine stoneflies. *Molecular Ecology*, **18**, 4073-4087.

4.1 Abstract

Wing reduction and flightlessness are common features of alpine and subalpine insects, and are typically interpreted as evolutionary adaptations to increase fecundity and promote local recruitment. Here we assess the impact of wing reduction on dispersal in stoneflies (Plecoptera: Gripopterygidae: *Zelandoperla*) in southern New Zealand. Specifically, we present comparative phylogeographic analyses (COI; H3) of strong-flying *Z. decorata* (144 individuals, 63 localities) versus the co-distributed but weak-flying *Z. fenestrata* species group (186 individuals, 81 localities). The latter group exhibits a variety of morphotypes, ranging from fully-winged to completely wingless. Consistent with its capacity for strong flight-mediated dispersal, *Z. decorata* exhibited no substantial phylogeographic differentiation across its broad South Island range. Conversely the weak-flying *fenestrata* species group exhibited substantial genetic structure across both fine and broad geographic scales. Intriguingly, the variable degrees of wing development observed within the *fenestrata* species group had no apparent impact on levels of phylogeographic structure, which were high regardless of morphotype, suggesting that even fully-winged specimens of this group do not fly. This finding implies that *Zelandoperla* flight-loss occurs independently of wing-loss, and might reflect underlying flight-muscle reduction.

4.2 Introduction

The evolution of flight in insects approximately 400 million years ago (Ma), coincided with exceptionally high rates of diversification (Wootton 1976, 1981; Carpenter & Burnham 1985; Grimaldi & Engel 2005). Flight conferred an increased ability to access resources, locate mates and escape predators (Denno *et al.* 2001), and has undoubtedly contributed to the success of insects. Despite the obvious advantages of flight, this dispersal capacity has been lost repeatedly (Roff 1990; Wagner & Liebherr 1992) in nearly all winged orders (Roff 1994b). The loss of flight, typically due to a reduction in wing length, has been attributed to the high energy expenditure required in the production and maintenance of flight apparatus, at the expense of other life history traits (Zera & Denno 1997). In wing-dimorphic insects, flightless females have been shown to have higher fecundity, reproduce at an earlier age, and produce more progeny compared to their flighted counterparts (Harrison 1980; Roff 1984; Zera 1984; Roff & Bradford 1996; Roff *et al.* 1999; Ikeda *et al.* 2008). Similarly, flightless males have been shown to acquire more matings and father more offspring than their flighted counterparts (Langellotto & Denno 2000).

The dispersal strategy adopted by an insect species is expected to reflect a balance between the advantage of having wings to access and exploit resources, and the increased reproductive success of flightless individuals (Roff 1990; Zera & Denno 1997; Denno *et al.* 2001). Habitat isolation is suggested to be one of the most important selective forces shaping insect dispersal strategies (Roff 1990; Denno *et al.* 1991; Denno *et al.* 2001), particularly on oceanic islands. It is suggested that consistent loss of flighted emigrants from islands should select locally against flight, though the spatial scale at which habitat isolation selects against flight has been debated (Roff 1990;

Wagner & Liebherr 1992; Denno *et al.* 2001). Similarly, at high altitudes small habitat size selects against flighted individuals (den Boer 1970; Harrison 1980). High winds may accelerate the process of wing loss, with local recruitment difficult to achieve for flighted alpine taxa (Jackson 1928; Hynes 1941; Carlquist 1974; Roff 1990).

Not all winged insects are capable of flight (Harrison 1980; Roff 1986; Roff & Fairbairn 1991); flight muscle reduction alone can cause flightlessness (Harrison, 1980). Flight muscle reduction is common in reduced winged morphs of a species, and this muscle reduction is thought to be more important than wing reduction in increasing fecundity (Zera & Denno 1997; Zera *et al.* 1999). Roff (1986) suggested that flight muscle reduction occurs before wing reduction in the evolution of flightless species, with flight muscle size polymorphic in many fully-winged taxa.

The New Zealand (NZ) biota is dominated by sedentary endemic taxa apparently descended from dispersive ancestors (Emerson & Wallis 1995; Waters & Wallis 2001; Waters & Craw 2006; Wallis & Trewick 2009). Flight loss is thought to be a major driver of speciation (Emerson & Wallis 1995, Bell *et al.* 2004) and is generally thought to be irreversible (but see Whiting *et al.* 2003). As a case in point, NZ's alpine region is home to an abundance of flightless insect taxa (Patrick 2003), and flightless stoneflies (Plecoptera) are particularly common (flightless adults recorded from 25 of 104 native stonefly species (McLellan 2006a)). In addition, levels of aptery vary within many NZ stonefly taxa, with some species exhibiting the full range of adult phenotypes: from fully-winged (macropterous), short winged (micropterous), to wingless (apterous) adults (McLellan 1977, 1996, 1997, 1999, 2006b). Although the majority of NZ's flightless stonefly species have relatively restricted geographic ranges, with many taxa apparently confined to a single mountain range (McLellan 1967, 1993), one flightless species is found throughout lower South Island: *Zelandoperla pennulata* (McLellan 1999). This widespread taxon ranges from Bluff in the south to Banks Peninsula north, and is located on both sides of the Southern Alps (McLellan 1999). *Z. pennulata* is placed in the "*fenestrata* species group" with the macropterous *Zelandoperla fenestrata* and *Zelandoperla tillyardi*, the latter having both macropterous and micropterous populations (McLellan 1999). Despite this variation within and between species, no polymorphism with respect to these wing morphotypes has ever been reported within populations (McLellan pers. comm.). These three taxa are closely related and show little or no difference in male genitalia (McLellan 1999). Indeed, the only clear morphological difference between *Z. fenestrata* and *Z. pennulata* is the wing reduction in the latter (Fig. 4.1; McLellan 1967, 1999). *Z. tillyardi* differs from these two species only in the presence of yellow bars on the adult femur and tibia (McLellan 1999). Despite usually being fully winged (Fig. 4.1), *Z. tillyardi* and *Z. fenestrata* adults appear to fly rarely or not at all. In contrast to the limited flight capabilities of the *fenestrata* species group, the congeneric and co-distributed *Zelandoperla decorata* is a stronger flyer (Ian McLellan, pers. comm.). For the purposes of this paper we treat *Z. decorata* and the *Z. fenestrata* species group as co-distributed biogeographic equivalents. The three nominal species of the latter assemblage have essentially parapatric distributions equivalent to that of *Z. decorata* as a whole.

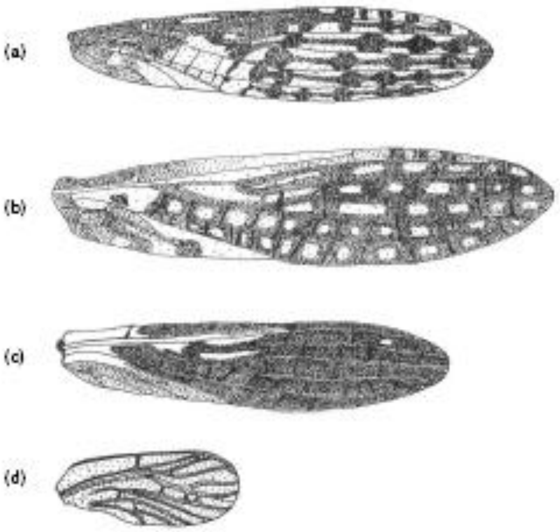


Fig. 4.1: ***Zelandoperla*** **forewings: (a)** ***Zelandoperla decorata,*** **and (b-d)** ***fenestrata*** **species group: (b)** ***fenestrata*****, (c)** ***tillyardi*** **and (d)** ***pennulata*****. Adapted from McLellan (1999), with permission of Publisher (© 1999 Royal Society of New Zealand).**

Stoneflies have a long aquatic larval stage followed by a much shorter terrestrial adult stage (Merritt & Cummins 1996). Larvae can potentially disperse by swimming and crawling through the stream, though it is believed that primary dispersal mechanism is by downstream larval drift (Waters 1965; Müller 1982; Dudgeon 1983; Palmer *et al.* 1996). Müller (1982) proposed that gravid female insects fly upstream to oviposit to compensate for the downstream larval drift. While this pattern has indeed been observed in some stream insect taxa (Neves 1979; Flecker & Allan 1988), most studies infer more complex patterns of adult movement, involving migration upstream, downstream and between streams (Bird & Hynes 1981; MacNeale *et al.* 2005).

The movement of adult stream insects is difficult to measure directly using traditional methods (Bunn & Hughes 1997), so the use of genetic markers to estimate dispersal is becoming more widespread (Schmidt *et al.* 1995; Hughes *et al.* 1998, 1999, 2000; Smith & Collier 2001; Hogg *et al.* 2002; Schultheis *et al.* 2002; Smith *et al.* 2006).

High rates of dispersal should be reflected by little genetic differentiation among populations, whereas low levels of movement will promote accumulation of substantial genetic differences among populations. The extent of genetic structure revealed by population genetic studies of aquatic insects varies greatly. Some species have extensive genetic structure (Hogg *et al.* 2002) while others are broadly homogeneous (Smith & Collier 2001). These contrasting results likely reflect both interspecific variation in dispersal ability and additional abiotic factors, such as selective sweeps or population expansions.

Within the last 5 Ma, NZ's South Island has experienced extensive glaciations, tectonic uplift, and horizontal plate displacement (Sutherland 1996), leading to numerous river reversal and river capture events (Koons 1995; Turnbull 2000; Craw & Norris 2003; Craw *et al.* 2007). These geological events have played significant roles in the biogeography and evolution of NZ's freshwater–limited fishes (Waters *et al.* 2000, 2001, 2006; Waters 2007; Burridge *et al.* 2006, 2007, 2008). As flightless stonefly species — like freshwater fishes — may have low dispersal potential, we predict that *Zelandoperla* species will exhibit strong spatial genetic structure and show genetic evidence of historic drainage alterations.

Here we use mitochondrial cytochrome oxidase I (COI) and nuclear histone 3 (H3) genes to assess phylogeographic relationships among flighted and flightless *Zelandoperla* populations. We hypothesize that taxa that are flightless (*Z. pennulata*), or have reduced flight (*Z. fenestrata*, *Z. tillyardi*) will have limited dispersal capabilities, leading to a high degree of genetic structure among populations. In contrast, we expect little genetic structure among populations of *Z. decorata*, consistent with its relatively strong flight capacity. We also expect the *fenestrata* species group stoneflies to show signals of historical vicariant events, such as river capture and river reversals, through phylogeographic analysis.

4.3 Materials and Methods

4.3.1 Sampling

Specimens of *Z. decorata* and the *fenestrata* species group were collected from 111 localities within South Island, NZ (Fig. 4.2a & 4.2b respectively; Appendix 4). Recently emerged adults were collected by hand from on and underneath stones at stream and river margins. Large *Zelandoperla* nymphs were typically collected by hand from under stones in riffle zones of moderate to high-gradient streams and rivers. Additionally, large nymphs were obtained from a few sites with the aid of electric fishing apparatus and a stop net. *Zelandoperla agnetis* and *Zelandoperla denticulata* were collected for use as outgroups. All specimens were stored in 95% ethanol. Each adult specimen was examined morphologically and characterized as macropterous, micropterous, or apterous (following McLellan 1999; see Appendix 4). We used a *t*-test to assess the significance of altitudinal partitioning of these distinct adult morphotypes.

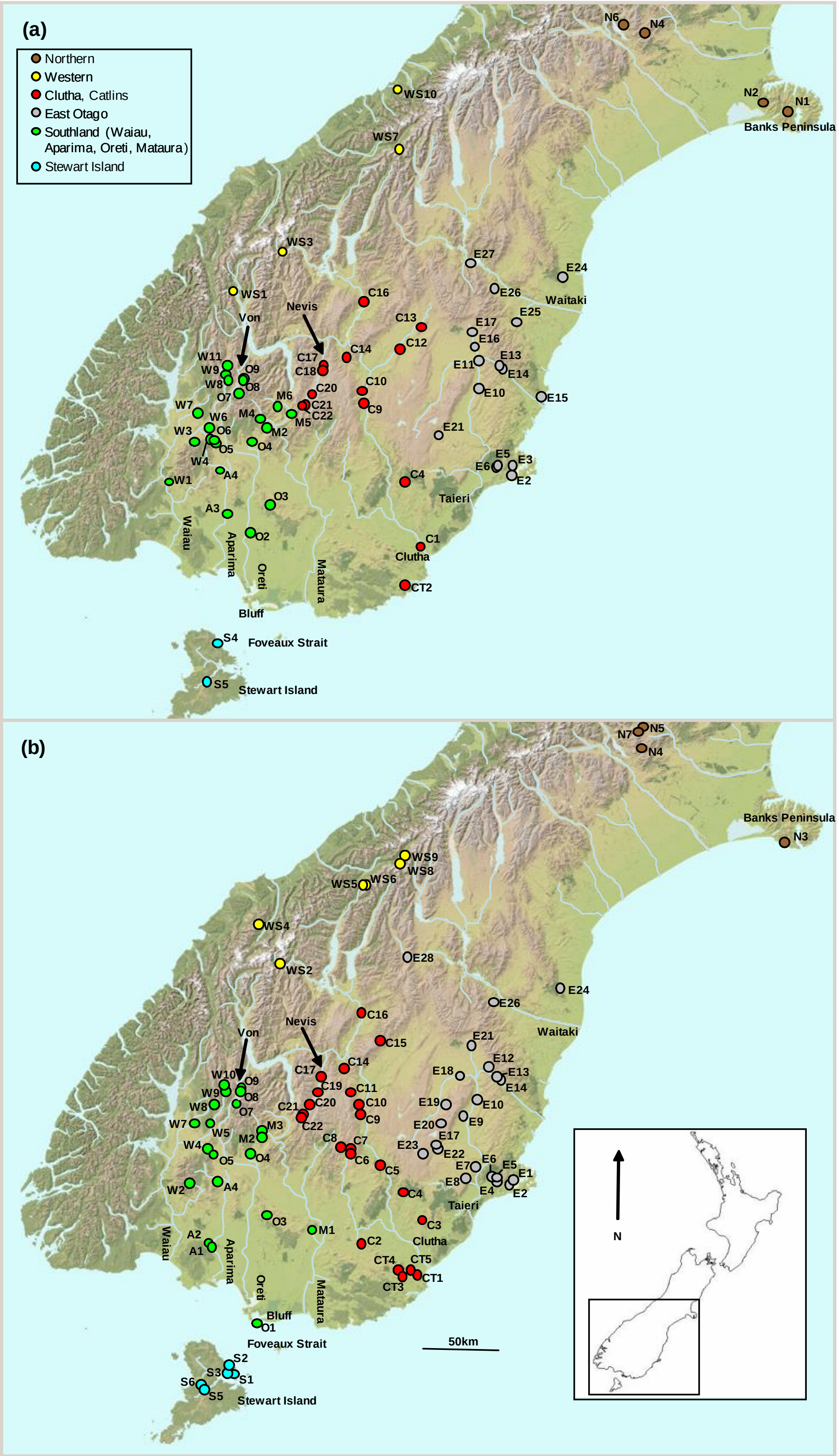


Fig 4.2: Sampling sites of (a) *Zelandoperla decorata* and (b) *fenestrata* species group collected from southern NZ (for details, see Appendix 4).

4.3.2 DNA extraction, amplification, and sequencing

DNA was extracted from the leg tissue using 5% Chelex solution (BioRad; (Walsh *et al.* 1991)) containing 20 µg proteinase K (Roche). A 644-bp portion of the COI was amplified using the primers LCO1490 (5’-ggtcaacaaatcataaagatattgg) and HCO2198 (5’- taaacttcagggtgaccaaaaaatca) (Folmer *et al.* 1994). One to five individuals were sequenced per site, with a total of 186 *fenestrata* species group and 144 *Z. decorata* specimens sequenced. Additionally a 322-bp portion of H3 was amplified from 64 *fenestrata* species group and 48 *Z. decorata* specimens, using the primers H3AF (5’ - atggctcgtaccaagcagacvgc) and H3AR (5’ - atatccttrggcatratrgtgac) (Colgan *et al.* 1998).

PCRs (25 µl) contained Taq buffer (200mM Tris pH 8.4, 500mM KCl), 1.5 mM $MgCl_2$, 200 µM of dATP, dGTP, dTTP, and dCTP, 1 pmol of each primer, 0.5 units of Taq DNA polymerase (BioTaq; Bioline) and c. 100 ng of template DNA. PCR was performed in a Mastercycler ep Gradient S (Eppendorf), with the following profile: 94°C for 2 min, 35 cycles of 94°C for 15 s, 48°C for 15 s, 72°C for 30 s, with a final extension for 4 min at 72°C. Sequencing reactions were performed using a capillary ABI3730 Genetic Analyzer (Applied Biosystems), with the forward primer.

4.3.3 Sequence analysis

Both COI and H3 coding sequences obtained were aligned by eye as length variation was absent. Discrete haplotypes were identified using phylogenetic software (PAUP 4.0b10; Swofford 2003). MODELTEST 3.7 (Posada & Crandall 1998) was used to select the most appropriate model of sequence evolution for our data using the AIC selection criterion. Pairwise sequence divergences were calculated using the Tamura and Nei + γ model of nucleotide sequence evolution implemented in PAUP 4.0b10 (Swofford 2003). Phylogenetic relationships were reconstructed via Bayesian analyses using MRBAYES v3.1.2 (Huelsenbeck & Ronquist 2001). Four Markov chains were run for a total of 5,000,000 generations, with chains sampled every 100 generations (the first 5,000 trees were discarded as burn-in). Posterior probabilities ≥ 0.95 were considered substantial node support. TRACER 1.1.1 (Rambaut & Drummond 2003) was used to check that the effective sample size was not too low, indicating that the MCMC mixed well and that the samples were independent. Sequences from the closest relatives of these taxa — the congeneric *Z. denticulata* and *Z. agnetis* — were included as outgroups.

Network analysis was performed to explore relationships among closely related COI haplotypes. Networks were constructed with TCS 1.21 (Clement *et al.* 2000), using a 95% likelihood criteria.

Population differentiation was assessed by analysis of molecular variance (AMOVA) using ARLEQUIN version 3.1 (Excoffier *et al.* 2005). The Tamura and Nei + γ model was used (ARLEQUIN's best estimate for the GTR + γ model). The significance of the resulting phi statistics was assessed by 100,000 permutations of individuals among populations, and populations among *a priori* geographic groupings. Due to the large number of rivers sampled, for the broad-scale analysis we grouped the samples into geomorphological regions (northern, western, Clutha, East Otago, Southland, Stewart Island), whereas fine-scale analysis of samples from the Southland region used distinct river drainages as groups (Waiau, Aparima, Oreti, Mataura).

4.4 Results

4.4.1 Z. decorata

Sequencing of 144 *Z. decorata* specimens yielded 45 discrete COI haplotypes (GenBank accession numbers GQ414666-GQ414710), with 47 variable nucleotide positions, of which 36 were parsimony informative. COI sequences lacked any ambiguous sites or stop codons, consistent with true mitochondrial origin (see Zhang & Hewitt 1996).

Z. decorata haplotypes formed a monophyletic group with respect to other members of the genus (Fig. 4.3). All 45 haplotypes could be assigned to a single network based on the 95% parsimony criterion (Fig. 4.4a). No obvious phylogeographic partitioning of genetic variation was observed, with eight haplotypes shared among two or more of the broad geographic regions. In particular, one common haplotype was identified from 25 sampling locations, representing five of the six regions sampled. Nine discrete H3 sequences were identified (GenBank accession numbers GQ414724-GQ414732) (Fig. 4.5). As with COI, no clear phylogeographic partitioning of variation was observed, with four of the nine sequences shared between broad geographic regions.

AMOVA analysis of *Z. decorata* showed that the majority (50.2%) of variation was found within-sites, with a similar amount found among sites within regions (41.4%; Table 4.1). In contrast, a non-significant component (8.4%, $P = 0.126$) was attributable to among-region differences. Similarly, no substantial phylogeographic structure was observed among drainages within the Southland region (1.2%, $P = 0.459$).

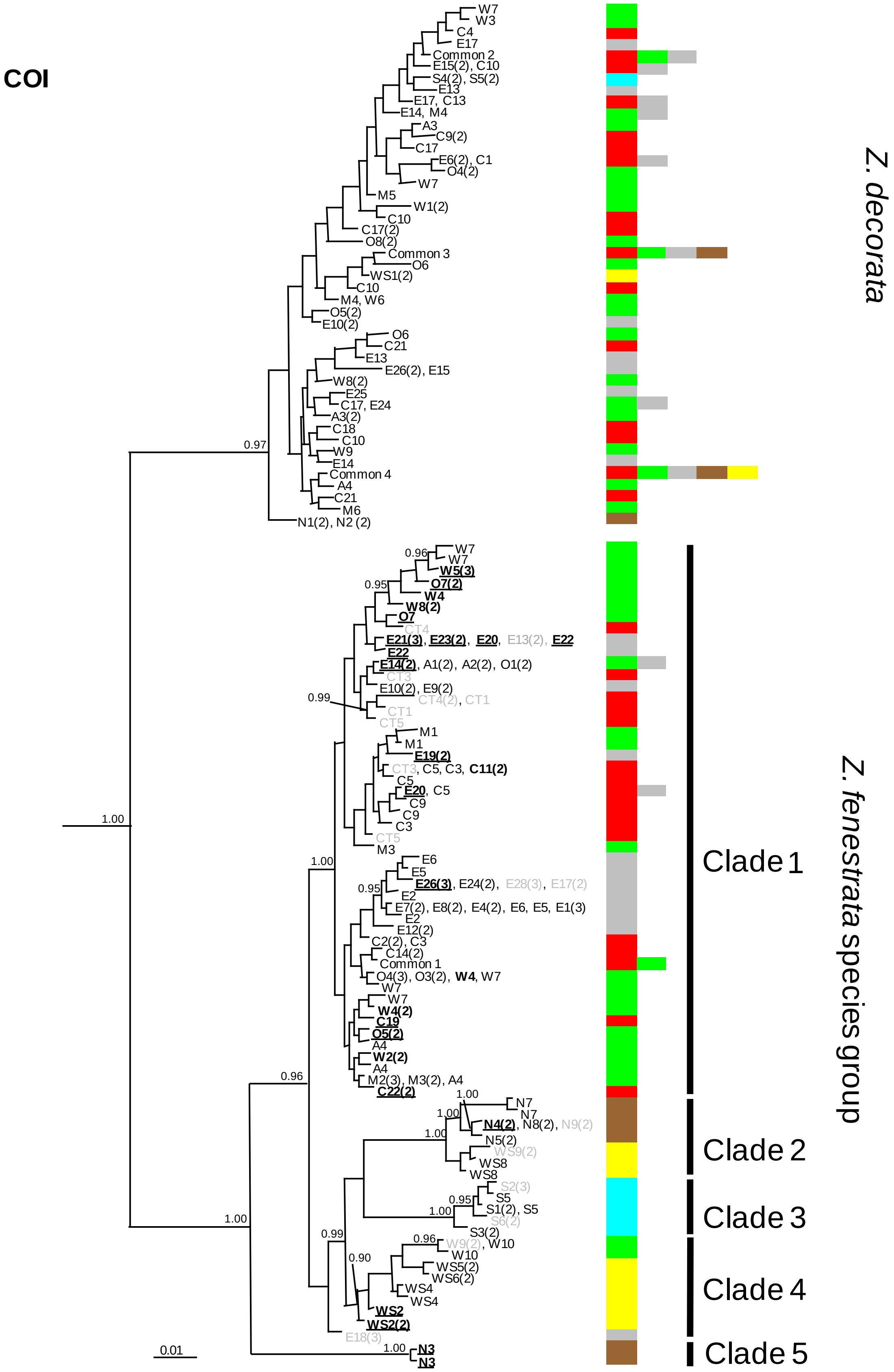

COI
Z. decorata
Z. fenestrata species group
Clade 1
Clade 2
Clade 3
Clade 4
Clade 5
W7
W3
C4
E17
Common 2
E15(2), C10
S4(2), S5(2)
E13
E17, C13
E14, M4
A3
C9(2)
C17
E6(2), C1
O4(2)
W7
M5
W1(2)
C10
C17(2)
O8(2)
Common 3
O6
WS1(2)
C10
M4, W6
O5(2)
E10(2)
O6
C21
E13
E26(2), E15
W8(2)
E25
C17, E24
A3(2)
C18
C10
W9
E14
Common 4
A4
C21
M6
N1(2), N2 (2)
0.97
1.00
0.96
W7
W7
W5(3)
0.95
O7(2)
W4
W8(2)
O7
CT4
E21(3), E23(2), E20, E13(2), E22
E22
E14(2), A1(2), A2(2), O1(2)
CT3
E10(2), E9(2)
0.99
CT4(2), CT1
CT1
CT5
M1
M1
E19(2)
CT3, C5, C3, C11(2)
C5
E20, C5
C9
C9
C3
CT5
M3
1.00
E6
E5
E26(3), E24(2), E28(3), E17(2)
0.95
E2
E7(2), E8(2), E4(2), E6, E5, E1(3)
E2
E12(2)
C2(2), C3
C14(2)
Common 1
O4(3), O3(2), W4, W7
W7
W7
W4(2)
C19
O5(2)
A4
W2(2)
A4
M2(3), M3(2), A4
C22(2)
0.96
1.00
N7
1.00
N7
N4(2), N8(2), N9(2)
1.00
N5(2)
WS9(2)
WS8
WS8
S2(3)
0.95
S5
1.00
S1(2), S5
S6(2)
1.00
S3(2)
0.99
0.96
W9(2), W10
W10
0.90
WS5(2)
WS6(2)
WS4
WS4
WS2
WS2(2)
E18(3)
0.01
1.00
N3
N3

Fig. 4.3: Bayesian phylogeny of *Z. decorata* and *fenestrata* species group COI haplotypes. Bayesian bipartition posterior probabilities (when exceeding 0.95) are shown above each node. The terminal descriptors identify the sample site/s (see Appendix 4) plus the number of specimens from that site (in parenthesis). Sample names in plain text represent macropterous specimens, bold indicates micropterous specimens, bold and underlined specimens are apterous, while grey indicates nymphs whose adult morphology is unknown. The coloured bar indicates the geographic origin of the specimens (see Fig. 4.2). Haplotype "common 1" is found in the following sites: C4(2), C6(2), C7(2), C8(2), C10(2), C15(2), C16(2), C17(2), C19(2), C20(2), C21(2), O8(4), O9(2), "common 2" in: C1, C10, C13(2), C16, W3, W6, W9, W11(2), O6, E15, E25, E27(2), "common 3" in: C9(2), C12(2), C14(2), C16, C21, M6, N6, E3(2), E5, E14, E16(2), E21(2), and "common 4" in: C4, C18(2), C20(2), C22(2), 09(2), E2(2), E5, E11(2), E13, E24, N4(2), N6, WS3(2), WS7(2), WS10(2), WS11(2), W4(2), W6(2), A4, O2(2), O3(2), O7(2), M2(2), M5, CT2(2). The phylogeny was rooted with *Z. agnetis*, *Z. decorata*, and *Z. denticulata* (not shown).

4.4.2 fenestrata species group

Sequencing of 186 *fenestrata* species group specimens yielded 71 discrete COI haplotypes (GenBank accession numbers GQ414595-GQ414665) and 109 variable nucleotide positions, of which 86 were parsimony informative. Of these 186 specimens, 92 were macropterous, 48 apterous, 16 micropterous, and 30 were early instar nymphs whose adult phenotype could not be easily identified (Appendix 4).

Phylogenetic analysis of COI (Fig. 4.3) indicated that the *fenestrata* species group was monophyletic with respect to other members of the *Zelandoperla* genus. Five divergent clades were identified (Fig. 4.3), corresponding to distinct geographic regions: Clade 1 contained specimens from Otago/Southland, Clade 2 from the northern part of South Island, Clade 3 from Stewart Island, Clade 4 from the western part of South Island, and Clade 5 from Banks Peninsula (Fig. 4.2b; Fig. 4.3). Sequence diversity within the clades was low, ranging from an average of 0.03% (within Clade 5) to 1.04% (within Clade 4). There was substantially more sequence diversity between the clades, ranging from an average of 1.89% (between clades 1 and 4) to 9.12% (between sequences in clades 2 and 5). Phylogenetic analysis of H3 produced broadly congruent results (GenBank accession numbers GQ414711-GQ414723), with similar phylogeographic partitioning of samples into regional haplogroups (Fig. 4.5). Sequence diversity within clades was minimal (maximum 0.70%), while sequence diversity between clades ranged from an average of 0.57% (between clades 1 and 4) to 1.24% (between clades 3 and 5). Micropterous and apterous specimens appear in numerous places throughout the COI tree (Fig. 4.3), including four of the five clades.

Table 4.1: AMOVA analysis of the *fenestrata* species group and *Z. decorata*. * indicates *P* < 0.001.

	Broad-scale geographic differentiation		
Genetic Structure to test	Among regions	Among sites within regions	Within sites
fenestrata species group	74.1*	21.9*	4.0*
macropterous	77.6*	19.2*	3.2*
micropterous+apterous	78.4*	16.3*	5.3*
Clade 1 *fenestrata*	26.4*	50.1*	20.5*
macropterous	28.1*	48.1*	23.8*
micropterous+apterous	27.1*	52.6*	20.3*
Z. decorata	8.4	41.4*	50.2 *
	Fine-scale geographic differentiation		
Genetic Structure to test	Among drainages	Among sites within drainages	Within sites
Southland *fenestrata* species group	15.4*	55.9*	28.7*
Southland *Z. decorata*	1.2	43.5*	55.3*

Reconstruction of COI haplotype relationships via network analysis revealed four distinct networks that could not be linked to each other under the 0.95 parsimony criterion (Fig. 4.4b). Northern South Island (WS8-9; N4-9), Stewart Island (S1-6) and Banks Peninsula (N3) haplotypes were assigned to distinct networks, whereas the majority of southern South Island haplotypes were included in a fourth network. Strong phylogeographic structure was revealed within this network, with only three haplotypes shared between the *a priori* defined geographic groupings. For instance, western haplotypes (WS2-6) formed a phylogenetically distinct assemblage, while there were also distinct network clusters within the Clutha, Southland, and East Otago regions. Even within the Southland region there was some phylogeographic structure, with distinct clusters of Waiau and Catlins haplotypes.

AMOVA analysis revealed that most of the genetic variation within the *fenestrata* species group could be attributed to among-region differences (74.1%), with smaller proportions attributable to differences among sites within regions (21.9%), or within site differences (4.0%) (Table 4.1). To determine whether this high level of population subdivision could be attributed to the wingless specimens within the *fenestrata* species group, additional AMOVA analyses were conducted separately on macropterous (assumed to be flight capable) versus micropterous + apterous (assumed to be flightless) specimens. These two different adult morphotypes exhibited essentially the same among-region genetic differentiation (77.6% and 78.4% respectively). When the analysis was restricted to Clade 1 *fenestrata* species group a smaller proportion of the variance (26.4%) was attributed to among-region differences, with the majority (50.1%) attributed to differences among sites within regions. Again, when macropterous and micropterous + apterous specimens were analysed separately they showed a similar amount of variance attributable to among-region differences (28.1% and 27.1% respectively).

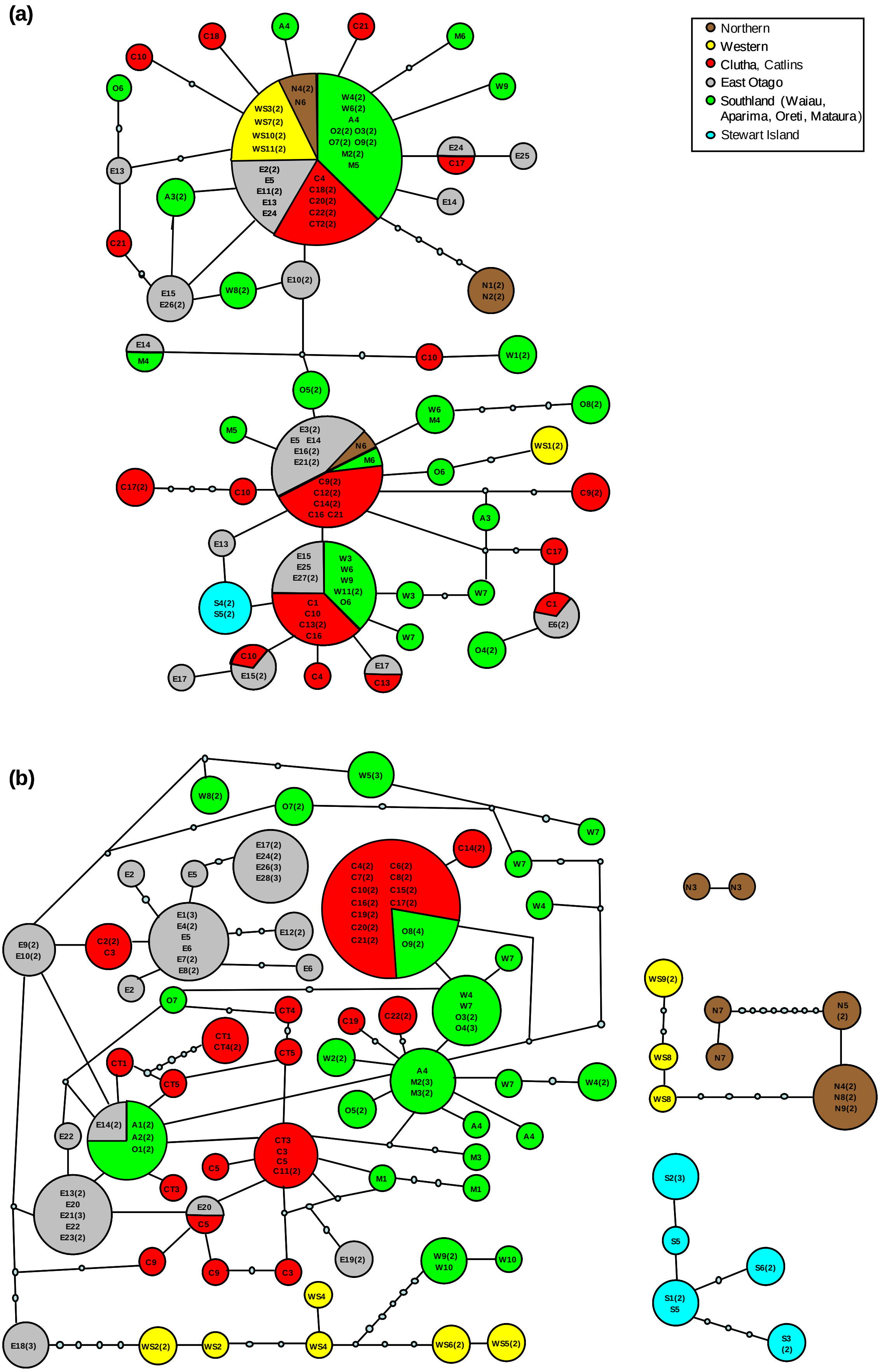

(a)
(b)
● Northern
● Western
● Clutha, Catlins
● East Otago
● Southland (Waiau, Aparima, Oreti, Mataura)
● Stewart Island
C18
A4
C21
M6
C10
O6
W9
N4(2) N6
WS3(2) WS7(2) WS10(2) WS11(2)
W4(2) W6(2) A4 O2(2) O3(2) O7(2) O9(2) M2(2) M5
E24 C17
E25
E13
A3(2)
E2(2) E5 E11(2) E13 E24
C4 C18(2) C20(2) C22(2) CT2(2)
E14
C21
N1(2) N2(2)
E10(2)
E15 E26(2)
W8(2)
E14 M4
C10
W1(2)
O5(2)
W6 M4
O8(2)
M5
E3(2) E5 E14 E16(2) E21(2)
N6
M6
WS1(2)
O6
C17(2)
C10
C9(2) C12(2) C14(2) C16 C21
C9(2)
A3
E13
C17
E15 E25 E27(2)
W3 W6 W9 W11(2) O6
W3
W7
S4(2) S5(2)
C1 C10 C13(2) C16
C1 E6(2)
W7
O4(2)
C10 E15(2)
E17
C4
E17 C13
W5(3)
W8(2)
O7(2)
W7
C14(2)
W7
E17(2) E24(2) E26(3) E28(3)
E2
E5
C4(2) C7(2) C10(2) C16(2) C19(2) C20(2) C21(2)
C6(2) C8(2) C15(2) C17(2)
O8(4) O9(2)
W4
N3
N3
E9(2) E10(2)
C2(2) C3
E1(3) E4(2) E5 E6 E7(2) E8(2)
E12(2)
E6
E2
W7
WS9(2)
O7
CT4
C19
C22(2)
W4 W7 O3(2) O4(3)
N7
N5 (2)
CT1 CT4(2)
CT5
W2(2)
CT1
CT5
A4 M2(3) M3(2)
W7
W4(2)
WS8
N7
WS8
N4(2) N8(2) N9(2)
O5(2)
E22
E14(2)
A1(2) A2(2) O1(2)
A4
A4
CT3 C3 C5 C11(2)
M3
C5
CT3
M1
M1
S2(3)
E13(2) E20 E21(3) E22 E23(2)
E20 C5
S5
C9
C9
C3
E19(2)
W9(2) W10
W10
S6(2)
S1(2) S5
WS4
E18(3)
WS2(2)
WS2
WS4
WS6(2)
WS5(2)
S3 (2)

Fig. 4.4: Statistical parsimony network of (a) *Z. decorata* and (b) *fenestrata* species group haplotypes (COI) from southern NZ. Circles represent haplotypes, scaled according to their observed frequency. Lines represent single-step mutations, with small circles representing hypothetical intermediate haplotypes not detected. Text within circles represents the geographic localities of each haplotype, with frequencies >1 indicated in parentheses.

To test for genetic structure at smaller geographic scales, AMOVA analysis was conducted on specimens from Southland using drainages (Waiau, Aparima, Oreti, Mataura; Fig. 4.2, Appendix 4) as *a priori* groupings. This separate analysis revealed that a significant amount of the variation (15.4%) could be attributed to among-drainage differences (Table 4.1).

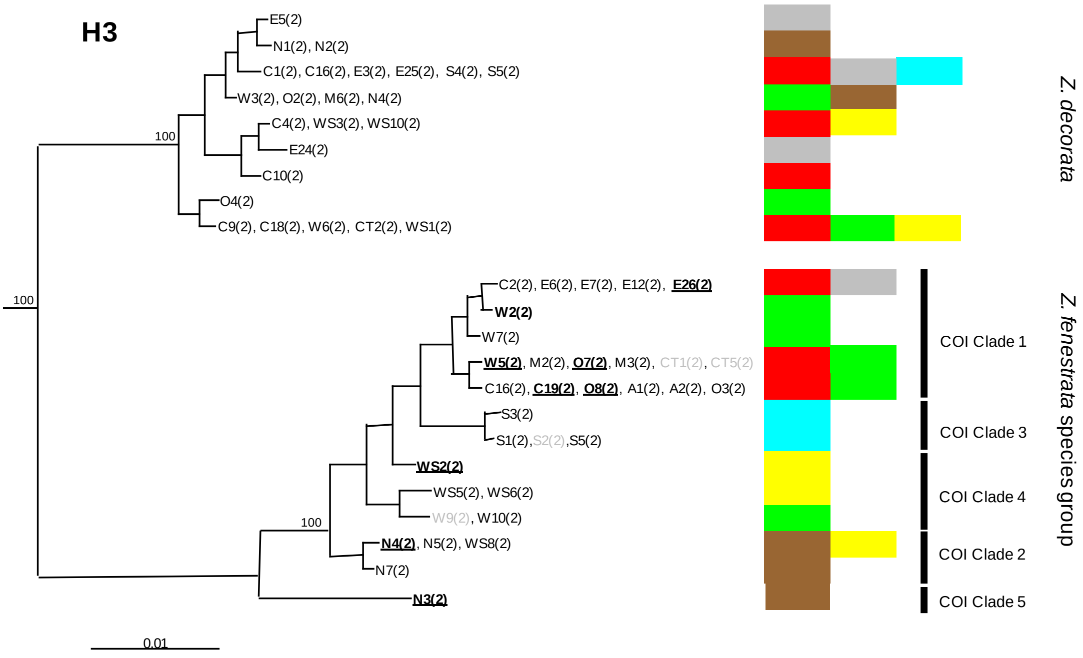


Fig 4.5: Bayesian phylogeny of *Z. decorata* and *fenestrata* species group H3 sequences. Bayesian bipartition posterior probabilities (when exceeding 0.95) are shown above each node. The terminal descriptors identify the sample site/s (see Appendix 4) plus number of specimens from the site (in parenthesis). Sample names in plain text represent macropterous specimens, bold indicates micropterous specimens, bold and underlined specimens are apterous, while grey indicates nymphs whose adult morphology is unknown. The coloured bar indicates the geographic origin of the specimens (see Fig. 4.2). Clades 1-5 correspond to the distinct COI haplogroups (Clades 1-5) (Fig. 4.3). The phylogeny was rooted with *Z. agnetis*, *Z. decorata*, and *Z. denticulata* (not shown).

Micropterous and apterous *fenestrata* species group morphotypes were located only at altitudes greater than 450 m, whereas macropterous specimens were generally found below this altitude (Fig. 4.6). Specifically, macropterous specimens were recorded at an average of 313 m above sea-level, micropterous specimens at 808 m and apterous specimens at 790m. While there was no significant difference in altitude between apterous and micropterous specimens ($P = 0.484$), there were significant differences between micropterous and macropterous ($P < 0.001$), and between apterous and macropterous specimens ($P < 0.001$).

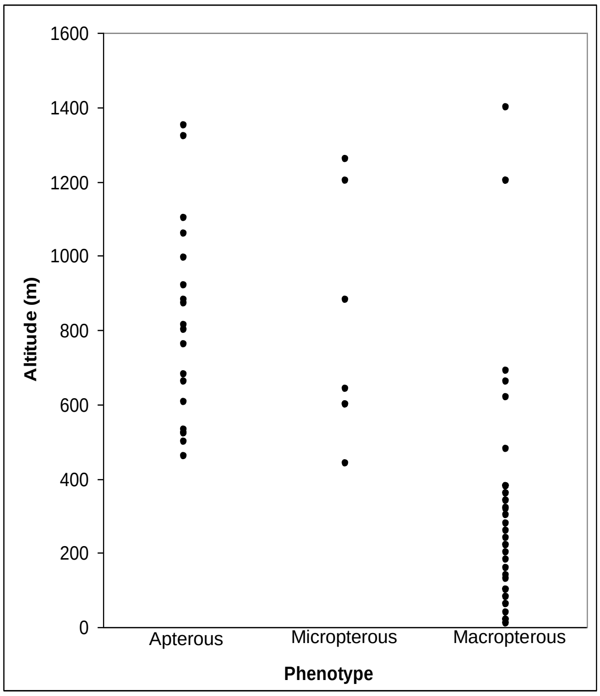


Fig 4.6: Altitude versus adult phenotype for *fenestrata* species group specimens collected.

4.5 Discussion

4.5.1 Flight ability and genetic differentiation

As predicted at the outset of our study, there is a strong relationship between flight-ability and population genetic differentiation. Specifically, whereas the weak-flying *fenestrata* species group shows strong phylogeographic structure both within and among clades (and regions), we observed no significant geographic partitioning of genetic variation within the strong-flying *Z. decorata*. The substantial and broad phylogeographic structure observed with the *fenestrata* species group is mirrored at smaller geographic scales (e.g. among adjacent Southland drainages). Overall, the clear phylogeographic distinction between the *fenestrata* species group and *Z. decorata* is consistent with their observed dispersal capacities. This correlation between dispersal ability and genetic structure is in keeping with a previous comparative study of gene flow involving four families across three orders of aquatic insects (Miller *et al.* 2002). Our study shows this effect within a monophyletic group of closely related taxa.

Intriguingly, the distinct *fenestrata* species group morphotypes analysed here (macropterus versus micropterous + apterous) both exhibited high (and essentially identical) levels of genetic variation among regions. This finding implies that both morphologies are associated with poor dispersal ability. In other words, flight has apparently been lost before wings themselves are reduced. The likely cause of the limited flight of the macropterous morph is a reduction in flight muscles, as a result of either arrested development of flight muscles or degeneration (histolysis) of originally functional muscles (Harrison 1980; Roff 1986). While there has been no quantitative research into the flight-ability of macropterous *fenestrata* species group, they have not been observed in flight.

If macropterous specimens are completely flightless, the reason for the retention of wings is not immediately obvious. Although muscle reduction is thought to be more effective than wing reduction in increasing fecundity in wing-reduced species (Zera & Denno 1997; Zera *et al.* 1999), there must still be some physiological cost associated with wing production. Roff (1986) suggested that wing development occurs at an earlier stage than flight muscles development in insects. Therefore, as the energetic cost of producing and maintaining wing-muscles is presumably greater than the cost of producing wings themselves, perhaps in a developing embryo flight muscle development is a secondary “decision” made only after the wing development stage (Roff, 1986). We also speculate that at high altitudes, where the costs associated with wing production may be greater (e.g. reduced local recruitment), the “decision” on flight might occur relatively early in stonefly development, leading to complete winglessness. Stoneflies are known for their rowing and skimming behaviour (Marden & Thomas 2003), which could represent a selective force maintaining wings. In addition, wings are thought have an important thermoregulatory function in many insects (Grimaldi & Engel 2005), which may explain the retention of wings despite the loss of flight ability.

4.5.2 Phylogeography of the fenestrata species group

Abiotic evolutionary factors are expected to generate phylogeographic congruence among ecologically similar co-distributed taxa (Avise 2000; Burridge *et al.* 2008). Along these lines, the strong phylogeographic partitioning of genetic variation detected in the current study (e.g. clades 1-5) is comparable to the findings of recent genetic studies of widespread freshwater invertebrates in NZ’s South Island. Specifically, Apte *et al.* (2007) identified distinct phylogeographic lineages of the freshwater crayfish *Paranephrops* consistent with Stewart Island (clade 3) and Eastern Otago (clade 1) *Z. fenestrata* group clades (Fig. 4.3).

Our *fenestrata* species group study reveals significant genetic differentiation between the Clutha and Southland drainages (16.1% of the genetic variance attributed to among region differences, $P < 0.001$; data not shown), with haplotypes generally restricted to one or other region. As with our findings for the *fenestrata* species group, strong freshwater phylogeographic structure has also been detected in co-distributed freshwater fishes (*Galaxias*), with broad differentiation between the Clutha and Southland drainages (Waters *et al.* 2001) and distinct lineages also present in eastern Otago (Waters & Wallis 2001).

Stewart Island *fenestrata* species group samples were genetically highly distinct from mainland samples (Fig. 4.4). This finding may seem surprising, given that Stewart Island has been connected with the mainland of NZ frequently throughout history during periods of Pleistocene low sea-level, the last of these ending ca. 12,000 years ago (Wallis & Trewick 2009). We suggest that the swampy, lowland connecting habitats (Craw *et al.* 2007) may have been ecologically unsuitable for these stoneflies, which prefer upland, high-gradient streams. In contrast to the *fenestrata* species group, *Z. decorata* exhibited similar haplotypes on Stewart Island and the mainland, consistent with the flying ability of *Z. decorata*.

Similarly, Banks Peninsula *fenestrata* species group specimens were genetically distinct from other mainland samples. Though now connected to the mainland, Banks Peninsula was an island until ca 20,000 ago. There are a number of invertebrate taxa endemic to this peninsula, including the stonefly species *Zelandobius wardi* (McLellan, 1999; Harding, 2003). Our data support McLellan's (1999) suggestion that *fenestrata* species group nymphs from Banks Peninsula may represent a distinct evolutionary unit.

If dispersal capacity of a freshwater species is low, such as within the *fenestrata* species group, the genetic structuring of the species might be shaped by vicariant processes such as river capture (Waters *et al.* 2001, 2006, 2007; Burridge *et al.* 2006, 2007, 2008). As a case in point, the Nevis River flowed south into the Mataura drainage (Southland) until a river reversal event occurred 0.3–0.5 Ma (Waters *et al.* 2001). A genetic signature of this vicariant event is provided by freshwater-limited *Galaxias* (Waters *et al.* 2001), but not for *Zelandoperla* stoneflies (current study). Specifically, the majority of Nevis River haplotypes (C17–C22) are distributed more broadly throughout the Clutha system. Similarly, our study provides no genetic evidence to support the Von River reversal hypothesis (Craw *et al.* 2007; Burridge *et al.* 2007): *fenestrata* species group samples from the Von river (O8-O9) yielded a common haplotype restricted to the Clutha system. The finding that both the Von and the Nevis river *fenestrata* species group have closest genetic affinities to other Clutha samples suggests that these stoneflies have historically had greater dispersal ability than freshwater fishes.

4.5.3 Systematics and taxonomy of flightless taxa

The presence of wingless (apterous) and reduced-winged (micropterous) specimens throughout both the COI (Fig. 4.3) and H3 (Fig. 4.5) phylogenies may imply repeated wing-loss in the *fenestrata* species group. The flightless *Z. pennulata* is apparently not a natural taxon, but a polyphyletic assemblage of convergent wing-reduced forms. Additionally, the finding that apterous and micropterous phenotypes are consistently associated with high altitudes (Fig. 4.6), suggests that a combination of high winds and a small habitat size may drive selection against winged forms, leading to the repeated formation of the apterous and micropterous populations within and among upland regions.

McLellan (1999) suggested that *Z. fenestrata*, *Z. tillyardi*, and *Z. pennulata* are closely related species, and accordingly placed them together in a distinct *fenestrata* species group. McLellan's hypothesis is supported by the combined monophyly of these taxa for both COI (Fig. 4.3) and H3 (Fig. 4.5). The three taxa recognized by McLellan (1999) are not, however, associated with reciprocally monophyletic clades: apterous and micropterous *Z. pennulata* specimens are located throughout the phylogenetic tree, whereas *Z. fenestrata* (clades 2 & 4) and *Z. tillyardi* (clades 1 & 3) samples are each represented by two distinct clades (Appendix 5). It seems likely that *Z. pennulata* represents an ecophenotype that has evolved independently on many occasions, and should be submerged into *Z. tillyardi* and *Z. fenestrata.* More broadly, the *fenestrata* species group apparently represents a species complex that exhibits variation in both wing length and coloration.

4.6 Acknowledgements

This paper is dedicated to the New Zealand stonefly expert Ian McLellan who passed away during the course of this study. Ian provided us with extremely valuable insights into stonefly biology and taxonomy. L. Anderson, C. Burridge, B. McCulloch, J. McCulloch, I. McLellan, D. Rowe, S. Waddingham, and M. Winterbourn assisted with *Zelandoperla* sampling. K. Miller assisted with figure production. This research was supported by a University of Otago PhD Scholarship to GAM, and Marsden contract UOO-0404 (Royal Society of New Zealand).

Chapter 5: General discussion

5.1 Biogeographic implications

5.1.1 Dispersal versus vicariance

Throughout the three chapters I have contrasted the relative importance of two key biogeographical processes – dispersal and vicariance. Given the supposed limited dispersal ability of stoneflies (Brundin 1967; Brundin 1972; Zwick 2000; Schultheis *et al.* 2002; Fochetti & Figueroa 2008), it was thought that vicariant events rather than dispersal events would predominate, although the analyses presented provide clear evidence supporting both biogeographic processes.

The formation of the stonefly sub-orders can be attributed to an ancient vicariant event: the tectonic splitting of Pangaea into supercontinents Gondwana and Laurasia (see Hypothesis 2a, Chapter 1). Subsequently, a northern Arctoperlaria lineage appears to have entered the southern hemisphere by dispersal, with the subsequent distribution of stonefly lineages throughout the southern hemisphere attributed to a combination of dispersal and vicariant events (see Hypothesis 2b). Most divergences between South American and Australian lineages are consistent with vicariant separation as a result of the rifting of Australia and South America (ca. 41 Ma; Scher & Martin 2006). While, in contrast, the estimated divergences between NZ lineages and their overseas sister taxa all postdate the split of NZ from the rest of Gondwana (ca. 82 Ma; Cooper & Millener 1993), with the estimated divergence dates broadly consistent with an Oligocene colonization of the landmass (see Hypothesis 2c). This finding suggests that these NZ lineages may have arrived relatively recently by long distance dispersal. Notwithstanding the importance of dispersal, in NZ there is strong evidence that relatively recent vicariance (via glaciation) drove simultaneous cladogenesis of alpine stoneflies across central South Island (see Hypothesis 3a). In addition, I provide evidence that a number of lineages have subsequently re-colonized the region extirpated by glacial activity, by dispersing into the region after the retreat of the glaciers ca. 10,000 years ago.

Limited dispersal ability is generally considered to make a lineage more susceptible to vicariant isolation events. Indeed it appears that limited dispersal ability of stoneflies may explain why stoneflies were affected by the ancient vicariant breakup of Pangaea, while other insect orders apparently were not. In addition, limited dispersal ability helps to maintain the ‘signature’ of ancient vicariant events, as recent dispersal events may overlie and obscure these more ancient events. This phenomenon is clearly seen in Chapter 3, where the ‘signature’ of the ancient vicariant isolation event is more obvious in the wingless taxa in comparison to the full-winged taxa (see Hypothesis 3b).

5.1.2 Larval versus adult dispersal

Dispersal through streams, probably as passive larval drift (see Palmer *et al.* 1996; Bilton *et al.* 2001), appears be an important dispersal mechanism for stoneflies. The *Zelandoperla fenestrata* species group exhibits a spectrum of wing morphotypes (McLellan 1999), and also has a broad geographic range in mainland NZ. However, I found that the level of genetic structure was nearly identical in macropterous versus apterous specimens, suggesting that both morphologies have similarly limited dispersal abilities (see Hypothesis 4b). Given these limited aerial dispersal abilities, dispersal of nymphs through streams may best explain the relatively large geographic range of this species complex. By comparison, NZ taxa with wingless adults and terrestrial larvae have relatively small species ranges. Within the wingless genus *Apteryoperla*, for instance, the species possessing aquatic larvae (*Apteryoperla illiesi*) has a far broader distribution relative to its non-dispersive (completely terrestrial) congeners, again implicating the importance of nymphal dispersal.

5.1.3 Wing loss and dispersal

Although the dispersal abilities of stoneflies are apparently limited – at least over ecological timescales – dispersal is clearly important over evolutionary timescales. In particular, my results suggest numerous dispersal events between NZ and other continents, and between NZ and its subantarctic (oceanic) islands. These newly emergent islands and volcanoes appear to have been colonized not long after their emergence. Similarly, the region extirpated by glaciations in central South Island has been colonized by a number of species since the end of the last glacial period ca. 10,000 years ago (Graham 2008).

Wing reduction or loss, a trait that is particularly common in NZ stoneflies (McLellan 2003), also appears to evolve relatively rapidly. *Fenestrata* species group specimens are commonly wingless at high altitudes (McLellan 1999), with these upland populations often genetically very similar to full-winged populations found at lower altitudes. Wing reduction and aptery apparently leads to reduced dispersal capacity, as observed in many of NZ’s stoneflies taxa (see Hypothesis 4a). As a result of this flight loss there are a number of species endemic to particular regions (e.g. Banks Peninsula, Stewart Island), and strong biogeographic structure is observed in a number of species (e.g. the *fenestrata* species group).

5.2 Taxonomic issues

This thesis is primarily concerned with the evolutionary relationships within and among plecopteran lineages, but there are plainly implications beyond biogeography, especially for taxonomy/systematics. Though formal taxonomy is beyond the scope of this study, the systematic relationships inferred within this study may be useful for subsequent formal taxonomic research.

5.2.1 Support for existing taxonomy

This molecular study validates the plecopteran sub-order delineations, Antarctoperlaria and Arctoperlaria, with these sub-orders recovered as monophyletic in the analysis. Similarly, all four Antarctoperlaria families were identified as monophyletic, as was the southern hemisphere Arctoperlaria family Notonemouridae, a family previously considered by many authors to be polyphyletic (Zwick 1981, 1990, 2000; McLellan 2000b; Fochetti & Figueroa 2008). Along similar lines, the two Eustheniidae sub-families, Eustheniidae and Stenoperlinae, were recovered as reciprocally monophyletic, consistent with taxonomy. In contrast none of the five Gripopterygidae sub-families were supported as monophyletic, although members of Zelandoperlinae and Antarctoperlinae did form strongly supported clades. Notably, if the genus *Vesicaperla* was reallocated to Zelandoperlinae, rather than the current placement within Antarctoperlinae, these sub-families would also be recovered as monophyletic in our analysis. More broadly, however, the Gripopterygidae sub-families are clearly in need of taxonomic revision (McLellan & Zwick 2007), and the molecular phylogeny presented in this study may provide a useful starting point for subsequent re-classification of these lineages.

5.2.2 Cryptic taxa

All genera studied were strongly supported as monophyletic, with the exception of *Dinotoperla* and *Acroperla*. Further molecular and morphological analysis should be carried out to determine the monophyly of these genera. Similarly, specimens from the same species generally formed monophyletic clades, with a few notable exceptions. For instance, phylogenetically distinct northern and southern clades of *H. angularis* were recovered, with the northern clade genetically closer to *Holcoperla jacksoni* than to the southern *H. angularis* clade. Further morphological analysis should be conducted to determine whether there are any consistent morphological differences between the two *H. angularis* clades that would support them being described as distinct species.

Similarly, the three members of the *fenestrata* species group - *Z. fenestrata*, *Z. tillyardi*, and *Z. pennulata* – were not recovered as monophyletic in any of the molecular analyses presented in this study. This is perhaps unsurprising, as these three taxa show little or no difference in male genitalia (McLellan 1999). These taxa appear to be part of a species complex which exhibits substantial variation in both wing length and coloration. This complex includes at least five strongly-supported regional clades. No major morphological differences between these clades are immediately apparent, although McLellan (1999) did note unusual spines on nymphs of *Z. pennulata* from Banks Peninsula, and suggested that these may represent a distinct *Zelandoperla* species. My preliminary molecular analysis of Banks Peninsula *Zelandoperla* specimens apparently supports this suggestion, as these are highly divergent from all other members of the complex. Extensive morphological analysis will be required to determine whether there are any additional distinctive morphological characters that define the remaining clades. In addition, work is currently being undertaken to test for reproductive isolation of distinct lineages in the regions where they come into contact.

5.2.3 Morphological plasticity within species

One of the described members of the *fenestrata* species group, *Z. pennulata*, was described purely on the basis of wing reduction (aptery or microptery) (McLellan 1999). However, this character is clearly phylogenetically labile, as apterous and micropterous '*Z. pennulata*' specimens are identified in each of the five clades reported in this study. This finding suggests that wing length alone is not a good character by which to define species; indeed many species (e.g. *Austroperla cyrene*, *Nesoperla fulvescens*) are known to exhibit substantial variation in wing-length. Similarly, additional species described primarily on the basis of their aptery (e.g. *Zelandobius brevicauda*) should be phylogenetically compared to their congeners to determine whether they indeed represent distinct species, or alternatively represent morphotypes within single species.

Another described member of the *fenestrata* species group, *Zelandoperla tillyardi*, is diagnosed primarily on the basis of its distinctive colour pattern: yellow bars on the adult femur and tibia (McLellan 1999). As with wing reduction, this colour pattern does not appear to be a good character on which to base species delineation, as specimens with this morphology occur in several parts of the *Zelandoperla* phylogeny. As this distinctive colour pattern has also been recorded from a variety of other NZ stoneflies, any taxa described solely on the basis of such patterns should be phylogenetically compared to their congeners to reassess their taxonomic status. Indeed *Taraperla pseudocyrene,* which is distinguished from the congeneric *Taraperla ancilis* on the basis of a similar colour polymorphism (yellow bars on femur and tibia), was found to differ by on a single base pair in COI sequence and possessed the identical H3 sequence as *T. ancilis* (data not shown in this study). McLellan (1999) speculated that these parallel colour variants may have evolved as a response to predation (mimicry).

It is hoped the taxonomic issues highlighted above will flow through to further systematic analysis, to ultimately bring the taxonomy of NZ stoneflies in line with the phylogeny.

References

Adams CJ (1981) Migration of late Cenozoic volcanism in the South Island of New Zealand and the Campbell Plateau. *Nature*, **294,** 153-155.

Alloway, BV, Lowe DJ, Barrell DJA, Newnham RM, Almond PC, Augustinus PC, Bertler NAN, Carter L, Litchfield NJ, McGlone MS, Shulmeister J, Vandergoes MJ, Williams PW (2007) Towards a climate event stratigraphy for New Zealand over the past 30,000 years (NZ-INTIMATE project). *Journal of Quaternary Science,* **22,** 9-35.

Allwood J, Gleeson D, Mayer G, Daniels S, Beggs JR, Buckley TR (2010) Support for vicariant origins of the New Zealand Onychophora. *Journal of Biogeography,* **37,** 669-681.

Apte S, Smith PJ, Wallis GP (2007) Mitochondrial phylogeography of New Zealand freshwater crayfishes, *Paranephrops* spp. *Molecular Ecology,* **16,** 1897-1908.

Arbogast BS, Kenagy GJ (2001) Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography,* **28,** 819-825.

Arensburger P, Simon C, Holsinger K (2004) Evolution and phylogeny of the New Zealand cicada genus *Kikihia* Dugdale (Homoptera: Auchenorrhyncha: Cicadidae) with special reference to the origin of the Kermadec and Norfolk Islands' species. *Journal of Biogeography,* **31,** 1769-1783.

Avise JC (1994) *Molecular Markers, Natural History and Evolution*. Chapman and Hall, New York.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA.

Avise JC (2004) What is the field of biogeography, and where is it going? *Taxon,* **53,** 893-898.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics,* **18,** 489-522.

Avise JC, Walker D, Johns GC (1998) Speciation durations and Pleistocene effects on vertebrate phylogeography. *Proceedings of the Royal Society of London, Series B: Biological Sciences,* **265,** 1707-1712.

Banarescu P (1990) *Zoogeography of Fresh Waters, Volume 1: General Distribution and Dispersal of Freshwater Animals.* Aula Verlag, Wiesbaden, Germany.

Batt GE, Braun J, Kohn BP, McDougall I (2000) Thermochronological analysis of the dynamics of the Southern Alps, New Zealand. *Bulletin of the Geological Society of America,* **112,** 250-266.

Baumann R (1975). Revision of the stonefly family Nemouridae (Plecoptera): A study of the world fauna at the generic level. *Smithsonian Contributions to Zoology,* **211**, 1-74.

Beaumont MA, Zhang W, Balding DJ (2002) Approximate Bayesian computation in population genetics. *Genetics,* **162,** 2025-2035.

Beck J, Kitching IJ (2007) Correlates of range size and dispersal ability: a comparative analysis of sphingid moths from the Indo-Australian tropics. *Global Ecology and Biogeography,* **16,** 341-349.

Beer-Stiller A, Zwick P (1995) Biometric studies of some stoneflies and a mayfly (Plecoptera and Ephemeroptera). *Hydrobiologia,* **299,** 169-178.

Beheregaray LB (2008) Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology,* **17,** 3754-3774.

Bell KL, Yeates DK, Moritz C, Monteith GB (2004) Molecular phylogeny and biogeography of the dung beetle genus *Temnoplectron* Westwood

(Scarabaeidae: Scarabaeinae) from Australia's wet tropics. *Molecular Phylogenetics and Evolution*, **31**, 741-753.

Bermingham E, Avise JC (1986) Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics*, **113,** 939-965.

Bermingham E, Moritz C (1998) Comparative phylogeography: concepts and applications. *Molecular Ecology,* **7,** 367-369.

Berthelemy C (1968) Contribution a la connaissance des Leuctridae. *Annales de Limnologie,* **4,** 175-98.

Bilton DT, Freeland JR, Okamura B (2001) Dispersal in freshwater invertebrates. *Annual Review of Ecology and Systematics,* **32,** 159-181.

Bird GA, Hynes HBN (1981) Movements of adult aquatic insects near streams in Southern Ontario. *Hydrobiologia,* **77,** 65-69.

Boyer SL, Baker JM, Giribet G (2007) Deep genetic divergences in *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi): a widespread mite harvestman defies DNA taxonomy. *Molecular Ecology,* **16,** 4999-5016.

Briggs JC (1987) Antitropical distribution and evolution in the Indo-West Pacific Ocean. *Systematic Zoology,* **36,** 237-247.

Briggs JC (1995) *Global biogeography*. Elsevier, New York, USA.

Briggs JC (2003) Fishes and birds: Gondwana life rafts reconsidered. *Systematic Biology,* **52,** 548-553.

Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences,* **91,** 6491-6495.

Brown B, Emberson RM, Paterson AM (1999) Phylogeny of "*Oxycanus*" lineages of hepialid moths from New Zealand inferred from sequence variation in the mtDNA COI and II gene regions. *Molecular Phylogenetics and Evolution,* **13,** 463-473.

Brown WM, George M, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences,* **76,** 1967-1971.

Brundin L (1966) Transantarctic relationships and their significance, as evidenced by chironomid midges. *Kungliga Svenska Vetenskapsakadamiens Handlingar*, **11,** 437-472.

Brundin L (1967) Insects and the problem of austral disjunctive distribution. *Annual Review of Entomology,* **12,** 149-168.

Brundin L (1972) Phylogenetics and biogeography. *Systematic Zoology,* **21,** 69-79.

Buckley TR, Simon C (2007) Evolutionary radiation of the cicada genus *Maoricicada Dugdale* (Hemiptera: Cicadoidea) and the origins of the New Zealand alpine biota. *Biological Journal of the Linnean Society,* **91,** 419-435.

Buckley TR, Simon C, Chambers GK (2001) Phylogeography of the New Zealand cicada *Maoricicada campbelli* based on mitochondrial DNA sequences: ancient clades associated with Cenozoic environmental change. *Evolution,* **55,** 1395-1407.

Buckley TR, Marske K, Attanayake (2009) Identifying glacial refugia in a geographic parthenogen using palaeoclimate modeling and phylogeography: the New Zealand stick insect *Argosarchus horridus* (White). *Molecular Ecology,* **18**, 4650-4663.

Bunn SE, Hughes JM (1997) Dispersal and recruitment in streams: Evidence from genetic studies. *Journal of the North American Benthological Society*, **16,** 338-346.

Burridge CP (2002) Antitropicality of Pacific fishes: molecular insights. *Environmental Biology of Fishes,* **65,** 151-164.

Burridge CP, Craw D, Jack DC, King TM, Waters JM (2008) Does fish ecology predict dispersal across a river drainage divide? *Evolution*, **62,** 1484-1499.

Burridge CP, Craw D, Waters JM (2006) River capture, range expansion, and cladogenesis: the genetic signature of freshwater vicariance. *Evolution*, **60,** 1038-1049.

Burridge CP, Craw D, Waters JM (2007) An empirical test of freshwater vicariance via river capture. *Molecular Ecology*, **16,** 1883-1895.

Burridge CP, McDowall RM, Craw D, Wilson MVH, Waters JM (201x) Reassessing a long-standing biogeographic controversy: the importance of considering ancestral life-history and distributional character states. *Syst Biol* (in review)

Burrows CJ (1965) Some discontinuous distributions of plants within New Zealand and their ecological significance. Part II: Disjunctions between Otago-Southland and Nelson-Marlborough and related distribution patterns. *Tuatara,* **13,** 9-29.

Campbell HJ (1998) Fauna and flora of the Chatham Islands: less than 4 my old. *Geology and Genes,* **97,** 15-16.

Campbell HJ, Andrews PB, Beu AG, Maxwell PA, Edwards AR, Laird MG, Hornibrook N, Mildenhall DC, Watters WA, Buckeridge JS, Lee DE, Strong CP, Wilson GJ, Hayward BW (1993) Cretaceous-Cenozoic geology and biostratigraphy of the Chatham Islands, New Zealand. *Institute of Geological and Nuclear Sciences Monograph,* **2,** 1-269.

Campbell HJ, Hutching GH (2007). *In Search of Ancient New Zealand*. Penguin Books, Auckland, New Zealand.

Campbell HJ, Landis CA (2001) New Zealand awash. *New Zealand Geographic,* **51,** 6-7.

Carlquist S (1974) *Island Biology*. Columbia University Press, New York and London.

Carpenter FM, Burnham L (1985) The geological record of insects. *Annual Review of Earth and Planetary Sciences*, **13,** 297-314.

Carstens BC, Knowles LL (2007) Shifting distributions and speciation: species divergence during rapid climate change. *Molecular Ecology,* **16,** 619-628.

Chamberlain CP, Poage MA (2000) Reconstructing the paleotopography of mountain belts from the isotopic composition of authigenic minerals. *Geology,* **28,** 115-118.

Chinn WG, Gemmell NJ (2004) Adaptive radiation within New Zealand endemic species of the cockroach genus *Celatoblatta* Johns (Blattidae): a response to Plio-Pleistocene mountain building and climate change. *Molecular Ecology,* **13,** 1507-1518.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology,* **9,** 1657-1659.

Cockayne L (1926). *Vegetation of New Zealand. 2nd Edition.* Engelmann Press, Leipzig, Germany.

Colgan DJ, McLauchlin A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology,* **46,** 419-437.

Cooper A, Cooper RA (1995) The Oligocene bottleneck and New Zealand biota: genetic record of a past environmental crisis. *Proceedings of the Royal Society of London, Series B: Biological Sciences,* **261,** 293-302.

Cooper A, Mourer-Chauvire C, Chambers GK, Von Haeseler A, Wilson AC, Pääbo S (1992) Independent origins of New Zealand moas and kiwis. *Proceedings of the National Academy of Sciences,* **89,** 8741-8744.

Cooper RA (1989) Early Paleozoic terranes of New Zealand. *Journal of the Royal Society of New Zealand,* **19,** 73–112.

Cooper RA, Millener PR (1993) The New Zealand biota: historical background and new research. *Trends in Ecology and Evolution,* **8,** 429-433.

Craw D, Burridge C, Anderson L, Waters JM (2007) Late Quaternary river drainage and fish evolution, Southland, New Zealand. *Geomorphology,* **84,** 98-110.

Craw D, Norris RJ (2003) Landforms. In: *The Natural History of Southern New Zealand* (eds Darby JT, Fordyce RE, Mark AF, Probert PK, Townsend CR), pp. 17-34. University of Otago Press, Dunedin.

Craw RC (1982) Phylogenetics, areas, geology and the biogeography of Croizat: a radical view. *Systematic Zoology,* **31,** 304-316.

Craw RC (1989) New Zealand biogeography: a panbiogeographic approach. *New Zealand Journal of Zoology,* **16,** 527-547.

Crisp MD, Cook LG (2005) Do early branching lineages signify ancestral traits? *Trends in Ecology and Evolution,* **20**, 122-128.

Croizat L (1958). *Panbiogeography*. The author, Caracas.

Croizat L, Nelson G, Rosen DE (1974) Centers of origin and related concepts. *Systematic Zoology,* **23,** 265-287.

Crowley TJ, North GR (1988) Abrupt climate change and extinction events in earth history. *Science,* **240,** 996-1002.

Cutten HNC (1979) Rappahannock Group: late Cenozoic sedimentation and tectonics contemporaneous with Alpine Fault movement. *New Zealand Journal of Geology and Geophysics,* **22,** 535-553.

Darlington, Jr, PJ (1957) *Zoogeography: the geographical distribution of animals.* John Wiley and Sons, New York.

Darwin C (1859) *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life.* D. Appleton, New York, USA.

Dawson MN, Louie KD, Barlow M, Jacobs DK, Swift CC (2002) Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition Zone. *Molecular Ecology,* **11,** 1065-1075.

De Candolle AP (1820) Geographie Botanique. *Dictionaire des sciences naturelles,* 18**,** 359-422.

den Boer PJ (1970) On the significance of dispersal power for populations of carabid-beetles (Coleoptera, Carabidae). *Oecologia*, **4,** 1-28.

Denno RF, Hawthorne DJ, Thorne BL, Gratton C (2001) Reduced flight capability in British Virgin Island populations of a wing-dimorphic insect: the role of habitat isolation, persistence, and structure. *Ecological Entomology*, **26,** 25-36.

Denno RF, Roderick GK, Olmstead KL, Dobel HG (1991) Density-related migration in planthoppers (Homoptera: Delphacidae): the role of habitat persistence. *American Naturalist*, **138**, 1513-1541.

DeSalle R, Freedman T, Prager EM, Wilson AC (1987) Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. *Journal of Molecular Evolution*, **26,** 157-164.

Donald KM, Kennedy M, Spencer HG (2005) Cladogenesis as the result of long-distance rafting events in South Pacific topshells (Gastropoda, Trochidae). *Evolution,* **59,** 1701-1711.

Douzery EJP, Snell EA, Bapteste E, Delsuc F, Philippe H (2004) The timing of eukaryotic evolution: Does a relaxed molecular clock reconcile proteins and fossils? *Proceedings of the National Academy of Sciences*, **101**, 15386-15391.

Drummond AJ, Ho SY, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology,* **4,** e88.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology,* **7,** 214.

Dudgeon D (1983) An investigation of the drift of aquatic insects in Tai Po Kau Forest Stream, New Territories, Hong Kong. *Archiv für Hydrobiologie,* **96,** 434-447.

Dumont HJ (2002) *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World*. Backhuys Publishers, Leiden, Netherlands.

Ebach MC, Humphries CJ, Williams DM (2003) Phylogenetic biogeography deconstructed. *Journal of Biogeography,* **30,** 1285-1296.

Eble JA, Toonen RJ, Bowen BW (2009) Endemism and dispersal: comparative phylogeography of three surgeonfishes across the Hawaiian Archipelago. *Marine Biology,* **156,** 689-698.

Emerson BC, Wallis GP (1995) Phylogenetic relationships of the *Prodontria* (Coleoptera; Scarabaeidae; subfamily Melolonthinae), derived from sequence variation in the mitochondrial cytochrome oxidase II gene. *Molecular Phylogenetics and Evolution,* **4,** 433-447.

Ericson PGP, Johansson US (2003) Phylogeny of Passerida (Aves: Passeriformes) based on nuclear and mitochondrial sequence data. *Molecular Phylogenetics and Evolution,* **29,** 126-138.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online,* **1,** 47-50.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* **39**, 783-791.

Felsenstein J (1988) Phylogenies from molecular sequences: inference and reliability. *Annual Review of Genetics,* **22,** 521-565.

Flecker AS, Allan JD (1988) Flight direction in some rocky mountain mayflies (Ephemeroptera), with observations of parasitism. *Aquatic Insects*, **10,** 33-42.

Fleischer RC, McIntosh CE, Tarr CL (1998) Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. *Molecular Ecology,* **7,** 533-545.

Fleming CA (1975) *The Geological History of New Zealand and its Biota*. Dr W Junk b.v. Publishers, The Hague, Netherlands.

Fochetti R, Figueroa JMT (2008) Global diversity of stoneflies (Plecoptera; Insecta) in freshwater. *Freshwater Animal Diversity Assessment,* **198,** 365-377.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* **3,** 294-299.

Fordyce R (1991) A new look at the fossil vertebrate record of New Zealand. In: *Vertebrate Palaeontology of Australasia* (eds Vickers-Rich P, Monaghan JM, Baird RF, Rich TH), pp. 1191-1316. Pioneer Design Studio and Monash University, Melbourne, Australia.

Gage M (1980). *Legends in the Rocks: an Outline of New Zealand Geology*. Whitcoulls, Christchurch, New Zealand.

Gibbs GW (2006) *Ghosts of Gondwana: The History of Life in New Zealand*. Craig Potton Publishing, Nelson, New Zealand.

Giller PS, Malmqvist B (1998). *The Biology of Streams and Rivers*. Oxford University Press, Oxford, USA.

Givnish TJ, Renner SS (2004) Tropical intercontinental disjunctions: Gondwana breakup, immigration from the boreotropics, and transoceanic dispersal. *International Journal of Plant Sciences,* **165,** 1-6.

Goldberg J, Trewick SA, Paterson AM (2008) Evolution of New Zealand's terrestrial fauna: a review of molecular evidence. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **363,** 3319-3334.

Graham IJ (2008). *A Continent on the Move: New Zealand Geoscience into the 21st Century.* The Geological Society of New Zealand in association with GNS Science, Wellington, New Zealand.

Greaves SNJ, Chapple DG, Gleeson DM, Daugherty CH, Ritchie PA (2007) Phylogeography of the spotted skink (*Oligosoma lineoocellatum*) and green skink (*O. chloronoton*) species complex (Lacertilia: Scincidae) in New Zealand reveals pre-Pleistocene divergence. *Molecular Phylogenetics and Evolution,* **45,** 729-739.

Grimaldi D, Engel MS (2005) *Evolution of the Insects*. Cambridge University Press, Cambridge, UK.

Haddrath O, Baker AJ (2001) Complete mitochondrial DNA genome sequences of extinct birds: ratite phylogenetics and the vicariance biogeography hypothesis. *Proceedings of the Royal Society of London, Series B: Biological Sciences,* **268,** 939-945.

Hagen H (1863) Neuropteren aus der Braunkohle von Rott im Siebengebirge. *Palaeontographica,* **10,** 247-269.

Harding JS (2003) Historic deforestation and the fate of endemic invertebrate species in streams. *New Zealand Journal of Marine and Freshwater Research,* **37**, 333-345.

Harrison RG (1980) Dispersal polymorphisms in insects. *Annual Review of Ecology and Systematics,* **11,** 95-118.

Heads M (1998) Biogeographic disjunction along the Alpine fault, New Zealand. *Biological Journal of the Linnean Society,* **63,** 161-176.

Heenan PB, Mitchell AD (2003) Phylogeny, biogeography and adaptive radiation of Pachycladon (Brassicaceae) in the mountains of South Island, New Zealand. *Journal of Biogeography,* **30,** 1737-1749.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role, in divergence and speciation. *Biological Journal of the Linnean Society,* **58,** 247-276.

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature,* **405,** 907-913.

Hickerson MJ, Dolman G, Moritz C (2006) Comparative phylogeographic summary statistics for testing simultaneous vicariance. *Molecular Ecology,* **15,** 209-223.

Hickerson MJ, Stahl E, Takebayashi N (2007) msBayes: Pipeline for testing comparative phylogeographic histories using hierarchical approximate Bayesian computation. *BMC Bioinformatics,* **8,** 268.

Hill KBR, Simon C, Marshall DC, Chambers GK (2009) Surviving glacial ages within the Biotic Gap: phylogeography of the New Zealand cicada *Maoricicada campbelli*. *Journal of Biogeography,* **36,** 675-692.

Hoernle K, White JDL, Van Den Bogaard P, Hauff F, Coombs DS, Werner R, Timm C, Garbe-Schönberg D, Reay A, Cooper AF (2006) Cenozoic intraplate volcanism on New Zealand: Upwelling induced by lithospheric removal. *Earth and Planetary Science Letters,* **248,** 350-367.

Hogg ID, Willmann-Huerner P, Stevens MI (2002) Population genetic structures of two New Zealand stream insects: *Archichauliodes diversus* (Megaloptera) and *Coloburiscus humeralis* (Ephemeroptera). *New Zealand Journal of Marine and Freshwater Research,* **36,** 491-502.

Hooker JD (1853). *Introductory Essay to the Flora of New Zealand*. London, Great Britain.

Hooker JD (1860) *Botany of the Antarctic voyage III: Flora Tasmaniae.* London, Great Britain.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, **17,** 754-755.

Hughes JM, Bunn SE, Cleary C, Hurwood DA (2000) A hierarchical analysis of the genetic structure of an aquatic insect *Bungona* (Baetidae: Ephemeroptera). *Heredity*, **85,** 561-570.

Hughes JM, Bunn SE, Hurwood DA, Cleary C (1998) Dispersal and recruitment of *Tasiagma ciliata* (Trichoptera: Tasimiidae) in rainforest streams, south-eastern Australia. *Freshwater Biology*, **39,** 117-127.

Hughes JM, Mather PB, Sheldon AL, Allendorf FW (1999) Genetic structure of the stonefly, *Yoraperla brevis*, populations: the extent of gene flow among adjacent montane streams. *Freshwater Biology*, **41,** 63-72.

Humphries CJ (2000) Form, space and time; which comes first? *Journal of Biogeography*, **27,** 11-15.

Hynes HBN (1941) The taxonomy and ecology of the nymphs of British Plecoptera with notes on the adults and eggs. *Transactions of the Royal Entomology Society of London*, **91**, 459-557.

Hynes HBN (1978) Annotated key to the stonefly nymphs (Plecoptera) of Victoria. *Australia Society for Limnology*, **2**, 1-63.

Hynes HBN (1981) Taxonomic notes on Australian Notonemouridae (Plecoptera) and a new species from Tasmania. *Aquatic Insects*, **3**, 147-166.

Hynes HBN (1989) Tasmanian Plecoptera. *Australian Society for Limnology*, **8**, 1-81.

Ikeda H, Kagaya T, Kubota K, Abe T (2008) Evolutionary relationships among food habit, loss of flight, and reproductive traits: life history evolution in the silphinae (Coleoptera: Silphidae). *Evolution*, **62**, 2065-2079.

Illies J (1965) Phylogeny and zoogeography of the Plecoptera. *Annual Review of Entomology,* **10,** 117-140.

Jackson, DJ (1928) The inheritance of long and short wings in the weevil (*Sitona hispidula*), with a discussion of wing reduction among beetles. *Transactions of the Royal Society of Edinburgh*, **55**, 665-735.

Johns GC, Avise JC (1998) Tests for ancient species flocks based on molecular phylogenetic appraisals of *Sebastes* rockfishes and other marine fishes. *Evolution,* **52,** 1135-1146.

Johnson NK, Cicero C (2004) New mitochondrial DNA data affirm the importance of Pleistocene speciation in North American birds. *Evolution,* **58,** 1122-1130.

Juan C, Oromi P, Hewitt GM (1995) Mitochondrial DNA phylogeny and sequential colonization of Canary Islands by darkling beetles of the genus *Pimelia* (Tenebrionidae). *Proceedings of the Royal Society of London, Series B: Biological Sciences,* **261**, 173-180.

Juan C, Oromi P, Hewitt GM (1996) Phylogeny of the genus *Hegeter* (Tenebrionidae, Coleoptera) and its colonization of the Canary Islands deduced from cytochrome oxidase I mitochondrial DNA sequences. *Heredity,* **76,** 392-403.

Kamp PJJ (1986) The mid-Cenozoic Challenger Rift System of western New Zealand and its implications for the age of Alpine fault inception. *Bulletin of the Geological Society of America,* **97,** 255-281.

Karol KG, Suh Y, Schatz GE, Zimmer EA (2000) Molecular evidence for the phylogenetic position of *Takhtajania* in the Winteraceae: inference from nuclear ribosomal and chloroplast gene spacer sequences. *Annals of the Missouri Botanical Garden,* **87,** 414-432.

Keilbach R (1982) Bibliographie und Liste der Arten tierischer Einschlüsse in fossilen Harzen sowie ihrer Aufbewahrungsorte. *Deutsche Entomologische Zeitschrift,* **29,** 129-286.

Kelly LC, Bilton DT, Rundle SD (2001) Population structure and dispersal in the Canary Island caddisfly *Mesophylax aspersus* (Trichoptera, Limnephilidae). *Heredity,* **86,** 370-377.

Kjer KM (2004) Aligned 18S and insect phylogeny. *Systematic Biology,* **53**, 506-514

King PR (2000) Tectonic reconstructions of New Zealand: 40 Ma to the present. *New Zealand Journal of Geology and Geophysics,* **43,** 611-638.

Klapalek F (1909). Plecoptera, Steinfliegen. In: *Die Susswasserfauna Deutschlands* (ed Brauer A), pp. 33-95. Gustav Fischer, Jena, Germany.

Knapp M, Mudaliar R, Havell D, Wagstaff SJ, Lockhart PJ (2007) The drowning of New Zealand and the problem of *Agathis*. *Systematic Biology,* **56,** 862-870.

Knapp M, Stöckler K, Havell D, Delsuc F, Sebastiani F, Lockhart PJ (2005) Relaxed molecular clock provides evidence for long-distance dispersal of *Nothofagus* (Southern Beech). *PLoS Biology,* **3,** e14.

Knowles LL (2000) Tests of Pleistocene speciation in montane grasshoppers (genus *Melanoplus*) from the sky islands of western North America. *Evolution,* **54,** 1337-1348.

Knowles LL (2001) Did the Pleistocene glaciations promote divergence? Tests of explicit refugial models in montane grasshoppers. *Molecular Ecology,* **10,** 691-701.

Koons PO (1995) Modeling the topographic evolution of collisional belts. *Annual Reviews of Earth and Planetary Sciences*, **23**, 375-408.

Korovchinsky NM (2006) The Cladocera (Crustacea: Branchiopoda) as a relict group. *Zoological Journal of the Linnean Society,* **147,** 109-124.

Krno I (2007) Impact of human activities on stonefly (Insecta, Plecoptera) ecological metrics in the Hron River (Slovakia). *Biologia*, **62,** 446-457.

Kubatko LS, Degnan JH (2007) Inconsistency of the phylogenetic estimates from concatenated data under coalescence. *Systematic Biology,* **56**, 17-24.

Landis CA, Campbell HJ, Begg JG, Midenhill DC, Paterson AM, Trewick SA (2008) The Waipounamu Erosion Surface: questioning the antiquity of the New Zealand land surface and terrestrial fauna and flora. *Geological Magazine,* **145,** 173-197.

Langellotto GA, Denno RF (2000) A trade-off between flight capability and reproduction in males of a wing-dimorphic insect. *Ecology,* **81,** 865-875.

Larkin MA, Blackshields G, Brown NP, Chenna R, Mcgettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. *Bioinformatics,* **23,** 2947-2948.

Leschen RB, Buckley TR, Harman HM, Shulmeister J (2008) Determining the origin and age of the Westland beech (*Nothofagus*) gap, New Zealand, using fungus beetle genetics. *Molecular Ecology,* **17,** 1256-1276.

Lindberg DR (1991) Marine biotic interchange between the northern and southern hemispheres. *Paleobiology,* **17,** 308-324.

Lockhart PJ, McLenachan PA, Havell D, Glenny D, Huson D, Jensen U (2001) Phylogeny, radiation, and transoceanic dispersal of New Zealand alpine buttercups: molecular evidence under split decomposition. *Annals of the Missouri Botanical Garden*, **88,** 458-477.

Macneale KH, Peckarsky BL, Likens GE (2005) Stable isotopes identify dispersal patterns of stonefly populations living along stream corridors. *Freshwater Biology,* **50,** 1117-1130.

Marden JH, Kramer MG (1994) Surface-skimming stoneflies: a possible intermediate stage in insect flight evolution. *Science,* **266,** 427-430.

Marden JH, Thomas MA (2003) Rowing locomotion by a stonefly that possesses the ancestral pterygote condition of co-occurring wings and abdominal gills. *Biological Journal of the Linnean Society,* **79,** 341-349.

Mark AF, Adams NM (1995) *New Zealand Alpine Plants*. Godwit, Auckland, New Zealand.

Marshall DC, Hill KBR, Fontaine KM, Buckley TR, Simon C (2009) Glacial refugia in a maritime temperate climate: Cicada (*Kikihia subalpina*) mtDNA phylogeography in New Zealand. *Molecular Ecology,* **18,** 1995-2009.

Marshall DC, Slon K, Cooley JR, Hill KBR, Simon C (2008) Steady Plio-Pleistocene diversification and a 2-Million-Year sympatry threshold in a New Zealand cicada radiation. *Molecular Phylogenetics and Evolution,* **48,** 1054-1066.

Matthew WD (1915) Climate and evolution. *Annals of the New York Academy of Science,* **24**, 171-318.

McCormick JE, Heled J, Delaney KS, Peterson AT, Knowles LL (2011) Calibrating divergence times on species trees versus gene trees: implications for speciation history of *Aphelocoma* Jays. *Evolution,* **65**, 184-202

McCulloch GA, Wallis GP, Waters JM (2009) Do insects lose flight before they lose their wings? Population genetic structure in subalpine stoneflies. *Molecular Ecology,* **18,** 4073-4087.

McCulloch GA, Wallis GP, Waters JM (2010) Onset of glaciation drove simultaneous vicariant isolation of alpine insects in New Zealand. *Evolution*, **64,** 2033-2043.

McDowall RM (2002) Accumulating evidence for a dispersal biogeography of southern cool temperate freshwater fishes. *Journal of Biogeography,* **29,** 207-219.

McGlone MS (1985) Plant biogeography and the late Cenozoic history of New Zealand. *New Zealand Journal of Botany,* **23,** 723-749.

McGlone MS (2002) The Late Quaternary peat, vegetation and climate history of the southern oceanic islands of New Zealand. *Quaternary Science Reviews,* **21,** 683-707.

McGlone MS (2005) Goodbye Gondwana. *Journal of Biogeography*, **32,** 739-740.

McGlone MS, Duncan RP, Heenan PB (2001) Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand. *Journal of Biogeography***, 28,** 199-216.

McGlone MS, Moar NT, Wardle P, Meurk CD (1997) Late-glacial and Holocene vegetation and environment of Campbell Island, far southern New Zealand. *The Holocene,* **7,** 1-12.

McGlone MS, Wilmshurst JM, Wiser SK (2000) Lateglacial and Holocene vegetation and climatic change on Auckland Island, Subantarctic New Zealand. *The Holocene,* **10,** 719-728.

McLellan ID (1967) New gripopterygids (Plecoptera) of New Zealand. *Transactions of the Royal Society of New Zealand,* **9,** 1-15.

McLellan ID (1975) The Freshwater Insects. In: *Biogeography and Ecology in New Zealand* (ed Kuschel G), pp.537-559 Monographiae Biologicae, The Hague, Netherlands.

McLellan ID (1977) New alpine and southern Plecoptera from New Zealand, and a new classification of the Gripopterygidae. *New Zealand Journal of Zoology*, **4,** 119-147.

McLellan ID (1983) A wingless alpine stonefly from New Zealand, and further information on genus *Holcoperla* (Plecoptera: Gripopterygidae). *New Zealand Journal of Zoology,* **10,** 263-266.

McLellan ID (1991) *Notonemouridae (Insecta: Plecoptera)*. Manaaki Whenua Press, Lincoln, New Zealand.

McLellan ID (1993) *Antarctoperlinae (Insecta: Plecoptera)*. Manaaki Whenua Press, Lincoln, New Zealand.

McLellan ID (1996) A revision of *Stenoperla* (Plecoptera: Eustheniidae) and removal of Australian species to *Cosmioperla* new genus. *New Zealand Journal of Zoology,* **23,** 165-182.

McLellan ID (1997) *Austroperla cyrene* Newman (Plecoptera: Austroperlidae). *Journal of the Royal Society New Zealand,* **27,** 271-278.

McLellan ID (1999) A revision of *Zelandoperla* Tillyard (Plecoptera: Gripopterygidae: Zelandoperlinae). *New Zealand Journal of Zoology,* **26,** 199-220.

McLellan ID (2000a) Additions to New Zealand notonemourid stoneflies. Insecta: (Plecoptera). *New Zealand Journal of Zoology,* **27,** 21-28.

McLellan ID (2000b) A revision of *Cristaperla* (Plecoptera: Notonemouridae) and some comments on Notonemouridae and its genetic groups. *New Zealand Journal of Zoology,* **27,** 233-244.

McLellan ID (2001) A revision of South American Austroperlidae (Plecoptera). *Aquatic Insects*, **23**, 233-251.

McLellan ID (2003) Six new species and a new genus of stoneflies (Plecoptera) from New Zealand. *New Zealand Journal of Zoology,* **30,** 101-114.

McLellan ID (2006a) Endemism and biogeography of New Zealand Plecoptera (Insecta). *Illiesia,* **2,** 15-23.

McLellan ID (2006b) The nymph of *Holcoperla angularis* (Wisely) (Plecoptera: Gripopterygidae) from New Zealand. *Illiesia,* **2,** 57-60.

McLellan ID, Zwick P (2007) New species of and keys to South American Gripopterygidae (Plecoptera). *Illiesia,* **3,** 20-42.

McLoughlin S (2001) The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany,* **49,** 271-300.

Merritt RW, Cummins KW (1996) *An Introduction to the Aquatic Insects of North America*. Kendall Hunt, Dubuque, USA.

Miller MP, Blinn DW, Keim P (2002) Correlations between observed dispersal capabilities and patterns of genetic differentiation in populations of four aquatic insect species from the Arizona White Mountains, U.S.A. *Freshwater Biology,* **47,** 1660-1673.

Müller K (1982) The colonization cycle of freshwater insects. *Oecologia,* **52,** 202-207.

Neiman M, Lively CM (2004) Pleistocene glaciation is implicated in the phylogeographical structure of *Potamopyrgus antipodarum*, a New Zealand snail. *Molecular Ecology,* **13,** 3085-3098.

Nelson G (1978) From Candolle to Croizat: comments on the history of biogeography. *Journal of the History of Biology,* **11,** 269-305.

Nelson G, Ladiges PY (2001) Gondwana, vicariance biogeography and the New York school revisited. *Australian Journal of Botany,* **49,** 389-409.

Nelson GJ, Platnick N (1981) *Systematics and biogeography: cladistics and vicariance*. Columbia University Press, New York, USA.

Neves RJ (1979) Movements of larval and adult *Pycnopsyche guttifer* (Walker) (Trichoptera: Limnephilidae) along Factory Brook, Massachusetts. *American Midland Naturalist,* **102,** 51-58.

Newnham RM, Lowe DJ, Williams PW (1999) Quaternary environmental change in New Zealand: a review. *Progress in Physical Geography,* **23,** 567-610.

Nylander JA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL (2004) Bayesian phylogenetic analysis of combined data. *Systematic Biology,* **53,** 47-67.

O'Neill SB, Chapple DG, Daugherty CH, Ritchie PA (2008) Phylogeography of two New Zealand lizards: McCann's skink (*Oligosoma maccanni*) and the brown skink (*O. zelandicum*). *Molecular Phylogenetics and Evolution,* **48,** 1168-1177.

Pagel M, Meade A (2004) A phylogenetic mixture model for detecting pattern-heterogeneity in gene sequence or character-state data. *Systematic Biology,* **53,** 571-581.

Palmer MA, Allan JD, Butman CA (1996) Dispersal as a regional process affecting the local dynamics of marine and stream benthic invertebrates. *Trends in Ecology and Evolution,* **11,** 322-326.

Patrick B (2003) Flightlessness and stream insects in southern New Zealand. In: *The Natural History of Southern New Zealand* (eds Darby JT, Fordyce RE, Mark AF, Probert PK, Townsend CR), pp. 387-388. University of Otago Press, Dunedin, New Zealand.

Phillips MJ, Gibb GC, Crimp EA, Penny D (2010) Tinamous and Moa flock together: mitochondrial analysis reveals independent losses of flight among ratites. *Systematic Biology,* **59,** 90-107.

Pole M (1994) The New Zealand flora-entirely long-distance dispersal? *Journal of Biogeography,* **21,** 625-635.

Pole M (2001) Can long-distance dispersal be inferred from the New Zealand plant fossil record? *Australian Journal of Botany,* **49,** 357-366.

Posada D (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution,* **25,** 1253.

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

Queiroz A (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology and Evolution,* **20,** 68-73.

Rambaut A, Drummond A (2003) *Tracer. MCMC trace analysis tool.* University of Oxford, Oxford, USA.

Raven PH, Axelrod DI (1972) Plate tectonics and Australasian paleobiogeography. *Science,* **176,** 1379-1386.

Riddle BR, Hafner DJ, Alexander LF, Jaeger JR (2000) Cryptic vicariance in the historical assembly of a Baja California Peninsular Desert biota. *Proceedings of the National Academy of Sciences,* **97,** 14438-14443.

Riek EF (1973) Fossil insects from the Upper Permian of Natal, South Africa. *Annals of the Natal Museum,* **21,** 513-532.

Riek EF (1976) New Upper Permian insects from Natal, South Africa. *Annals of the Natal Museum,* **22,** 755-789.

Roff DA (1984) The cost of being able to fly: a study of wing polymorphism in two species of crickets. *Oecologia*, **63,** 30-37.

Roff DA (1986) The genetic basis of wing dimorphism in the sand cricket, *Gryllus firmus* and its relevance to the evolution of wing dimorphisms in insects. *Heredity*, **57,** 1-3.

Roff DA (1990) The evolution of flightlessness in insects. *Ecological Monographs*, **60,** 389-421.

Roff DA (1994a) Habitat persistence and the evolution of wing dimorphism in insects. *American Naturalist*, **144**, 772-798.

Roff DA (1994b) The evolution of flightlessness: is history important? *Evolutionary Ecology,* **8,** 639-657.

Roff DA, Bradford MJ (1996) Quantitative genetics of the trade-off between fecundity and wing dimorphism in the cricket *Allonemobius socius*. *Heredity*, **76,** 178-185.

Roff DA, Fairbairn DJ (1991) Wing dimorphisms and the evolution of migratory polymorphisms among the Insecta. *Integrative and Comparative Biology*, **31**, 243-251.

Roff DA, Tucker J, Stirling G, Fairbairn DJ (1999) The evolution of threshold traits: effects of selection on fecundity and correlated response in wing dimorphism in the sand cricket. *Journal of Evolutionary Biology*, **12,** 535–546.

Rogers AR (1995) Genetic evidence for a Pleistocene population explosion. *Evolution*, **49,** 608-615.

Rosen DE (1978) Vicariant patterns and historical explanation in biogeography. *Systematic Biology,* **27,** 159.

Rundle SD, Foggo A, Choiseul V, Bilton DT (2002) Are distribution patterns linked to dispersal mechanism? An investigation using pond invertebrate assemblages. *Freshwater Biology,* **47,** 1571-1581.

Sage RD, Wolff JO (1986) Pleistocene glaciations, fluctuating ranges, and low genetic variability in a large mammal (*Ovis dalli*). *Evolution*, **40**, 1092-1095.

Sanders KL, Lee MS (2007) Evaluating molecular clock calibrations using Bayesian analyses with soft and hard bounds. *Biology Letters*, **22**, 275-279.

Sanmartin I, Ronquist F (2004) Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology,* **53,** 216-243.

Schaal BA, Hayworth DA, Olsen KM, Rauscher JT, Smith WA (1998) Phylogeographic studies in plants: problems and prospects. *Molecular Ecology,* **7,** 465-474.

Scher HD, Martin EE (2006) Timing and climatic consequences of the opening of Drake Passage. *Science,* **312,** 428-430.

Schmidt SK, Hughes JM, Bunn SE (1995) Gene flow among conspecific populations of *Baetis sp*. (Ephemeroptera): Adult flight and larval drift. *Journal of the North American Benthological Society*, **14,** 147-157.

Schultheis AS, Weigt LA, Hendricksn AC (2002) Gene flow, dispersal, and nested clade analysis among populations of the stonefly *Peltoperla tarteri* in the southern Appalachians. *Molecular Ecology,* **11,** 317-327.

Shepard DB, Burbrink FT (2008) Lineage diversification and historical demography of a sky island salamander, *Plethodon ouachitae,* from the interior highlands. *Molecular Ecology,* **17,** 5315-5335.

Shepard DB, Burbrink FT (2009) Phylogeographic and demographic effects of Pleistocene climatic fluctuations in a montane salamander, *Plethodon fourchensis*. *Molecular Ecology,* **18,** 2243-2262.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, **87,** 651-701.

Simpson GG (1940) Mammals and land bridges. *Journal of the Washington Academy of Sciences,* **30**, 137-163

Sinichenkova ND (1997) Palaeontology of stoneflies. In *Ephemeroptera & Plecoptera: Biology–Ecology–Systematics* (eds Landolt P, Sartoni M, Fribourg M), pp. 561-565. Mauron, Tinguely and Lachat SA, Fribourg, Switzerland.

Sinitshenkova ND (1987) Historical development of the stoneflies. *Trudy Paleontologicheskogo Instituta, Academiya Nauk SSSR,* **221,** 1-143.

Sinitshenkova ND (2004) New stoneflies of the family Palaeonemouridae from the Upper Permian of Udmurtiya and the Orenburg region (Insecta: Perlida= Plecoptera). Journal of *Paleontology*, **38,** 164–172.

Smissen RD, Breitwieser I, Ward JM, McLenachan PA, Lockhart PJ (2003) Use of ISSR profiles and ITS-sequences to study the biogeography of alpine cushion plants in the genus *Raoulia* (Asteraceae). *Plant Systematics and Evolution,* **239,** 79-94.

Smith PJ, Collier KJ (2001) Allozyme diversity and population genetic structure of the caddisfly *Orthopsyche fimbriata* and the mayfly *Acanthophlebia cruentata* in New Zealand streams. *Freshwater Biology,* **46,** 795-805.

Smith PJ, McVeagh SM, Collier KJ (2006) Genetic diversity and historical population structure in the New Zealand mayfly *Acanthophlebia cruentata*. *Freshwater Biology*, **51,** 12-24.

Stark BP, Gaufin AR (1976) The nearctic genera of Perlidae (Plecoptera). *Miscellaneous Publications of the Entomological Society of America,* **10,** 1-80.

Stark BP, Lentz DL (1992) *Dominiperla antigua* (Plecoptera: Perlidae), the first stonefly from Dominican amber. *Journal of the Kansas Entomological Society***,** **65,** 93-96.

Stöckler K, Daniel I, Lockhart P (2002) New Zealand Kauri (*Agathis australis* (D. Don) Lindl., Araucariaceae) survives Oligocene drowning. *Systematic Biology,* **51,** 827-832.

Suggate RP (1982) Low-rank sequences and scales of organic metamorphism. *Journal of Petroleum Geology,* **4,** 377-392.

Suggate RP (1990) Late Pliocene and Quaternary glaciations of New Zealand. *Quaternary Science Reviews,* **9,** 175–197.

Sutherland R (1994) Displacement since the Pliocene along the southern section of the Alpine Fault, New Zealand. *Geology,* **22,** 327-330.

Sutherland R (1996) Transpressional development of the Australia-Pacific boundary through southern South Island, New Zealand: constraints from Miocene-

Pliocene sediments, Waiho-1 borehole, South Westland. *New Zealand Journal of Geology and Geophysics*, **39,** 251-264.

Sutherland R (1999) Basement geology and tectonic development of the greater New Zealand region: an interpretation from regional magnetic data. *Tectonophysics,* **308,** 341-362.

Swofford DL (2003) *PAUP*. Phylogenetic analysis using parsimony (* and other methods). Ver. 4b10.* Sinauer Associates, Sunderland, MA, USA.

Terry MD, Whiting MF (2005) Mantophasmatodea and phylogeny of the lower neopterous insects. *Cladistics,* **21,** 240-257.

Thomas MA, Walsh KA, Wolf MR, McPheron BA, Marden JH (2000) Molecular phylogenetic analysis of evolutionary trends in stonefly wing structure and locomotor behavior. *Proceedings of the National Academy of Sciences,* **97,** 13178-13183.

Torsvik TH, Rousse S, Labails C, Smethurst MA (2009) A new scheme for the opening of the South Atlantic Ocean and the dissection of an Aptian salt basin. *Geophysical Journal International,* **177,** 1315-1333.

Treviranus GR (1803). *Biologie, oder Philosophie der lebenden*. Rower, Gottingen, Germany.

Trewick SA (1997) Flightlessness and phylogeny amongst endemic rails (Aves:Rallidae) of the New Zealand region. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **352,** 429-446.

Trewick SA (2001) Scree weta phylogeography: surviving glaciation and implications for Pleistocene biogeography in New Zealand. *New Zealand Journal of Zoology,* **28,** 291-298.

Trewick SA, Morgan-Richards M (2005) After the deluge: mitochondrial DNA indicates Miocene radiation and Pliocene adaptation of tree and giant weta (Orthoptera: Anostostomatidae). *Journal of Biogeography,* **32,** 295-309.

Trewick SA, Paterson AM, Campbell HJ (2007) Hello New Zealand. *Journal of Biogeography,* **34,** 1-6.

Trewick SA, Wallis GP (2001) Bridging the "beech-gap": New Zealand invertebrate phylogeography implicates Pleistocene glaciation and Pliocene isolation. *Evolution***, 55,** 2170-2180.

Trewick SA, Wallis GP, Morgan-Richards M (2000) Phylogeographical pattern correlates with Pliocene mountain building in the alpine scree weta (Orthoptera, Anostostomatidae). *Molecular Ecology,* **9,** 657-666.

Turnbull IM (2000) *Geology of the Wakatipu area.* Institute of Geological & Nuclear Sciences 1: 250 000 geological map 18. Institute of Geological and Nuclear Sciences, Lower Hutt, New Zealand.

Veblen TT, Young KR, Orme AR (2007). *The Physical Geography of South America.* Oxford University Press, Oxford,USA.

Vera A, Camousseight A (2006) Estado de conocimiento de los plecópteros de Chile. *Gayana,* **70,** 57-64.

Villet MH (2000) *A Catalogue of the Insects of South Africa*. Rhodes University, Grahamstown, South Africa.

Voje KL, Hemp C, Flagstad O, Saetre GP, Stenseth NCHR (2009) Climatic change as an engine for speciation in flightless Orthoptera species inhabiting African mountains. *Molecular Ecology,* **18,** 93-108.

Wagner DL, Liebherr JK (1992) Flightlessness in insects. *Trends in Ecology and Evolution*, **7,** 216-220.

Wallis GP, Trewick SA (2001) Finding fault with vicariance: a critique of Heads (1998). *Systematic Biology,* **50,** 602-609.

Wallis GP, Trewick SA (2009) New Zealand phylogeography: evolution on a small continent. *Molecular Ecology,* **18,** 3548-3580.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques,* **10,** 506-513.

Wardle P (1988) Effects of glacial climates on floristic distribution in New Zealand 1. A review of the evidence. *New Zealand Journal of Botany,* **26,** 541-555.

Waters JM (2007) Geological dates and molecular rates: rapid divergence of rivers and their biotas. *Systematic Biology,* **56,** 271-282.

Waters JM, Allibone RM, Wallis GP (2006) Geological subsidence, river capture, and cladogenesis of galaxiid fish lineages in central New Zealand. *Biological Journal of the Linnean Society,* **88,** 367-376.

Waters JM, Craw D (2006) Goodbye Gondwana? New Zealand biogeography, geology, and the problem of circularity. *Systematic Biology,* **55,** 351-356.

Waters JM, Craw D (2008) Evolution and biogeography of New Zealand's longjaw galaxiids (Osmeriformes: Galaxiidae): the genetic effects of glaciation and mountain building. *Freshwater Biology,* **53,** 521-534.

Waters JM, Craw D, Youngson JH, Wallis GP (2001) Genes meet geology: fish phylogeographic pattern reflects ancient, rather than modern, drainage connections. *Evolution,* **55,** 1844-1851.

Waters JM, Lopez JA, Wallis GP (2000) Molecular phylogenetics and biogeography of galaxiid fishes (Osteichthyes: Galaxiidae): dispersal, vicariance, and the position of *Lepidogalaxias salamandroides*. *Systematic Biology,* **49,** 777-795.

Waters JM, Saruwatari T, Kobayashi T, Oohara I, McDowall R, Wallis GP (2002) Phylogenetic placement of retropinnid fishes: data set incongruence can be reduced by using asymmetric character state transformation costs. *Systematic Biology*, **51,** 432-449.

Waters JM, Wallis GP (2001) Cladogenesis and loss of the marine life-history phase in freshwater galaxiid fishes (Osmeriformes: Galaxiidae). *Evolution*, **55**, 587-597.

Waters TF (1965) Interpretation of invertebrate drift in streams. *Ecology*, **46,** 327-334.

Weir JT, Schluter D (2004) Ice sheets promote speciation in boreal birds. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **271,** 1881-1887.

Wellman HW (1979) An uplift map for the South Island of New Zealand, and a model for uplift of the Southern Alps. In: *The Origin of the Southern Alps* (eds Walcott RI, Cresswell MM), pp. 18. Royal Society of New Zealand Bulletin, New Zealand.

Wheeler WC, Whiting M, Wheeler QD, Carpenter JM (2001) The phylogeny of the extant hexapod orders. *Cladistics*, **17,** 113-169.

Whiting MF (2002) Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zoologica Scripta*, **31**, 93-104.

Whiting MF, Carpenter JC, Wheeler QD, Wheeler WC (1997) The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic Biology*, **46**, 1-68.

Whiting MF, Bradler S, Maxwell T (2003) Loss and recovery of wings in stick insects. *Nature*, **421,** 264-267.

Whittaker RJ, Fernández-Palacios JM (2007). *Island Biogeography: Ecology, Evolution, and Conservation*. Oxford University Press, Oxford, USA.

Wilgenbusch JC, Warren DL, Swofford DL (2004) AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. *Website http://ceb.csit.fsu.edu/awty [Accessed 17 June 2006]*.

Willett RW (1950) The New Zealand Pleistocene snow line, climatic conditions, and suggested biological effects. *New Zealand Journal of Science and Technology,* **32,** 18-48.

Winkworth RC, Wagstaff SJ, Glenny D, Lockhart PJ (2002) Plant dispersal NEWS from New Zealand. *Trends in Ecology and Evolution,* **17,** 514-520.

Winterbourn MJ, Gregson KLD, Dolphin CH (2006) *Guide to the aquatic insects of New Zealand 4th edition*. Entomological Society of New Zealand, NZ.

Wootton RJ (1976) *The Fossil Record and Insect Flight*. Blackwell Scientific Publications, Oxford, USA.

Wootton RJ (1981) Palaeozoic Insects. *Annual Review of Entomology*, **26**, 319-344.

Zajic J (2000) Vertebrate zonation of the non-marine Upper Carboniferous - Lower Permian basins of the Czech Republic. *Courier Forschungsinstitut Senckenberg*, **223**, 563-575.

Zera AJ (1984) Differences in survivorship, development rate and fertility between the longwinged and wingless morphs of the waterstrider, *Limnoporus canaliculatus*. *Evolution*, **38**, 1023-1032.

Zera AJ, Denno RF (1997) Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology*, **42**, 207-231.

Zera AJ, Sall J, Otto K (1999) Biochemical aspects of flight and flightlessness in *Gryllus*: flight fuels, enzyme activities and electrophoretic profiles of flight muscles from flight-capable and flightless morphs. *Journal of Insect Physiology*, **5**, 275-285.

Zhang DX, Hewitt GM (1996) Nuclear integrations: Challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution*, **11**, 247-251.

Zhang LB, Renner S (2003) The deepest splits in Chloranthaceae as resolved by chloroplast sequences. *International Journal of Plant Science*, **164,** 383-392.

Zwick P (1973) *Insecta: Plecoptera. Phylogenetisches System und Katalog. Das Tierreich 94*. Walter de Gruyter and Co., Berlin, Germany.

Zwick P (1979) Revision of the stonefly family Eusthniidae (Plecoptera), with emphasis on fauna of the Australian region. *Aquatic Insects*, **1**, 17-50.

Zwick P (1981) *Plecoptera. Ecological Biogeography in Australia***.** Dr W. Junk, The Hague, Netherlands.

Zwick P (1990) Transantarctic relationships in the Plecoptera. *In: Mayflies and Stoneflies, Life History and Biology* (ed Campbell IC), pp. 141-148. Kluwer Academic Pub, Netherlands.

Zwick P (2000) Phylogenetic system and zoogeography of the Plecoptera. *Annual Review of Entomology,* **45,** 709-746.

Zwick P (2003) Biogeographical patterns. In: *Encyclopedia of Insects* (eds Resh VH, Carde RT), pp. 94-103. Academic Press, Elsevier Science, USA**.**

Zwick P (2009) The Plecoptera - who are they? The problematic placement of stoneflies in the phylogenetic system of insects. *Aquatic insects,* **31**, 181-194.

Zwickl DJ (2006) *Genetic Algorithm approaches for the phylogenetic analysis of large biological datasets under the maximum likelihood criterion.* Ph.D., The University of Texas.

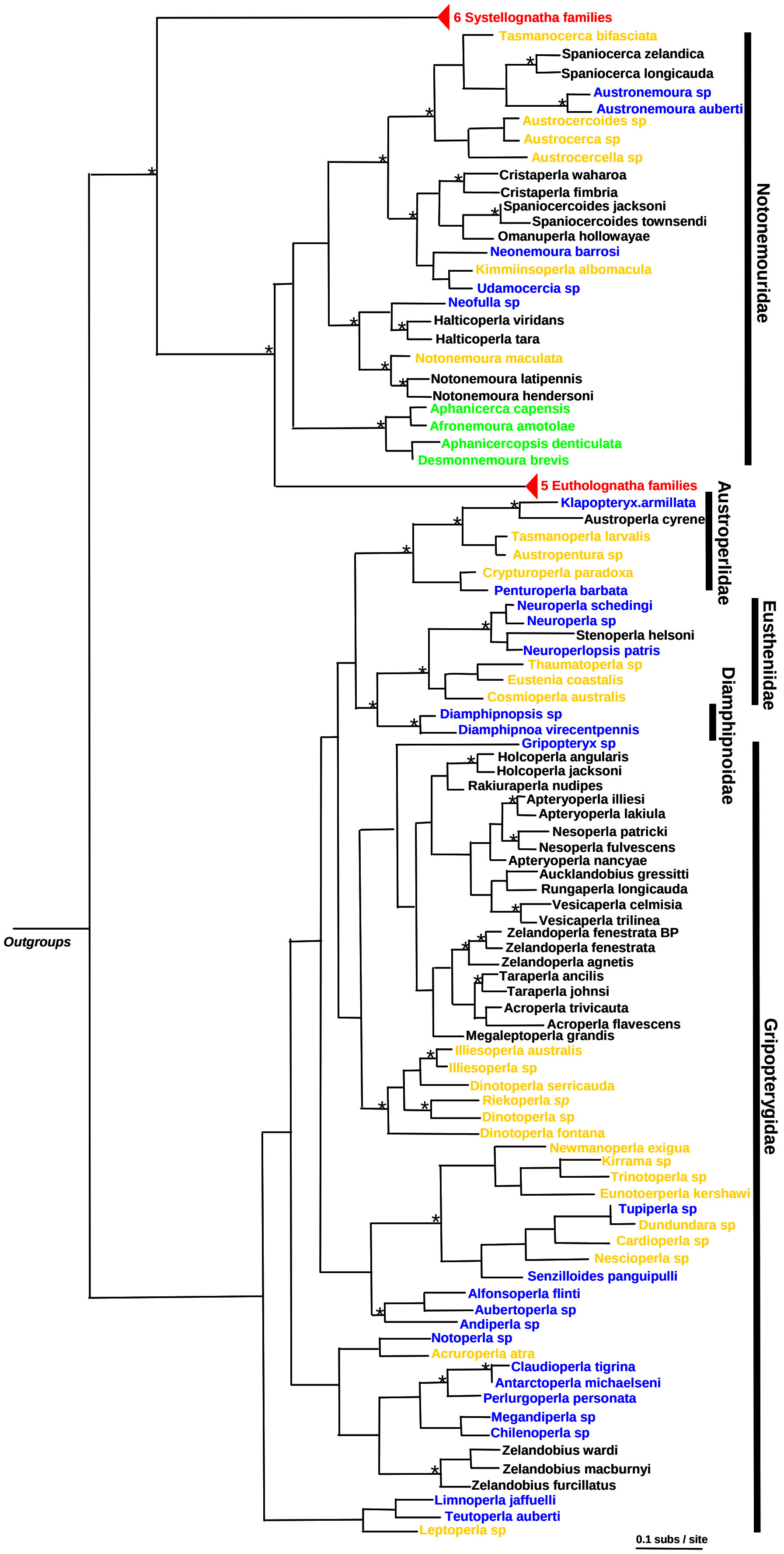
Appendix 1: Sampling sites and NZAC numbers (if applicable) of the plecopteran specimens from Chapter 2.

Family	Species	Country	NZAC Record	Site	Northing	Easting
Notonemouridae	*Kimminsoperla albomacula*	Australia		Lake Dixon trib, Tasmania	n/a	n/a
	Tasmanocerca bifasciata	Australia		Wandle River, Tasmania	n/a	n/a
	Austrocerca rieki	Australia		Kermandie River, Tasmania	n/a	n/a
	Austrocercoides zwicki	Australia		Liffey Falls, Tasmania	n/a	n/a
	Austrocercella sp.	Australia		Watchbed Creek, Victoria	n/a	n/a
	Notonemoura maculata	Australia		Erskine Falls, Victoria	n/a	n/a
	Neonemoura barrosi	Chile		Chiloe, Llanquihue	n/a	n/a
	Udamocercia sp.	Chile		Antillanca, Puyehue	n/a	n/a
	Austronemoura sp.	Argentina		Bariloche, Rio Negro	n/a	n/a
	Austronemoura auberti	Chile		Coimallin, Malleco	n/a	n/a
	Neofulla sp.	Chile		Rio Chaquiguan, Llanquihue	n/a	n/a
	Afronemoura amatolae	South Africa		Buffalo River, Eastern Cape	n/a	n/a
	Aphanicerca capensis	South Africa		Skeleton Gorge, Eastern Cape	n/a	n/a
	Desmonemoura brevis	South Africa		Berg River, Western Cape	n/a	n/a
	Aphanicercopsis denticulata	South Africa		Eerste River, Eastern Cape	n/a	n/a
	Cristaperla waharoa	NZ		Cap Burn	2289835	5437250
	Cristaperla fimbria	NZ		Humboldt Creek	2123200	5600700
	Omanuperla hollowayae	NZ	NZAC03003859	Mount Matthews	2678700	5982300
	Spaniocercoides jacksoni	NZ		Haast River	2219950	5679082
	Spaniocercoides townsendi	NZ	NZAC03003568	Cobb Reservoir	2483890	6011420
	Spaniocerca zelandica	NZ	NZAC03002386	Cuckoo Creek	2402900	5781500
	Spaniocerca longicauda	NZ		Carsons Creek	2231288	5475967
	Halticoperla tara	NZ	NZAC03003865	Cascade Stream	2116658	5426337
	Halticoperla viridans	NZ	NZAC03003751	Cropp River	2344450	5790171
	Notonemoura latipennis	NZ	NZAC03003622	Rahu River	2438300	5879700
	Notonemoura hendersoni	NZ		Cascades, Gates of Haast	2219776	5679082
Diamphipnoidae	*Diamphipnoa virecentpennis*	Chile		Puerto Montt, Llanquihue	n/a	n/a
	Diamphipnosis sp.	Chile		Panguipulli, Valdivia	n/a	n/a
Eustheniidae	*Neuroperlopsis patris*	Chile		Rio Triu-Triu, Rinihue	n/a	n/a
	Neuroperla sp.	Argentina		Nequen River, Nequen	n/a	n/a
	Neuroperla schedingi	Chile		Lago Puyehue, Osorno	n/a	n/a
	Cosmioperla australis	Australia		Barrington River, NSW	n/a	n/a
	Eusthenia coastalis	Australia		Robertson Stream, Tasmania	n/a	n/a
	Thaumatoperla sp.	Australia		White Falls, NSW	n/a	n/a
	Stenoperla helsoni	NZ	NZAC03004226	Goings Creek	2462800	5869400
Austroperlidae	*Klapopteryx* sp.	Argentina		Futaleufu River, Chubut	n/a	n/a
	Penturoperla barbata	Chile		Lake Pillaifa, Cautin	n/a	n/a
	Austropentura sp.	Australia		New Town Rivulet, Tasmania	n/a	n/a
	Tasmanoperla larvalis	Australia		Wedge River, Tasmania	n/a	n/a
	Crypturoperla paradoxa	Australia		Lake Dobson trib, Tasmania	n/a	n/a
	Austroperla cyrene	NZ	NZAC03004017	Lake Moke trib.	2155900	5562500
Gripopterygidae	*Notoperla* sp.	Argentina		Futaleufu River, Chubut	n/a	n/a
	Megandiperla sp.	Chile		Monte Jervis, Magallanes	n/a	n/a
	Chilenoperla sp.	Chile		Antillanca, Osorno	n/a	n/a
	Antarctoperla michaelsoni	Argentina		Percy Stream, Chubut	n/a	n/a
	Claudioperla tigirina	Peru		Lima, Puna	n/a	n/a
	Pelurgoperla personata	Chile		Pellaifa, Valdivia	n/a	n/a
	Limnoperla jaffueli	Argentina		Percy Stream, Chubut	n/a	n/a
	Teutoperla auberti	Chile		Las Cabras, Nuble	n/a	n/a
	Gripopteryx sp.	Brazil		Petropolis, Rio de Janerio	n/a	n/a
	Alfonsoperla flinti	Chile		El Purgatorio, Nuble	n/a	n/a
	Aubertoperla sp	Argentina		El Comisario stream, Chubut	n/a	n/a
	Andiperla sp.	Argentina		Upsala Glacier, Santa Cruz	n/a	n/a
	Senzilloides panguipulli	Argentina		Futaleufu River, Chubut	n/a	n/a
	Tupiperla sp.	Argentina		Arroyo Coati, Misiones	n/a	n/a

Acruroperla atra	Australia		Sawpit Creek, NSW	n/a	n/a
Leptoperla sp.	Australia		Lake Dobson trib, Tasmania	n/a	n/a
Newmanoperla exigua	Australia		Margaret River, WA	n/a	n/a
Kirrama sp.	Australia		Bloomfield River, Queensland	n/a	n/a
Trinotperla nivata	Australia		Fitzroy Falls, Victoria	n/a	n/a
Eunotoperla kershawi	Australia		Belgrave, Victoria	n/a	n/a
Dinotoperla fontana	Australia		Lees Creek, Victoria	n/a	n/a
Riekoperla sp.	Australia		Alpine Creek, NSW	n/a	n/a
Dinotoperla sp.	Australia		Cotter River, Tasmania	n/a	n/a
Dinotoperla serricauda	Australia		Liffey Falls, Tasmania	n/a	n/a
Illiesoperla australis	Australia		McCarrs Creek, NSW	n/a	n/a
Illiesoperla sp.	Australia		Aire River, Victoria	n/a	n/a
Nescioperla sp.	Australia		Bloomfield River, Queensland	n/a	n/a
Cardioperla sp.	Australia		Marakoopa Stream, Tasmania	n/a	n/a
Dundundara sp.	Australia		Birthday Creek, Queensland	n/a	n/a
Zelandobius macburnyi	NZ	NZAC03000753	Johnsonville River	2660100	5995800
Zelandobius wardi	NZ		Armstrong Reservoir	2510400	5707800
Zelandobius furcillatus	NZ	NZAC03004113	Rough Creek	2165100	5629000
Taraperla johnsi	NZ		Mt Maungatua	2285810	5476790
Taraperla ancilis	NZ		Nevis River	2181300	5524600
Acroperla trivicauta	NZ		Lee Stream	2297300	5487400
Acroperla flavescens	NZ	NZAC03002997	Ashton Burn	2139044	5525261
Megalandoperla grandis	NZ		Strathconan Stream	2331300	5672500
Zelandoperla agnetis	NZ		Hapiata Stream	2567000	6011300
Zelandoperla fenestrata.BP	NZ		Peraki Creek	2497415	5705450
Zelandoperla fenestrata	NZ		Haast River	2219950	5679082
Vesicaperla celmisia	NZ	NZAC03004252	Old Man Range	2213662	5531325
Vesicaperla trilinea	NZ	NZAC03004256	Pisa Range	2204323	5586178
Apteryoperla nancayae	NZ		Mount Lyell	2431979	5937058
Nesoperla patricki	NZ	NZAC03004254	Swampy Summit	2314176	5488596
Nesoperla fulvescens	NZ		Denniston	2409716	5939409
Apteryoperla illiesi	NZ	NZAC03001227	Homer Saddle	2114583	5593171
Apteryoperla lakiula	NZ		Mount Rakeahua	2120591	5349877
Holcoperla angularis	NZ	NZAC03001591	Gertrude Valley	2115621	5594984
Holcoperla jacksoni	NZ		Mount Travatore	2462800	5869400
Rakiuraperla nudipes	NZ	NZAC03002980	Mt Rakeahua	2120591	5349877
Aucklandobius gressitti	Auckland Is.	NZAC03003021	Port Ross	n/a	n/a
Rungaperla longicauda	Campbell Is.	NZAC03002264	Camp Stream	n/a	n/a

Appendix 2: Maximum likelihood phylogeny of the relationships among Plecoptera.

Bootstrap proportions exceeding 90 are indicated by a * above each node.



Appendix 3: Sampling sites and NZAC numbers (if applicable) of the six stonefly genera from Chapter 3.

Genus	Location	Species	NZAC accession no	Site	Northing	Easting
Apteryoperla	*1*	*lakiula*	NZAC03001770	Mount Rakeahua	2120591	5349877
	2	*ramsayi*	NZAC03001631	Furket Pass	2054285	5484773
	3			Pleasant Range	2054000	5484930
	4	*monticola*		Wairaki Peaks	2112834	5484615
	5		NZAC03001575	Family Peaks	2103870	5488189
	6	*illiesi*		Gertrude Saddle	2114480	5596363
	7			Triangle Peak	2114300	5580500
	8			Homer Saddle	2114583	5593171
	9		NZAC03001243	Homer Tunnel	2113610	5592494
	10		NZAC03001232	Cascade Saddle	2162290	5627620
	11			Albert Burn Saddle	2179640	5637013
	12			Tamatea Peak	2056090	5485274
	13	*nancyae*	NZAC03001633	Mount Lyell	2431979	5937058
	14			St Andrew's Basin	2428494	5953275
Holcoperla	*1*	*angularis*		Tamatea Peak	2056400	5486000
	2			Mount Burns	2072631	5479616
	3		NZAC03001574	Family Peaks	2103870	5488189
	4			Stuart Mountains	2085182	5563058
	5		NZAC03001591	Gertrude Valley	2115621	5594984
	6			Gertrude Saddle	2114480	5596363
	7	*magna*	NZAC03001586	Helen Peaks	2143853	5521996
	8		NZAC03001582	Eyre Mountains	2155597	5536730
	9			Cecil Peak	2168135	5555119
	10			West James Peak	2183700	5543100
	11			Corner Peak	2219188	5630939
	12	*"sp"*		Stewarts Knob	2219821	5679651
	13		NZAC03001386	Mount Clarke	2254300	5701300
	14	*jacksoni*		Mount Barron	2389637	5817780
	15		NZAC03001401	Doubtful Range	2446300	5850900
	16		NZAC03001403	Moss Pass	2479700	5904800
	17			Mount Trovatore	2462800	5869400
	18			Otira Valley	2392600	5810133
Vesicaperla	*1*	*dugdalei*	NZAC03004311	Wilmot Pass	2055820	5506140
	2			Wilmot Pass	2056266	5504850
	3	*celmisia*	NZAC03004252	Old Man Range	2213562	5531325

	4			Titan Rocks	2196817	5512159
	5			Umbrella Mountains	2206070	5508080
	6	*trilinea*	NZAC03004256	Pisa Range	2204323	5586178
	7			North Dunstan Mountains	2247791	5590138
	8	*townsendi*	NZAC03003307	Louisa Peak	2279695	5749408
	9			Alex Knob	2279546	5749676
	10	*substirpes*	NZAC03004217	Duffy Creek Saddle	2432302	5881091
	11	*eylesi*	NZAC03004318	Richmond Saddle	2544414	5970375
Cristaperla	*1*	*eylesi*		Excelsior Creek	2104107	5497593
	2		NZAC03003579	Borland Burn	2073500	5480700
	3			Telford Burn	2106776	5483337
	4			Snowy Creek	2155943	5623886
	5	*waharoa*		Benger Burn	2217682	5506472
	6			West Diadem Creek	2250253	5638971
	7		NZAC03003889	Gem Lake trib.	2206070	5508080
	8			Cap Burn	2289835	5437250
	9			Burns Creek	2313710	5487330
	10	*fimbria*		Humboldt Creek	2123200	5600700
	11			Duggans Creek	2461405	5861594
	12			Gypsy Creek	2166486	5675727
	13			Depot Creek	2208772	5687664
	14			Maruia River trib.	2460870	5869891
	15			Poulter River trib	2416196	5813271
	16			Poverty Stream trib.	2412290	5793652
	17		NZAC03003680	Mill Stream	2305368	5484215
	18			Pigroot Creek	2307724	5553683
Halticoperla	*1*	*tara*		Snowy Creek	2157500	5619300
	2			Dart River trib.	2152000	5621700
	3			Whitbourn River	2154300	5625700
	4		NZAC03003871	Whitestone River trib.	2124072	5534217
	5		NZAC03003865	Cascade Stream	2116658	5426337
	6			Matukituki River	2164300	5626600
	7			Aston Burn	2139044	5525261
	8			Afton Burn	2151795	5555012
	9			Rastus Burn	2179927	5564937
	10	*viridans*	NZAC03003751	Cropp River	2344450	5790171
	11			Rappahannock River	2448638	5901628
	12			Little Kaituna Stream	2484836	6050688
Spaniocerca	*1*	*longicauda*		Carsons Creek	2231288	5475967

2			Ravine Creek	2223961	5468065
3		NZAC03002325	Rees River	2154700	5619000
4			Borland Burn	2075704	5480903
5			Excelsior Creek	2104107	5497593
6			Hut Creek	2114300	5580500
7			Mount Richmond tarn	2123300	5522800
8			Pig Creek	2156420	5522704
9			Donald Stuart Creek	2254066	5583305
10			Rob Roy Stream	2171223	5625954
11			Mill Stream	2305368	5484215
12			McQuilkans Creek	2310109	5484429
13			Billy Creek	2155597	5536730
14	*zwicki*		Makarora River trib.	2204650	5651382
15			Gorge Creek	2218300	5530300
16		NZAC03002557	Shingle Creek	2219800	5524900
17			Black Creek	2208082	5505660
18			Lug Creek	2281083	5527659
19	*acuata*	NZAC03002533	Lake Ohau trib.	2255100	5662000
20			Temple Stream	2255200	5673200
21			Dorcy Stream	2261457	5661876
22			Solitude Stream	2222700	5686837
23	*hamishi*		Bluewater Creek	2267000	5725400
24		NZAC03002204	Architect Creek	2259000	5728100
25		NZAC03002203	Copland River	2256900	5728200
26	*minor*		Opuha River	2334047	5702488
27			Greyney Creek	2394949	5800848
28		NZAC03002599	Neave Creek	2346184	5778577
29			Cropp River	2363102	5791907
30	*zelandica*		Toi Creek	2526598	5977239
31			Boakes Creek	2460321	5859185
32			Cuckoo Creek	2402900	5781500
33		NZAC03002369	Porirua Stream	2660135	5997127
34			Whakaki Stream	2692600	6378500
35			Abbots Creek	2698723	6008527
36	*bicornuta*		Silvermine Creek	2458787	5993374
37			Clay Creek	2476480	6050227
38		NZAC03002533	Ferris Creek	2455482	6001346
39			Myttons Creek	2475013	6011910
40			Upper Twin Creek	2392870	5810328

41	NZAC03002214	Scotties Creek	2432600	5934600
42		Sentinel Hill Stream	2472700	5963500
43		Rough Creek	2450600	5869600

Appendix 4: Sampling location of *Z. decorata* and *fenestrata* species group specimens from Chapter 4, plus number of specimens sequenced per site for COI and the adult phenotype of the *fenestrata* species group specimens (**A**pterous, **M**icropterous, fully-**W**inged). * indicates specimens deposited in the New Zealand Arthropod Collection. Specific NZAC accession codes are as follows: N2 (NZAC03004711), N3 (NZAC03004714), N4 (NZAC03004712), WS1 (NZAC03004733), WS (NZAC03004721), WS5 (NZAC03004732), C1 (NZAC03004741), C2 (NZAC03004742), C6 (NZAC03004738), C22 (NZAC03004699), CT2(NZAC03004698), E1 (NZAC03004751), E11 (NZAC03004752), W5 (NZAC03004757), W9 (NZAC03004758), M3 (NZAC03004753), M5 (NZAC03004759), O2 (NZAC03004761), A1 (NZAC03004763), A3 (NZAC03004762), S1 (NZAC03004770), S4 (NZAC03004771).

Region	River system	Site	Northing	Easting	*fenestrata*	morph	*decorata*	Code
Northern	coastal	Okuti River	2494480	5713297			2	N1
		Kaituna River	2485346	5719368			2	N2*
	coastal	Peraki Creek	2497415	5705450	2	A		N3*
	Waimakariri	Porter River trib.	2400500	5769400	2	A	2	N4*
		Upper Craigieburn	2404868	5787571	2	W		N5
	Rakaia	Lake Evelyn trib.	2387297	5771617			2	N6
		Ryton River	2398531	5779707	2	W		N7
	Wairau	Wairau River	2503200	5921500	2	W		N8
	coastal	Takaka River	2498734	6019443	2	?		N9
Western	Clutha	Route Burn	2136031	5599196			2	WS1*
		Brides Vail Stream	2167500	5628400	3	A		WS2*
		Downs Creek	2170076	5624695			2	WS3
		Lake Greaney trib.	2172800	5670400	2	W		WS4
	Haast	Haast River	2219950	5678990	2	W		WS5*
		Haast River	2219776	5679082	2	W		WS6
		Landsborough trib.	2242200	5692500			2	WS7
		Fraser Creek	2241300	5692800	2	W		WS8
		Landsborough trib.	2245316	5694699	2	?		WS9
	coastal	Te Naihi Creek	2238275	5730880			2	WS10
	coastal	Wanganui River	2434061	5980831			2	WS11
Clutha	Clutha	Clutha trib.	2257928	5434458			2	C1*

		Waipahi River	2220206	5440838	2	W		C2*
		Tokomairiro	2267922	5451873	3	W		C3
		Bowlers Creek	2247400	5476000	2	W	2	C4
		Clutha River trib.	2232300	5494300	3	W		C5
		Jordan River	2211072	5507673	2	M		C6*
		Jordan River	2211154	5507843	2	M		C7
		Gem Creek	2206271	5503030	2	A		C8
		Shingle Creek	2219800	5524900	2	W	4	C9
		Gorge Creek	2218300	5530300	2	W	5	C10
		Ranch Creek	2212500	5541300	2	M		C11
		Manuherikia River	2241663	5560822			2	C12
		Manuherikia River	2256504	5576008			3	C13
		Shepherds Creek	2207790	5556965	2	W	2	C14
		Thomsons Creek	2232698	5576075	2	A		C15
		Poison Creek	2220179	5594395	2	W	2	C16
	Nevis(Clutha)	Nevis River	2195400	5551700	2	W	4	C17
		Schoolhouse Creek	2184170	5517975			3	C18
		Nevis trib.	2190500	5542900	3	A		C19
		Drummond Creek	2186800	5533300	2	A	2	C20
		Nevis River	2182100	5525700	2	M	3	C21
		Nevis River	2181300	5524600	2	A	2	C22*
	coastal	Burnt Scrub Stream	2259453	5418767	2	?		CT1
	Catlins	McLaren Creek	2245894	5408397			2	CT2*
	Owaka	Tuck Stream	2248568	5418205	2	?		CT3
	coastal	Puerua River trib.	2243700	5421800	3	?		CT4
		Puerua River trib.	2251273	5425241	2	?		CT5
East Otago	coastal	Lindsay Creek	2319816	5483719	3	W		E1*
		Water of Leith	2316642	5480801	2	W	2	E2
		Careys Creek	2320400	5486800			2	E3
	Taieri	McQuilkans Creek	2309834	5484472	2	W		E4
		Whare Creek	2308361	5484827	2	W	2	E5
		Whare Creek	2307987	5483992	2	W	2	E6
		Lee Stream	2297300	5487400	2	W		E7
		Silver Stream	2289000	5484200	2	W		E8
		Lug Creek	2286921	5527343	2	W		E9

		Six Mile Creek	2293643	5536257	2	W	2	E10
		Kye Burn	2294595	5558549			2	E11*
		Swin Burn	2300967	5560112	2	W		E12
	Shag	Shag River	2305322	5555636	2	?	3	E13
		Pigroot Creek	2307638	5553605	2	A	3	E14
	coastal	Trotters Gorge	2336177	5531372			4	E15
	Taieri	Kye Burn	2293248	5565548			2	E16
		Deep Stream	2291124	5575624	2	?	2	E17
		Taieri River trib.	2284509	5554861	3	?		E18
		Rock & Pillar Creek	2275200	5533600	2	A		E19
		Logan Burn	2272348	5522177	2	A		E20
		Ratty Creek	2268262	5508917	3	A	2	E21
		Sutton Stream	2269731	5506240	2	A		E22
		Ailsa Creek	2259050	5503070	2	A		E23
	coastal	Waimate Creek	2349817	5609533	2	W	2	E24
	Waitaki	Maerewhenua River	2317663	5581439			2	E25
		Awakino River	2301057	5601866	3	A	2	E26
		Otematata River trib.	2288068	5618817			2	E27
		Ahuriri River trib.	2249259	5632326	3	?		E28
Southland	Waiau	Ligar Creek	2094000	5477500			2	W1
		Telford Burn	2106833	5483643	2	M		W2
		Elm Tree Creek	2109300	5500700			2	W3
		Gorge Creek	2120700	5505500	4	M	2	W4
		Mararoa trib.	2123300	5522800	3	A		W5*
		Mararoa River	2120600	5512100			4	W6
		Whitestone River	2111200	5523400	5	W	2	W7
		Whitestone River	2124576	5534085	2	M	2	W8
		North Mavora Lake	2131600	5541400	2	?	2	W9*
		North Mavora Lake	2130300	5546600	2	W		W10
		Mararoa River	2131000	5551900			2	W11
	Mataura	Whisky Creek	2188184	5452139	2	W		M1
		Eyre Creek	2154800	5513500	3	W	2	M2
		Ross Creek	2156100	5516700	3	W		M3*
		Eyre Creek	2154800	5514300			2	M4
		Mataura River	2172730	5521519			2	M5*

		Mataura River	2164200	5525600			2	M6
	Oreti	Bluff	2152038	5389139	2	W		O1
		Oreti River	2146716	5443075			2	O2*
		Warwick Downs	2159862	5459667	2	W	2	O3
		Acton Stream	2146400	5503900	3	W	2	O4
		Weydon Burn	2124800	5502800	2	A	2	O5
		Weydon Burn	2123600	5504700			3	O6
		Gorge Burn	2138200	5534200	3	A	2	O7
	Von (Clutha)	Von River trib.	2142900	5541100	4	A	2	O8
		Bush Creek trib.	2141200	5541800	2	A	2	O9
	Aparima	South Fern Burn	2120862	5442507	2	W		A1*
		North Fern Burn	2119862	5443188	2	W		A2
		Aparima River	2131806	5455788			3	A3*
		Kowhai Creek	2125352	5484147	3	W	2	A4
Stewart Is.	coastal	Mill Creek	2136355	5357244	2	W		S1*
		Little River	2136883	5360598	3	?		S2
		Kaipipi walk	2133706	5356663	2	W		S3
		Christmas Village	2126322	5372475			2	S4*
	Rakeahua	Rakeahua River trib.	2122637	5347525	2	W	2	S5
	Rakeahua	Rakeahua River trib.	2120481	5349603	2	?		S6
Total					186		144	

Appendix 5: Bayesian phylogeny of the *fenestrata* species group COI haplotypes (see Fig. 4.3). The coloured bar indicates the particular species represented by each haplotype.