ASSESSING DISPERSAL AMONG HABITATS FOR THREE NEW ZEALAND AQUATIC INSECTS USING MITOCHONDRIAL DNA (COI) SEQUENCES

A thesis

Submitted in partial fulfilment

of the requirements for the Degree

of

Master of Science

in Biological Sciences

at

The University of Waikato

By

MORGAN ALEXANDRA RIDING

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

2016

ABSTRACT

Dispersal of aquatic insects is a key process allowing colonization of new habitat and linking existing populations among habitats. Studying the dispersal of freshwater taxa directly is logistically challenging and assessments of population genetic structure using DNA sequence fragments have provided an alternative means to examine the dispersal and connectivity of natural populations. Here, I used mitochondrial cytochrome c oxidase subunit I (COI) DNA sequences to assess the genetic similarity for aquatic insect species across both small (<50km) and large (>1000km) spatial scales. For the first study, I examined genetic similarity among source and restored stream habitats in Taranaki Region, New Zealand. Three common species were targeted based on differences in dispersal capabilities: *Archichauliodes diversus* (Megaloptera; active larvae, weak flight), and *Hydropsyche colonica* and *Pycnocentrodes aeris* (Trichoptera; sessile larvae, strong flight). *Archichauliodes diversus* showed the greatest sequence diversity (19 haplotypes) followed by *P. aeris* (14 haplotypes) and *H. colonica* (4 haplotypes). Despite relatively high levels of genetic diversity, *A. diversus* was genetically similar throughout the study area, suggesting adequate dispersal among source and restored habitats. Individuals of *H. colonica,* with much lower levels of haplotype diversity, were also genetically similar between source and restored habitats. In contrast, *P. aeris* had one haplotype that was more common in source versus restored habitats. I concluded that taxa were relatively well connected between source and restored habitats and that COI sequences can provide a useful indicator for tracking restoration efforts within relatively small geographic areas. In the second study, I focused on large-scale patterns of diversity for *A. diversus* which is

widespread throughout both the North and the South Islands of New Zealand and compared this with available sequences from a congeneric Australian species. I found sequence divergences between the New Zealand *A. diversus* and Australian *Archichauliodes sp* (11.8% divergence), and between New Zealand North Island and South Island *A. diversus* (3% divergence). In the South Island, there were 8 haplotypes restricted to individual sites and 4 haplotypes that were shared among sites. For the North Island, individuals there were 21 haplotypes restricted to one of more sites and 1 common haplotype shared among Taranaki sites. There were 12 missing mutational steps between haplotypes from the North Island and the South Islands. The Australian *Archichauliodes sp* was further separated by 70 missing mutational steps from the South Island of New Zealand. Differentiation was also observed within the South Island with shared haplotypes observed at distances of > 150 km. On this basis, I suggested that despite potentially weak flight, individuals of *A. diversus* can regularly disperse distances of up to 150 km. However, they also appear limited by geographic barriers such as the Cook Strait between the North and South Islands (<25 km). Molecular clock estimates between New Zealand and Australian *Archichauliodes sp.* suggested that isolation of individuals occurred around 5 Mya and isolation between the North and South *A. diversus* occurred within the last 2 Mya; corresponding with the Pliocene and Pleistocene, respectively. On the basis of both studies, I concluded that COI gene sequences can provide a useful method for assessing the genetic similarity of aquatic insect populations on both small (<50km) and large (>1000km) spatial scales and that these data can then be used to test ecological and evolutionary hypotheses.

ACKNOWLEDGEMENTS

First and foremost I would like to thank my supervisor A. Prof Ian Hogg. You have been incredibly supportive, your patience and enthusiasm have kept me going. I couldn't have asked for a better supervisor. You pushed me when I needed a push and picked me up when I was down. For the late night emailing of manuscripts, the hours of editing and the endless support, I thank you. I'd further like to thank you for the opportunities you have given me, attending the International Barcode of Life conference in Canada was an incredible experience that I gained so much from. It's not every day you get to discuss your research with some of the biggest names in genetics. For everything you have done for me, I thank you.

I would like to thank Dr. Richard Storey and Brian Smith from National Institute of Atmospheric and Water Research (NIWA), it has been a pleasure working with you both. I thoroughly enjoyed being out in the field with you Brian and your expertise and wealth of knowledge in aquatic invertebrates was well appreciated. Your ability to target different taxa in differing habitats made sampling run smoothly. Richard, thank you for organizing the meetings, making sure I was on track and the direction you gave me throughout my research. Thank you NIWA for the funding, this thesis would not have been possible without it.

To the PBRL lab manager Stacey Meyer, you have been more than a lab manger to me, you have been a friend. I'd like to thank you for all your help in the lab and the emotional support you gave me throughout my research year. To the people of PBRL, especially Clare Beet, Erin Doyle and Gemma Collins, I would like to thank you for all your assistance and logistical support.

Dr. Chrissen Gemmill, I would like to thank you for your support, you were always eager to help with any question at any time. Even if you didn't have an answer you always pointed me in the right direction. Lee Laboyrie, I'd also like to thank you for assisting me with getting my field equipment together, with my supervisor and lab manager away your help was greatly appreciated.

Thank you to the University of Waikato for awarding me the University of Waikato Masters Research Scholarship allowing me to complete my MSc degree.

Last but definitely not least, to my parents, John and Gill Riding. I would like to thank you wholeheartedly for all the support you have given me throughout my years at Waikato University. Words cannot thank you enough for both the emotional and physical support. You both have gone above and beyond to help in every way. Mum, thank you for the endless routine 'walk to university' phone calls and all advice when I needed it. Dad, as your favourite daughter, I'd like to thank you for all your inspiration, your hardworking, dedicated attitude has been and will always be something I look up to. I'd like to thank you both for the opportunities you provided to me at such a young age that have shaped who I am today. The completion of this thesis is as much your achievement as it is mine.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER III

LIST OF FIGURES

Chapter II

individuals represented. Top left figure demonstrates that dispersal is occurring from upstream to downstream.

Figure 5. Haplotype network showing 4 unique haplotypes for *Hydropsyche colonica* (Trichoptera) when separated into upper (Sites 1A, 1B, 2A, 3A 4A) and lower stream sites (Sites 1C, 1D, 2B, 2C, 3B). Size of circles is proportional to the number of individuals represented. Top left figure represents locations for upper and lower sites on a topographical map of Mount Taranaki.

Figure 6. Haplotype networks for individuals of *Pyncocentrodes aeris* (Trichoptera) when separated into upper (Sites 1A, 1B, 2A, 3A 4A) and lower stream sites (Sites 1C, 1D, 2B, 2C, B). Size of circles is proportional to the number of individuals represented. Top left figure represents locations for upper and lower sites on a topographical map of Mount Taranaki.

Figure 7. Hypothetical dispersal of *Pyncocentrodes aeris* (Trichoptera) among sites based on the distribution of a unique haplotype.

Chapter III

the number of individuals of each haplotype. The numbers between haplotype groups are the number of mutational steps missing between the respective haplotypes.

Figure 7: Phylogeographic distribution of haplotypes from sites on the North and South Island New Zealand

Figure 8: Maximum likelihood phylogeny showing the distribution of unique haplotypes at the North and the South Island sites.

Figure 9: Hypothetical dispersal of the Cook Strait during the Pliocene (5 Mya) and Pleistocene glaciations (2 Mya). **A**) Suggests *A. diversus* populations might be allopatric on older Islands before moving between Island through oversea dispersal during the Pliocene, before remaining specific to the two Islands (North Island and South Island) following the LGM. **B**) Suggests that one population of *A. diversus* exists on one Island, expanding the range (Cook Strait land bridge) during the LGM and then becoming separated as sea level rose. Figures adapted from Trewick et al. (2011).

LIST OF APPENDENDICS

CHAPTER I

Thesis Introduction

Aquatic insects are key components in freshwater ecosystems providing key trophic links between the primary producers and secondary consumers (Cummins, 1973; Kleine et al. 2005). The majority of their life cycle is spent as nymphs or larvae in the aquatic realm (McCafferty et al. 1983) and consequently, they are often used as sensitive indicators of environmental quality. The adult (reproductive) life stage usually occurs within the terrestrial realm and can last from as long as a few hours (e.g. mayflies) to several weeks (e.g. stoneflies, caddisflies). The dispersal of aquatic insects drives species' distributions and links populations across geographic boundaries within and among freshwater environments (Malmqvist, 2002). Within lotic habitats (streams and rivers), aquatic insects can disperse as nymphs or larvae by walking/crawling upstream or downstream via active, or passive drift in the water current (Müller, 1982; Humphries et al. 2002; Parkyn et al. 2011). Among-habitat dispersal is primarily achieved by the winged adults, although "accidental" carriage by birds is also possible (Green et al. 2005). Accordingly, dispersal abilities can influence species' distributions as well as community composition within aquatic habitats. Although mechanisms of dispersal for aquatic insects are relatively well understood, there is still a need for data that directly examines inter-habitat dispersal (Templeton et al. 1990; Alp et al. 2012; Tonkin et al. 2014). Unfortunately, dispersal in freshwater taxa is difficult to study directly meaning rare but significant dispersal events may remain undetected (Bilton et al. 2001). Understanding the effects of connectivity within and among populations is essential for determining the effects of both natural and anthropogenic barriers among ecosystems (Hughes et al. 2008). Previously, research inferring dispersal has used malaise traps to estimated dispersal of aquatic insects (e.g. Petersen et al.

2004). Mark and recapture studies using stable isotopes have also been used to estimate dispersal (e.g. Macneale et al. 2005). The application of molecular techniques such as allozyme and DNA sequence based analyses have provided an alternative approach to examine population connectivity and frequency of dispersal events (Williams et al. 2003; Hughes et al. 2011). Genetic markers such as allozymes have been used extensively to assess population genetic structures for a number of aquatic insects (e.g. Scribner et al. 1998; Monaghan et al. 2002; Hogg et al. 2002; Hughes, 2007). More recently, DNA-based approaches such as analyses of microsatellite loci and mitochondrial gene sequences have been used (e.g. Mesnick et al. 2010; Bock et al. 2011; Finn et al. 2011). Mitochondrial genes have a relatively low mutation rate and have provided a useful method for assessing species and genetic diversity (Smith et al. 2002). In particular, sequence fragments of the mitochondrial cytochrome c oxidase subunit I (COI) allow for species-level identification while providing enough variability to detect genetic diversity within natural populations of the same species through subtle variation among sequences (Hebert et al. 2003; Hughes et al. 2011). For example, sequence fragments from the COI gene have been used to examine the effects of habitat fragmentation on large biogeographic scales (Macey et al. 1998; Vandergast et al. 2004). Variation in the level of mtDNA diversity may be used to infer population expansion from an area of high diversity towards one of low diversity and suggests that the CO1 gene could be useful for studying the dispersal patterns of aquatic insects (Trewick et al. 2011). This variation among COI sequences can be assessed using a variety of methods including phylogenetic trees and haplotype networks. Phylogenetic trees can be constructed using several methods including Neighbour Joining (NJ), Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian analyses. Neighbour joining trees provide a straightforward method

using distance data while ML, MP and Bayesian analyses use more complex evolution models for phylogenetic analysis (Saitou et al. 1987). Sequences with differences in one or more nucleotides are termed haplotypes (Chung et al. 2003). Haplotype networks provide a graphical representation of the mutational linkages among haplotypes and offer a powerful method for analysing populations and determining the association from which these subtle genetic differences arise (Stephens et al. 2001). Despite the ability of the COI gene to detect genetic diversity within natural populations of the same species, the geographic distances by which variability can be detected is relatively unknown. Accordingly, scale is an important consideration when examining dispersal among habitats. Bergsten et al. (2012) examined the effect of increasing the geographical scale of sampling for the performance of COI gene sequences to distinguish between ecological and historical processes. Based on an assessment of *Agabini* sp. beetles, they concluded that smaller geographical scales deliver higher accuracy when inferring ecological processes (dispersal) and larger geographical scales deliver higher accuracy when inferring historical processes (speciation). In order to improve our understanding of connectivity among natural habitats and to examine the utility of COI sequences for determining potential dispersal, I focused on three aquatic insects, *Archichauliodes diversus* (Megaloptera*), Hydropsyche colonica* and *Pycnocentrodes aeris* (Trichoptera). This thesis consists of two research chapters and focuses on both small (<50km) and large (>1000km) spatial scales. Genetic similarity among locations was used as a measure of gene flow (and hence dispersal) and to examine the effects of both recent (small scale) and historical (large scale) historical habitat fragmentation.

The first research chapter (Chapter II) investigates connectivity of sites within four streams representing both source and restored streams in the Taranaki Region of New Zealand. Selection of targeted insect species was based on varying levels of adult dispersal (flight) capability and larval drift propensity: *Archichauliodes diversus* is considered to have moderate levels of larval in-stream drift potential, although poor flight capabilities as an adult (Hogg et al. 2002; Heilviel et al. 2004), whereas both *H. colonica* and *P. aeris* have active flight as adults although they differ slightly in their propensity to drift (Smith et al. 2002; Didham et al. 2012). The free living *H. colonica* form fixed retreats during larval stages and tend to remain in situ even during high flow conditions (Edington, 1968; Elliot, 2003). In contrast, the cased *P. aeris* are stationary filter feeders although subject to some drift particularly during increased stream discharge events (Watson, 1971; Collier et al. 1997). Restoration projects primarily restore habitat, with often little consideration for invertebrate dispersal pathways and/or recovery time (Bond et al. 2002; Parkyn et al. 2011). Furthermore, stream restoration often occurs on smaller spatial scales, resulting in isolated patches of restored habitat such as that found in Taranaki Region, New Zealand. It is recognized that fragmentation and isolation with lower connectivity may result in an extremely slow recovery rate and in some cases the failure to recover (Lake et al. 2007). Even if habitat conditions were suitable, the re-colonisation of aquatic invertebrates will not occur unless there is a potential source population (Hughes, 2007). Accordingly, knowledge of the dispersal pathways between habitats is essential to ensure invertebrate recovery. The purpose of this chapter was to investigate genetic differentiation within source and restored habitats using COI gene sequences. I predicted that dispersal by drift would reduce genetic differentiation within streams and that any dispersal by flight would reduce

genetic differentiation among streams. Further, I predicted that *A. diversus* would show higher levels of genetic differentiation among site locations owing to its potentially limited adult dispersal.

The second research chapter (Chapter III) focuses specifically on *Archichauliodes diversus*. Here, I used COI gene sequences to determine genetic variability among various locations around the North Island and the South Island of New Zealand. I also compared these individuals with available sequences from an Australian congener to determine the level of divergence between New Zealand and Australian *Archichauliodes* species. New Zealand has a dynamic geological past and large scale isolation events have strongly influenced the biogeography of terrestrial and freshwater invertebrates (Winkworth et al. 2005; Hogg et al. 2006; Pratt et al. 2008; Trewick et al. 2011). Despite isolation of New Zealand's landmass from Australia dating back around 80 Mya, research has suggested that insect species assemblages in New Zealand originated as recently as the Pliocene 5 Mya (Winkworth et al. 2005; Goldberg et al. 2008). It was during this time that New Zealand experienced significant climatic fluctuations and extensive crustal uplift resulting in the formation of the Southern Alps, the Taupo Volcanic Zone and land extension between the North and the South Island (Suggate, 1990). Large glacial and interglacial oscillations resulted in an extension of the Cook Strait 'land bridge' periodically connecting the North and South Islands of New Zealand (Proctor et al. 1989; Alloway et al, 2007; Boyer et al. 2009). As new habitat became available, long-distance dispersal between the North and the South Islands would have been possible. Towards the end of the last glacial maximum (LGM) sea levels rose and high tidal amplitudes caused a breach in the Cook Strait 'land bridge' separating the North Island and the South Island (Lewis et al.

1994; Alloway et al. 2007; Marske et al. 2009). The geographic isolation of populations is a well understood mechanism of speciation (Mayr, 1940) and a species' dispersal abilities will influence genetic differentiation among populations and ultimately speciation (Shafer et al. 2013). In this chapter, I obtained COI sequences for *Archichauliodes diversus* and used phylogeographic methods to assess divergence over a large spatial scale (>1000km).

The final chapter of my thesis provides a summary and conclusion of the findings within my two research chapters and suggests potential avenues for future research.

LITERATURE CITED

Alloway, B.V., Lowe, D.J., Barrell, D.J.A., Newnham, R.M., Almond, P.C., Augustinus, P.C., Bertler, N.A.N., Carter, L., Litchfield, N.J., McGlone, M.S., Shulmeister, J., Vandergoes, M.J and Williams, P. W. 2007. Towards a climate event stratigraphy for New Zealand over the past 30 000 years (NZ-INTIMATE project). Journal of Quaternary Science. **22**(1): 9-35. doi:10.1002/jqs.1079.

Alp, M., Keller, I., Westram, A. M and Robinson, C.T. 2012. How river structure and biological traits influence gene flow: A population genetic study of two stream invertebrates with differing dispersal abilities. Freshwater Biology. **57**(5): 969-981. doi:10.1111/j.1365-2427.2012.02758.x.

Bergsten, J., Bilton, D.T., Fujisawa, T., Elliott, M., Monaghan, M.T., Balke, M., Hendrich, L., Geijer, J., Herrmann, J., Foster, G.N, Ribera, I., Nilsson, A.N., Barraclough, T.G and Vogler, A.P. 2012. The effect of geographical scale of sampling on DNA barcoding. Systematic Biology. **61**(5): 851-869. doi:10.1093/sysbio/sys037.

Bilton, D.T., Freeland, J.R and Okamura, B. 2001. Dispersal in freshwater invertebrates Annual Review of Ecology and Systematics. **32**(1): 159-181. doi:10.1146/annurev.ecolsys.32.081501.114016.

Bock, D.G., Zhan, A., Lejeusne, C., MacIsaac, H.J and Cristescu, M.E. 2011. Looking at both sides of the invasion: Patterns of colonization in the violet tunicate Botrylloides violaceus. Molecular Ecology. **20**(3): 503-516. doi:10.1111/j.1365-294X.2010.04971.x.

Bond N.R. and Lake P.S. 2003. Local habitat restoration in streams: Constraints on the effectiveness of restoration for stream biota. Ecological

Management and Restoration. **4**(3): 193–198. doi: 10.1046/j.1442-8903.2003.00156.x.

Boyer, S.L and Giribet, G. 2009. Welcome back New Zealand: Regional biogeography and gondwanan origin of three endemic genera of mite harvestmen (Arachnida, Opiliones, Cyphophthalmi). Journal of Biogeography. **36**(6): 1084-1099. doi:10.1111/j.1365-2699.2009.02092.x.

Chung, R. H and Gusfield, D. 2003. Perfect phylogeny haplotyper: Haplotype inferral using a tree model. Bioinformatics. **19**(6): 780-781. doi:10.1093/bioinformatics/btg078.

Collier, K.J and Smith, B. J. 1997. Dispersal of adult caddisflies (Trichoptera) into forests alongside three New Zealand streams. Hydrobiologia. **361**(1). 53-65. doi:10.1023/A:1003133208818.

Cummins, K.W. 1973. Trophic relations of aquatic insects. Annual Review of Entomology. **18**: 183-206. doi: 10.1146/annurev.en.18.010173.001151.

Didham, R.T., Rapheal, K., Blakely, T.J., Tanya, J., Ewers, R.M., Hitchings, T.R., Ward, J.B and Winterbourn, M.J. 2012. Horizontal and vertical structuring in the dispersal of adult aquatic insects in a fragmented landscape. Fundamental and Applied Limnology. **180**(1): 27-40. doi: http://dx.doi.org/10.1127/1863-9135/2012/0243.

Edington, J.M. 1968. Habitat preferences in net-spinning caddis larvae with special reference to the influence of water velocity. Journal of Animal Ecology. **37**(3). 675-692.

Elliot, J.M. 2003. A comparative study of the dispersal of 10 species of stream invertebrates. Freshwater Biology. **48**(9): 1652-1668. doi: 10.1046/j.1365-2427.2003.01117.x.

Finn, D.S., Bonada, N., Múrria, C and Hughes, J.M. 2011. Small but mighty: Headwaters are vital to stream network biodiversity at two levels of organization. Journal of the North American Benthological Society. **30**(4), 963-980. doi:10.1899/11-012.1.

Goldberg, J., Trewick, S. A and Paterson, A.M. 2008. Evolution of New Zealand's terrestrial fauna: A review of molecular evidence. Philosophical Transactions of the Royal Society B: Biological Sciences. **363**(1508): 3319-3334. doi:10.1098/rstb.2008.0114.

Green, A. J and Figuerola, J. 2005. Recent advances in the study of long-distance dispersal of aquatic invertebrates via birds: Dispersal of invertebrates via waterbirds. Diversity and Distributions. **11**(2): 149-156. doi:10.1111/j.1366-9516.2005.00147.x.

Hebert, P.D.N., Cywinska A and Ball S.L. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B. Biological Sciences. **270**: 313-321.

Heilviel, S.J. 2004. Mating Habits, Dispersal and Postglacial Range Expansion of Nigronia serricornis (Say) (Megaloptera; Corydalidae). Ph.D. Thesis, Department of Entomology, University of Illinois at Urbana-Champaign, Illinois, United States.

Hogg, I., Willmann-Huerner, P and Stevens, M. 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**(3): 491-501. doi:10.1080/00288330.2002.9517104.

Humphries, S. 2002. Dispersal in drift-prone macroinvertebrates: A case for density-independence. Freshwater Biology. **47**(5): 921-929. doi:10.1046/j.1365-2427.2002.00819.x.

Hughes, J. M. 2007. Constraints on recovery: Using molecular methods to study connectivity of aquatic biota in rivers and streams. Freshwater Biology. **52**(4): 616-631. doi:10.1111/j.1365-2427.2006.01722.x.

Hughes J.M., Huey, J, A., McLean, A, J and Baggiano, O. 2011. Aquatic insects in Eastern Australia: A window on ecology and evolution of dispersal in streams. Insects. **2**(4): 447-461; doi:10.3390/insects2040447.

Hughes, J.M, Schmidt, D., McLean, A. J and Wheatley, A. 2008. Population genetic structure in stream insects: What have we learned? Aquatic insects. (Challenges to populations, Proceedings of the Royal Entomological Society's 24th Symposium. 268-288).

Kleine, P and Trivinho-Strixino, S. 2005. Chironomidae and other aquatic macroinvertebrate of a first order stream: Community response after habitat fragmentation. Acta Limnologica Brasiliensia. **17**(1): 81–90.

Lake, P.S., Bond, N and Reich, P. 2007. Linking ecological theory with stream restoration. Freshwater Biology. **52**(4): 597-615. doi:10.1111/j.1365-2427.2006.01709.x.

Lewis, K.B., Carter, L., and Davey, F.J. 1994. The opening of Cook Strait: Interglacial tidal scour and aligning basins at a subduction to transform plate edge. Marine Geology. **116**(3): 293-312. doi:10.1016/0025-3227(94)90047-7.

Parkyn, S.M., Davies-Colley, R.J., Halliday, N.J., Costley, K.J and Croker, G. F. 2003. Planted riparian buffer zones in New Zealand: Do they live up to

expectations? Restoration Ecology. **11**(4): 436-447. doi:10.1046/j.1526-100X.2003.rec0260.x.

Parkyn, S. M and Smith, B. J. 2011. Dispersal constraints for stream invertebrates: Setting realistic timescales for biodiversity restoration. Environmental Management. **48**(3): 602-614. doi:10.1007/s00267-011-9694-4.

Petersen, I., Masters, Z., Hildrew, A.G and Ormerod, S.J. 2004. Dispersal of adult aquatic insects in catchments of differing land use. Journal of Applied Ecology. **41**(5): 934-950. doi: 10.1111/j.0021-8901.2004.00942.x.

Pratt, R. C., Morgan-Richards, M and Trewick, S. A. 2008. Diversification of New Zealand weta (orthoptera: Ensifera: Anostostomatidae) and their relationships in Australasia .Philosophical Transactions of the Royal Society B: Biological Sciences. **363**(1508): 3427-3437. doi:10.1098/rstb.2008.0112.

Proctor, R and Carter, L. 1989. Tidal and sedimentary response to the Late Quaternary closure and opening of Cook Strait, New Zealand: Results from numerical modelling. Paleoceanography. **4**(2): 167-180. doi: 10.1029/PA004i002p00167.

Saitou, N and Tadashi, I. 1989. Relative efficiencies of the Fitch-Margoliash, Maximum Parsimony, Maximum-Likelihood, Minimum-Evolution, and Neighbour-joining methods of phylogenetic tree construction in obtaining the correct tree. Molecular Biology and Evolution. **6**(5): 514-525.

Scribner, K.T., Crane, P.A., Spearman, W.J and Seeb, L.W. 1998. DNA and allozyme markers provide concordant estimates of population differentiation: Analyses of U.S. and Canadian populations of Yukon River fall-run chum salmon (Oncorhynchus keta). Canadian Journal of

Fisheries and Aquatic Sciences. **55**(7): 1748-1758. doi:10.1139/cjfas-55-7-1748.

Shafer, A.B.A., Wolf, J.B.W and Wiens, J. 2013. Widespread evidence for incipient ecological speciation: A meta-analysis of isolation-by-ecology. Ecology Letters. **16**(7): 940-950. doi:10.1111/ele.12120.

Smith, J.M. and Smith, N.H. 2002. Recombination in animal mitochondrial DNA. Molecular Biology and Evolution. **19**(12): 2330-2332. doi:10.1093/oxfordjournals.molbev.a004058.

Stephens, M., Smith, N. J and Donnelly, P. 2001. A new statistical method for haplotype reconstruction from population data. The American Journal of Human Genetics. **68**(4): 978-989. doi:10.1086/319501.

Suggate R.P. 1990. Late Pliocene and Quaternary glaciations of New Zealand. Quaternary Science Reviews. **9**(2): 175-197. doi:10.1016/0277-3791(90)90017-5.

Templeton, A. R., Shaw, K., Routman, E and Davis, S. K. 1990. The genetic consequences of habitat fragmentation. Annals of the Missouri Botanical Garden. **77**(1): 13-27. doi:10.2307/2399621.

Tonkin, J. D., Stoll, S., Sundermann, A and Haase, P. 2014. Dispersal distance and the pool of taxa, but not barriers, determine the colonisation of restored river reaches by benthic invertebrates. Freshwater Biology. **59**(9): 1843-1855. doi:10.1111/fwb.12387.

Trewick, S.A, P. Wallis, P.G and Morgan-Richards, M. 2011. The Invertebrate Life of New Zealand: A Phylogeographic Approach. Insects. **2**: 297-325 doi:10.3390/insects2030297.

Macneale, K.H., Peckarsky, B.L and Likens, G.E. 2005. Stable isotopes identify dispersal patterns of stonefly populations living along stream corridors. Freshwater Biology. **50**(7): 1117-1130. doi:10.1111/j.1365-2427.2005.01387.x.

Macey, J.R., Schulte, J.A., Ananjeva, N.B., Larson, A., Rastegar-Pouyani, N., Shammakov, S.M and Papenfuss, T.J. 1998. Phylogenetic relationships among agamid lizards of the Laudakia caucasia species group: Testing hypotheses of biogeographic fragmentation and an area cladogram for the iranian plateau. Molecular Phylogenetics and Evolution. **10**(1): 118-131. doi:10.1006/mpev.1997.0478.

Malmqvist, B. 2002. Aquatic invertebrates in riverine landscapes. Freshwater Biology. **47**(4): 679-694.

Marske, K.A., Leschen, R.A.B., Barker, G.M and Buckley, T.R. 2009. Phylogeography and ecological niche modelling implicate coastal refugia and trans-alpine dispersal of a New Zealand fungus beetle. Molecular Ecology. **18**(24): 5126. doi:10.1111/j.1365-294X.2009.04418.x.

Mayr, E. 1940. Speciation phenomena in birds. The American Naturalist. **74**(752): 249-278. doi:10.1086/280892.

McCafferty, W.P and Provonsha, A,V. 1983. Aquatic entomology: the fisherman's and ecologists' illustrated guide to insects and their relatives. Jones and Bartlett publishers. United States of America.

Mesnick, S.L., Taylor, B.L., Archer, F.I., Martien, K.K., Trevino, S.E., Hancock-Hanser, B.L and Morin, P.A. 2011. Sperm whale population structure in the eastern and central north pacific inferred by the use of single-nucleotide polymorphisms, microsatellites and mitochondrial DNA.

Molecular Ecology Resources. **11**(S1), 278-298. doi:10.1111/j.1755-0998.2010.02973.x.

Monaghan, M.T., Spaak, P., Robinson, C.T and Ward, J.V. 2002. Population genetic structure of three alpine stream insects: Influences of gene flow, demographics, and habitat fragmentation. Journal of the North American Benthological Society. **21**(1): 114-131. doi: 10.2307/1468304.

Müller, K. 1982. The colonization cycle of freshwater insects. Oecologia. **52**(2): 202-207. doi:10.1007/BF00363837.

Vandergast, A. G., Gillespie, R. G and Roderick, G. K. 2004. Influence of volcanic activity on the population genetic structure of Hawaiian Tetragnatha spiders: fragmentation, rapid population growth and the potential for accelerated evolution. Molecular Ecology. **13**(7): 1729-1743. doi:10.1111/j.1365-294X.2004.02179.x.

Watson W.G. 1971. Drift of Stream Invertebrates. Tane. **17**: 97-212.

Williams, B. L., Brawn, J. D. and Paige, K. N. 2003. Landscape scale genetic effects of habitat fragmentation on a high gene flow species: Speyeria idalia (nymphalidae): Genetics of fragmentation in *Speyeria Idalia*. Molecular Ecology. **12**(1): 11-20. doi:10.1046/j.1365-294X.2003.01700.x.

Winkworth, R., Wagstaff, S., Glenny, D and Lockhart P. 2005. Evolution of the New Zealand mountain flora: Origins, diversification and dispersal. Organisms Diversity & Evolution. **5**: 237–247.

CHAPTER II

Using DNA barcodes to assess aquatic insect dispersal among source and restored forest patches on Mount Taranaki, New Zealand

*Submitted under the same title, as: Riding, MA, Hogg ID, Smith BJ, Storey RG

ABSTRACT

The recovery of aquatic invertebrate communities following stream habitat restoration is dependent on connectivity to source populations. Here, we used mitochondrial DNA (COI) sequences to assess the genetic similarity (as a measure of connectivity) for individuals of aquatic insects among 10 sites from four streams representing both source and restored streams in the Taranaki Region, New Zealand. Three common aquatic insect species were targeted based on differences in dispersal capabilities: *Archichauliodes diversus* (Megaloptera; active larvae, poor flight), and *Hydropsyche colonica* and *Pycnocentrodes aeris* (Trichoptera; sessile larvae, strong flight). *A. diversus* showed the greatest sequence diversity (19 haplotypes) followed by *P. aeris* (14 haplotypes) and *H. colonica* (4 haplotypes). Despite relatively high levels of genetic diversity, *A. diversus* was genetically similar throughout the study area, suggesting adequate dispersal among source and restored habitats. Individuals of *H. colonica*, with much lower levels of haplotype diversity, were also genetically similar between source and restored habitats. In contrast, *P. aeris* had one haplotype that was more common in source versus restored habitats. We conclude that taxa were relatively well connected between source and restored habitats and that COI sequences can provide a useful indicator for tracking restoration efforts within relatively small geographic areas.

Key words: aquatic macroinvertebrates, dispersal, Megaloptera, population connectivity, stream restoration, Trichoptera

INTRODUCTION

Riverine ecosystems consist of a mosaic of habitats that are linked through a range of physical and biological processes and support highly diverse biotic communities (Malmqvist, 2002; Lake et al. 2007). Aquatic insects are key trophic components of these systems and are sensitive indicators of environmental integrity (Kleine et al. 2005). Anthropogenic activities in the vicinity of riverine systems have largely resulted in the degradation of riparian flora and loss of lotic fauna habitats which often require remediation (Zwick, 1992; Brederveld et al. 2011). However, following any restoration efforts, the colonisation of restored habitat by aquatic invertebrates is dependent on dispersal/recruitment from a suitable source habitat (Lake et al. 2003).

Aquatic invertebrates vary in their ability to disperse and, even with a winged adult stage, habitat recolonization may be limited despite close proximity to source populations (Bilton et al. 2001; Lake et al. 2007; Hughes, 2007). The ability to disperse is dependent on the mechanism of dispersal (flight and drift) and the proximity of the restored habitats to source populations (Humphries, 2002; Hughes, 2007). As restoration often results in a mosaic of forested patches that vary on both temporal and spatial scales, recovery of aquatic insect populations is often limited by connectivity to source populations (Brederveld et al. 2011). Although the mechanisms of dispersal for aquatic invertebrates are relatively well understood there is still a need for more data directly examining connectivity of restored habitats to potential source populations (Templeton et al. 1990; Tonkin et al. 2014).

Genetic analyses can provide a useful approach for assessing connectivity among natural habitats (Slatkin, 1987; Hughes, 2007). Genetic markers such as allozymes

have previously been used to assess population genetic structure for a wide range of aquatic invertebrates (Scribner et al. 1998; Monaghan et al. 2002; Hughes, 2007). More recently, DNA-based approaches such as microsatellites and mitochondrial DNA have also been used (Mesnick et al. 2010; Bock et al. 2011; Finn et al. 2011). For example, sequence fragments from the cytochrome c oxidase subunit I (COI) gene have been used to examine the effects of habitat fragmentation on large biogeographic scales (Macey et al. 1998; Vandergast, et al. 2004). Moritz and Cicero (2004) found that within many species there was sufficient genetic diversity to allocate individuals to a particular geographic location. Variation in the level of mtDNA diversity may also be used to infer population expansion from an area of high diversity towards one of low diversity (Trewick et al. 2011). This suggests that the CO1 gene could be useful for studying the dispersal patterns of aquatic insects on relatively small spatial scales. Here, we assessed use of the COI gene to assess patterns of within-species genetic variability across a small (<50km) spatial scale. We then used these data to examine genetic similarity among locations to determine connectivity of aquatic insect populations among source and restored fragments. Mount Taranaki on the west coast of New Zealand's North Island provides an ideal environment to study the effects of fragmentation and genetic diversity on a relatively small geographical scale. The mountain is a 2518m high symmetrical volcano (Procter et al. 2010), with a series of "radial" streams flowing from native forest around the summit to a surrounding ring plain of cleared farm land below. Streams that flow through this ring plain are subject to intensive pastoral land use (dairy, sheep and beef farming) and are thus highly modified. There have been extensive restoration efforts including riparian planting (over 1765 km of streams replanted) and stock exclusion fencing along stream margins (3538 km fenced since 1990). This has resulted in a

patchwork of forested, reforested and exposed habitats in the ring plain with varying levels of connectivity to source populations in the higher altitude native forests. We selected three taxa, *Archichaulidoes diversus* (Megaloptera), *Hydropsyche colonica* and *Pycnocentrodes aeris* (Trichoptera) and tested the hypothesis that genetic differentiation would occur among source and restored habitats. We predicted that dispersal by drift would reduce genetic differentiation within streams and dispersal by flight would reduce genetic differentiation among streams. Further, we predicted that *A. diversus* would show higher levels of genetic differentiation among site locations owing to potentially limited adult dispersal, relative to *H. colonica* and *P. aeris*.

MATERIALS AND METHODS

Four streams around Mt Taranaki were selected: Kapuni (Stream 1), Kaupokonui (Stream 2), Punehu (Stream 3) and Waiwhakaiho (Stream 4). Stream locations were chosen on the basis of distance between sites. Stream 1 and Stream 2 were located in close proximity (<4km). Stream 3 was located >25 km from Streams 1 and 2 and Stream 4 was situated on the opposite side of the mountain (>5 km). A total of 10 sites were chosen from the four stream locations (Fig. 1).

We targeted three widespread and common aquatic insect taxa with varying levels of adult dispersal (flight) capability and larval drift propensity: *Archichaulidoes diversus* (Megaloptera), *Hydropsyche colonica* and *Pycnocentrodes aeris* (Trichoptera). *Archichauliodes diversus* is considered to have moderate levels of larval in-stream drift potential, although poor flight capabilities as an adult (Hogg et al. 2002; Heilviel et al. 2004). In contrast, both *H. colonica* and *P. aeris* have active flight as adults although they differ slightly in their propensity to drift (Smith et al. 2002; Didham et al. 2012): the free living *H. colonica* form fixed retreats during larval stages and tend to remain *in situ* even during high flow conditions (Edington, 1968; Elliot, 2003). In contrast, the cased *P. aeris* are stationary filter feeders though subject to some drift particularly during increased stream discharge events (Watson, 1971; Collier et al. 1997). Previous records available on the Barcode of Life Datasystems (BOLD) database (boldsystems.org; Ratnasingham and Hebert, 2007), suggested that all species possessed sufficient sequence diversity (> 4 haplotypes in all cases) to potentially facilitate comparisons among locations.

In-stream sampling of larval insects was conducted 20-22 January 2015 using a standard, 30 cm width, D- net (Stark et al. 2001). Multiple samples were taken

haphazardly from riffle habitats at each site and contents were placed into white 20 x 30 cm sorting trays with water from the same stream. Contents of each sample where then visually examined for target taxa and individuals were removed with forceps and placed directly into 100% ethanol. Sampling continued until roughly 40 individuals for each species were collected from each site. Samples were kept on ice for return to the lab. Once in the lab, ethanol in the samples was replaced with fresh 100% ethanol and samples were stored at 4^{o}C until needed for genetic analyses. Sampling nets and other equipment were physically cleaned to remove all animals/debris and disinfected (using a detergent) between sites.

Once in the lab, species identifications were confirmed with the aid of a dissecting microscope (50X magnification). Up to 15 individuals were selected from each site for each of the three target species (*A. diversus*, *H. colonica* and *P. aeris*). A 4 mm sample of leg tissue was taken from each individual using sterilised (flamed) forceps and added to a single well of a 96-well plate. DNA sequencing was conducted at the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph, Canada following standard protocols (Ivanova et al. 2006). A 658 bp region of the mitochondrial cyctochrome c oxidase subunit I (COI) gene was PCR amplified using the primer pair LepF1 (5' ATTCAACCAATCATAAAGATATTGG-3') and LepR1 (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') (Hebert et al. 2004). Genomic DNA was extracted following the AcroPrepTM PALL Glass Fibre plate method using a total mix of 5 ml insect lysis buffer (0.5 ml of Proteinase K, 20 mg/ml per 96-well plate) (Ivanova et al. 2006). A total of 5 µl of the DNA extraction product was used for PCR. PCR thermal cycling conditions were: initial denaturation of

samples at 94ºC for 1 minute, followed by five cycles of 94ºC for 30 seconds. Annealing occurred at 48°C for 1.5 minutes with the extension occurring at 72°C for 1 minute, 35 cycles of 94°C at 30 seconds, 52°C for 1 minute with the final extension occurring at 72°C for 10 minutes. Successful PCR products were cleaned using Sephadex® and then sequenced using an ABI3730xl DNA analyser. All data have been added to the Barcode of Life Datasystems (BOLD) database (Ratnasingham & Hebert, 2007) under project NZDIS (Dispersal of New Zealand invertebrates).

Sequences were aligned using MUSCLE implemented in Geneious 7.1.9 (Biomatters, Auckland) and trimmed to exclude primer regions. *Archichaulidoes diversus* sequences were further trimmed to 591bp, *Hydropsyche colonica* sequences to 656bp and *Pycnocentrodes aeris* sequences to 654bp. PAUP* 4.0 (Swofford, 2002) was used to identify parsimony-informative sites. Chi square tests implemented in PAUP* 4.0 were used to determine if base frequencies were equal among all sites and among first, second or third codon positions. Neighbour Joining (NJ) trees were constructed using MEGA v5.2.2 (Tamura et al. 2011). As we were interested in the intraspecific differences among sites (rather than evolutionary relationships), we did not employ outgroups which would have compressed the trees and obscured any differences among the individual locations. Haplotype networks for each individual species were produced using TCS v1.2 (Clement et al. 2000), and based on a comparison of source (upper) and restored (downstream) sites. Sites were categorized into upper (Sites 1A, 1B, 2A, 3A, 4A) and lower (Sites 1C, 1D, 2B, 2C, 3B). A pairwise distance matrix was also constructed using MEGA v5.2.2 and used for a Multidimensional Scaling (MDS) analysis. MDS plots were produced using STATISTICA 12 (Statsoft,

2014) to visualize the level of similarity among individuals from the various site locations.

RESULTS

We sequenced 380 individuals and obtained sequences (>500 nucleotides) from 294 for a success rate of 77%. This included 109 *Archichauliodes diversus*, 81 *Hydropsyche colonica*, and 108 *Pycnocentrodes aeris*. Of the 618 nucleotide positions for *A. diversus*, 602 positons were constant and 16 were variable with seven parsimony uninformative and nine parsimony informative. There was an overall A-T base frequency bias of 65.9% (A=27.0%, C=17.4%, G=16.7%, T=38.9%). Base frequencies were homogeneous across sequences at all sites (x^2 =0.53, df=315, p=1.0). Homogeneity was accepted for variable sites (x^2 =19.9, df=315, p=1.0) and parsimony informative sites (x^2 =30.9, df=315, p=1.0). First (x^2 =0.24, df=315, p=1.0) second (x^2 =0.24, df=315, p=1.0) and third (x^2 =0.02, df=315, p=1.0) codon positions were all homogeneous. Nucleotide composition for *H. colonica* (664 nucleotide positions) showed that 418 positions were constant, six were parsimony uninformative and 240 were parsimony informative. A-T base frequency bias was 70.2% (A=30%, C=16.9%, G=12.6%, T=40.2%), and base frequencies were homogenous across sequences (x^2 =58.0, df=354, p=1.0) as were variable sites (x^2 =213.4, df=354, p=1.0) and parsimony informative sites (x^2 =213.9, df=354, p=1.0). Sequences were homogeneous at first (x^2 =85.9, df=354, p=1.0) second (x^2 =93.3, df=354, p=1.0) and third (x^2 =, df=354, p=1.0) codon positions. Of the 657 characters for *P. aeris,* 642 were constant with eight parsimony uninformative and seven parsimony informative. The A-T base frequency bias was 70.6% (A=30.3%, C=15.7%, G=13.7%, T=40.3%). Base frequencies were homogeneous across all sequences and sites (x^2 =2.4, df=309, p=1.0). Homogeneity was also found for both variable sites (x^2 =61.2, df=309, p=1.0) and parsimony informative sites (x^2 =117.1 df=309, p=1.0)

and at all codon positions (first: x^2 =2.5 df=309, p=1.0; second: x^2 =0.4.1 df=309, p=1.0; third: x^2 =35.2 df=309, p=1.0).

The NJ tree for the 109 *A. diversus* sequences showed evidence for intraspecific variation over our small geographic sampling range (Fig. 3A). Within-species divergences averaged 0.71% (range 0.23%-0.98%) and three branches were observed on the NJ tree. However, there were no obvious differences related to the various site locations (e.g. different streams or source versus restored habitats) (Fig. 3A). For *H. colonica*, pairwise sequence divergences for the 81 individuals ranged from 0.0%-0.17%. The NJ tree showed two branches also with no obvious relationship with sites (Fig. 3B). Pairwise divergences for the 104 *P. aeris* sequences ranged from 0.0% - 1.9%. The maximum divergence (1.9%) resulted from a single sequence at Site 2C, and all other pairwise divergences were < 0.9%. The NJ tree showed four branches, the first of which corresponded to a single individual from Site 2C. The second branch included individuals found primarily at the upper sites with the exception of one individual from Site 3B. Ten individuals from this branch were found only at Site 4A. The third branch included the upper and lower sites at Stream 1 and Stream 2.The fourth branch included the majority of individuals and had representatives from all site locations except Site 4A (Fig. 3C).

From the 109 *A. diversus* sequences, we recovered 19 unique haplotypes which was highest of the three species. There was one common haplotype and several less common haplotypes all distributed evenly among the upper (Sites 1A, 1B 2A, 3A, 4A) and lower sites (Sites 1C, 1D, 2B 2C, 3B) (Fig. 5; Fig. S1). The haplotype network for *H. colonica* showed a common haplotype shared among most sites and no obvious differences relative to site locations (Fig. 6; Fig. S2). For the 104 *P. aeris* sequenced individuals, there was a common haplotype shared

among upper and lower sites as well as a haplotype from Site 4A that was shared with individuals from other upper sites (sites 1A, 2A) and at one lower site (Site 3B) (Fig. S3).

The MDS plots of pairwise sequence divergences for *A. diversus* showed some within-species differences and a large central cluster representing the majority of individual divergences (n=72) with 14 additional clusters outside the central cluster. There was some differentiation apparent among upper and lower sites. For example, there were five clusters associated with upper sites and five clusters associated with the lower sites. The remaining four clusters contained individuals from both upper and lower site locations (Fig. S4). The MDS plot for *H. colonica* showed three distinct clusters and showed little differentiation between upper and lower sites. However, two clusters corresponded to individuals within the same stream. Cluster 1 contained individuals found at upper and lower sites from Stream 1 (n=2) and Cluster 2 contained individuals from the upper and lower sites from Stream 2 (n=3) (Figure S5). MDS plots for *P. aeris* showed a large cluster containing 81 individuals representative of all sites. There were five clusters which showed some differentiation between upper and lower sites. Two clusters were specific to upper sites and one specific to lower sites and one cluster was specific to Stream 1 (2 individuals). The remaining cluster containing 18 individuals was shared between upper and lower locations. Here, 89% of the individuals represented the upper (source) locations and of these 65% were specific to Site 4A; only 11% were from lower sites (Fig. S6).

DISCUSSION

Our COI analyses of three New Zealand macroinvertebrate species have shown that COI gene sequences can provide a useful indicator of genetic differentiation across a relatively small spatial scale and can also be applied to assess connectivity among source and restored habitats. *Archichauliodes diversus* showed the greatest within-species diversity over the small geographic range. Although A. *diversus* was expected to show higher levels of genetic differentiation among locations (owing to weaker flight capabilities as an adult e.g. Hogg et al. 2002), we found no obvious patterns of differentiation among our sites. This suggests that dispersal was occurring among local sites. Mechanisms maintaining the genetic diversity of *A. diversus* over such a small geological area may include volcanic events over the last 135 000 years which have created a dynamic and fractured landscape (McGlone et al. 1988). This would promote and maintain genetic variability among small isolated populations. For example, Vandergast et al. (2004) found that patterns of neutral genetic variation reflected patterns of volcanic activity for some *Tetragnatha* (Arachnidae) species. Trewick et al. (2011) suggested that the effect of volcanic activity in the central North Island Taupo Volcanic Zone in New Zealand less than 2000 years ago may have obscured and overwritten previous population genetic structures for insect species. Mount Taranaki remains an active volcano with a rough periodicity of 300–500 years between larger-scale eruptions (Turner et al. 2008). Accordingly, it is possible that this dynamic environment could maintain the within-species diversity we found for *A. diversus* as well as 'overwrite' any previous population genetic structures. Thus, we suggest that within-species diversity shown by *A.*

diversus is due to relatively recent gene flow from previously isolated populations.

Hydropsyche colonica showed limited COI diversity within our study area. However, all sites were genetically similar suggesting *H. colonica* was dispersing among the sites. Monaghan et al. (2002) also found that the caddisfly *Allogamus auricollis* (Limnephilidae) did not display genetic differentiation among site locations and suggested that dispersal was occurring throughout the geographic range of their study (<100 km). Lower genetic diversity is expected within smaller populations due to the effects of genetic drift (Lacy, 1987; Templeton et al. 1990, Watanabe et al. 2008). It is possible that the *H. colonica* populations were comparatively small and restricted to a single source population isolated in the forested national park above the ring plain of the mountain. Trewick et al (2011) suggested that low diversity within areas of fairly homogeneous habitat can occur as a result of smaller populations. Gene flow as a consequence of range expansion to restored habitats would still reflect the low diversity. Our data therefore suggest that individuals of *H. colonica* are dispersing well within and among the restored streams.

For *Pycnocentrodes aeris*, we found evidence for some genetic differentiation between upper and lower sites locations suggesting that dispersal may be limited among some sites. For example, a single haplotype found most commonly at Site 4A was predominantly associated with other higher altitude sites. This suggests that there may be some isolation or selection limiting this haplotype to the upper (source) locations. Petersen et al. (2004) described vegetative corridors as being the main 'highways' for dispersal. Accordingly, it is possible that the boundary of the forested national park was providing a corridor which facilitated dispersal among the upper sites on the mountain. However, this haplotype was also found

(albeit in low numbers) at the lower sites of Streams 2 and 3 suggesting larval drift and/or adult flight between sites 2A and 2B and flight between Sites 2B and 3B. We hypothesize dispersal pathways for *P. aeris* is by flight from Sites 4A to 1A to 2A followed by drift or flight to Site 2B and flight to Site 3B (see Fig 7). Bergsten et al. (2012) examined the effect of increasing the geographical scale of sampling for the performance of DNA barcoding to distinguish between ecological and historical processes. Based on an assessment of *Agabini* beetles, they concluded that smaller geographical scales deliver higher accuracy when inferring ecological processes (dispersal). Within our study we found that COI analyses can provide a useful indicator of genetic differentiation and a proxy for dispersal across a relatively small spatial scale (<50km). However, we caution that application of the COI gene may be limited to particular taxa. For example, we had originally chosen *Coloburiscus humeralis* as a target taxon. Unfortunately, we were unable to obtain reliable sequences from these individuals likely due to our choice of primers – a methodological issue that could be resolved with ongoing research. Further, some species may have little or no variability for COI gene sequences, which would then limit the ability to assess any genetic differences among locations. However, for the three species we ultimately used in the study, the COI gene provided sufficient genetic variability in order to assess differences among locations. On this basis, we conclude that COI gene sequences can provide a useful indicator of genetic similarity within relatively small geographic areas and that these data can be used to track dispersal among source and restored habitats

ACKNOWLEDGEMENTS

We are grateful to the New Zealand National institute of Water and Atmospheric Research (NIWA) for funding, the University of Guelph and Canadian Centre for DNA Barcoding for sequencing, and M. de Wit and N. Groot for assistance with sampling. C. Fowles and G. Hughes, from Taranaki Regional Council provided helpful advice as well as the catchment restoration GIS maps. G. Priovolos assisted with mapping.

LITERATURE CITED

Bergsten, J., Bilton, D.T., Fujisawa, T., Elliott, M., Monaghan, M.T., Balke, M., Hendrich, L., Geijer, J., Herrmann, J., Foster, G.N, Ribera, I., Nilsson, A.N., Barraclough, T.G and Vogler, A.P. 2012. The effect of geographical scale of sampling on DNA barcoding. Systematic Biology. **61**(5): 851-869. doi:10.1093/sysbio/sys037.

Bilton, D.T., Freeland, J.R and Okamura, B. 2001. Dispersal in freshwater invertebrates Annual Review of Ecology and Systematics. **32**(1): 159-181. doi:10.1146/annurev.ecolsys.32.081501.114016.

Brederveld, R.J., Jähnig, S.C., Lorenz, A.W., Brunzel, S and Soons, M.B. 2011. Dispersal as a limiting factor in the colonization of restored mountain streams by plants and macroinvertebrates. Journal of Applied Ecology. **48**(5): 1241-1250. doi:10.1111/j.1365-2664.2011.02026.x.

Bock, D.G., Zhan, A., Lejeusne, C., MacIsaac, H.J and Cristescu, M.E. 2011. Looking at both sides of the invasion: Patterns of colonization in the violet tunicate *Botrylloides violaceus*. Molecular Ecology. **20**(3): 503-516. doi:10.1111/j.1365-294X.2010.04971.x.

Collier, K.J and Smith, B. J. 1997. Dispersal of adult caddisflies (Trichoptera) into forests alongside three New Zealand streams. Hydrobiologia. **361**(1): 53-65. doi:10.1023/A:1003133208818.

Clement, M., Posada, D and Crandall, K. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology. **9**(10): 1657-1660.

deWaard, J,R., Ivanova, N,V., Hajibabaei, M and Hebert, P.D.N. 2008. Assembling DNA barcodes. Methods in Molecular Biology. **410**: 275-294.

Didham, R.T., Rapheal, K., Blakely, T.J., Tanya, J., Ewers, R.M., Hitchings, T.R., Ward, J.B and Winterbourn, M.J. 2012. Horizontal and vertical structuring in the dispersal of adult aquatic insects in a fragmented landscape. Fundamental and Applied Limnology. **180**(1): 27-40. doi: http://dx.doi.org/10.1127/1863-9135/2012/0243.

Edington, J.M. 1968. Habitat preferences in net-spinning caddis larvae with special reference to the influence of water velocity. Journal of Animal Ecology. **37**(3). 675-692.

Elliot, J.M. 2003. A comparative study of the dispersal of 10 species of stream invertebrates. Freshwater Biology. **48**(9): 1652-1668. doi: 10.1046/j.1365-2427.2003.01117.x.

Hebert, P.D.N., Cywinska, A., Ball, S.L and deWaard, J,R. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences. **270**(1512): 313–321.

Finn, D.S., Bonada, N., Múrria, C and Hughes, J.M. 2011. Small but mighty: Headwaters are vital to stream network biodiversity at two levels of organization. Journal of the North American Benthological Society. **30**(4): 963-980. doi:10.1899/11-012.1.

Hebert, P.D.N., Penton, E.H., Burns, J., Janzen, D.H., and Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly, *Astraptes fulgerator*. Proceedings of the National Academy of Sciences. **101**(41): 14812-14817.

Heilviel, S.J. 2004. Mating Habits, Dispersal and Postglacial Range Expansion of *Nigronia serricornis* (Say) (Megaloptera; Corydalidae). Ph.D. Thesis, Department of Entomology, University of Illinois at Urbana-Champaign, Illinois, United States.

Hogg, I., Willmann-Huerner, P and Stevens, M. 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**(3): 491-501. doi:10.1080/00288330.2002.9517104.

Humphries, S. 2002. Dispersal in drift-prone macroinvertebrates: A case for density-independence. Freshwater Biology. **47**(5): 921-929. doi:10.1046/j.1365-2427.2002.00819.x.

Hughes, J.M. 2007. Constraints on recovery: Using molecular methods to study connectivity of aquatic biota in rivers and streams. Freshwater Biology. **52**(4): 616-631. doi:10.1111/j.1365-2427.2006.01722.x.

Ingvarsson, P.K. 1997. The effect of delayed population growth on the genetic differentiation of local populations subject to frequent extinctions and recolonizations. Evolution. **51**(1): 29-35.

Ivanova, N.V., Dewaard, J.R and Hebert, P.D.N. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology. **6**(4): 998–1002.

Kleine, P and Trivinho-Strixino, S. 2005. Chironomidae and other aquatic macroinvertebrate of a first order stream: Community response after habitat fragmentation. *Acta Limnologica Brasiliensia*. **17**(1): 81–90.

Lacy, R.C. 1987. Loss of genetic diversity from managed populations: Interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology. **1**(2): 143-158. doi:10.1111/j.1523-1739.1987.tb00023.x.

Lake, P.S., Bond, N., and Reich, P. 2007. Linking ecological theory with stream restoration. Freshwater Biology. **52**(4): 597-615. doi:10.1111/j.1365-2427.2006.01709.

Macey, J.R., Schulte, J.A., Ananjeva, N.B., Larson, A., Rastegar-Pouyani, N., Shammakov, S.M and Papenfuss, T.J. 1998. Phylogenetic relationships among agamid lizards of the *Laudakia caucasia* species group: Testing hypotheses of biogeographic fragmentation and an area cladogram for the iranian plateau. Molecular Phylogenetics and Evolution. **10**(1): 118-131. doi:10.1006/mpev.1997.0478.

McGlone, M.S., Neall, V.E and Clarkson, B. D. 1988. The effect of recent volcanic events and climatic changes on the vegetation of Mt Egmont (Mt Taranaki), New Zealand. New Zealand Journal of Botany. **26**(1): 123-144. doi:10.1080/0028825X.1988.10410105.

Malmqvist, B. 2002. Aquatic invertebrates in riverine landscapes. Freshwater Biology. **47**(4): 679-694. doi:10.1046/j.1365-2427.2002.00895.x.

Mesnick, S.L., Taylor, B.L., Archer, F.I., Martien, K.K., Trevino, S.E., Hancock-Hanser, B.L and Morin, P.A. 2011. Sperm whale population structure in the eastern and central north pacific inferred by the use of single-nucleotide polymorphisms, microsatellites and mitochondrial DNA. Molecular Ecology Resources. **11**(S1): 278-298. doi:10.1111/j.1755-0998.2010.02973.x.

Monaghan, M.T., Spaak, P., Robinson, C.T and Ward, J.V. 2002. Population genetic structure of three alpine stream insects: Influences of gene flow, demographics, and habitat fragmentation. Journal of the North American Benthological Society. **21**(1): 114-131. doi: 10.2307/1468304.

Moritz, C and Cicero, C. 2004. DNA barcoding: Promise and pitfalls. PLoS Biology, **2**(10): 1529-1531. doi:10.1371/journal.pbio.0020354.

Palmer, M. A., Ambrose, R. F and Poff, N. L. 1997. Ecological theory and community restoration ecology. Restoration Ecology. **5**(4): 291-300. doi:10.1046/j.1526-100X.1997.00543.x.

Parkyn, S.M and Smith, B.J. 2011. Dispersal constraints for stream invertebrates: Setting realistic timescales for biodiversity restoration. Environmental Management. **48**(3): 602-614. doi:10.1007/s00267-011-9694-4.

Pearson, W.D and Kramer, R.H. 1972. Drift and production of two aquatic insects in a mountain stream. Ecological Monographs. **42**(3): 365-385. doi:10.2307/1942214.

Petersen, I., Masters, Z., Hildrew, A. G and Ormerod, S. J. 2004. Dispersal of adult aquatic insects in catchments of differing land use. Journal of Applied Ecology. **41**(5): 934-950. doi:10.1111/j.0021-8901.2004.00942.x.

Procter, J.N., Cronin, S.J., Platz, T., Patra, A., Dalbey, K., Sheridan, M and Neall, V. 2010. Mapping block-and-ash flow hazards based on titan 2D simulations: A case study from Mt. Taranaki, NZ. Natural Hazards. **53**(3): 483-501. doi:10.1007/s11069-009-9440-x.

Ratnasingham, S and Hebert, P. D. N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology. **7**: 355-364. doi: 10.1111/j.1471-8286.2006.01678.x.

Scribner, K.T., Crane, P.A., Spearman, W.J and Seeb, L.W. 1998. DNA and allozyme markers provide concordant estimates of population differentiation: Analyses of U.S. and Canadian populations of Yukon River fall-run chum salmon (*Oncorhynchus keta*). Canadian Journal of

Fisheries and Aquatic Sciences. **55**(7): 1748-1758. doi:10.1139/cjfas-55-7-1748.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science. **236**(4803): 787-792. doi:10.1126/science.3576198.

Smith. B.J., Collier, K.J and Halliday, J.N. 2002. Composition and flight periodicity of adult caddisflies in New Zealand hill-country catchment of contrasting land use. New Zealand Journal of Marine and Freshwater Research. **39**(4): 863-878. doi:10.1080/00288330.2002.9517138.

Statsoft. 2014. Multidimensional Scaling. DELL Textbook. Tulsa, Oklahoma. Available from http://www.statsoft.com/Textbook/Multidimensional-Scaling.

Stark, J., Boothroyd, I., Harding, J., Maxted, J and Scarsbrook, M. 2001. Protocols for sampling macroinvertebrates in wadeable streams. New Zealand Macroinvertebrate Working Group Report no.1 ISSN 1175-7791.

Swofford, D. 2002. PAUP*: phylogenetic analysis using parsimony, version 4.0b10 for Macintosh. Sinauer Associates Inc Publishers, Sunderland.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology Evolution. **28**(10): 2731–2739. doi:10.1093/molbev/msr121.

Templeton, A. R., Shaw, K., Routman, E and Davis, S. K. 1990. The genetic consequences of habitat fragmentation. Annals of the Missouri Botanical Garden. **77**(1): 13-27. doi:10.2307/2399621.

Tonkin, J. D., Stoll, S., Sundermann, A and Haase, P. 2014. Dispersal distance and the pool of taxa, but not barriers, determine the colonisation of

restored river reaches by benthic invertebrates. Freshwater Biology. **59**(9): 1843-1855. doi:10.1111/fwb.12387.

Trewick, A, S; P. Wallis, P, G and Morgan-Richards, M. 2011. The invertebrate life of New Zealand: a Phylogeographic approach. Insects. **2**: 297-325 doi:10.3390/insects2030297.

Turner, M.B., Cronin, S.J., Bebbington, M.S and Platz, T. 2008. Developing probabilistic eruption forecasts for dormant volcanoes: A case study from Mt Taranaki, New Zealand. Bulletin of Volcanology. **70**(4): 507-515. doi:10.1007/s00445-007-0151-4.

Vandergast, A. G., Gillespie, R. G and Roderick, G. K. 2004. Influence of volcanic activity on the population genetic structure of Hawaiian *Tetragnatha* spiders: fragmentation, rapid population growth and the potential for accelerated evolution. Molecular Ecology. **13**(7): 1729-1743. doi:10.1111/j.1365-294X.2004.02179.x.

Watson W.G. 1971. Drift of Stream Invertebrates. *Tane*. **17**: 97-212.

Wiens, J.A. 1989. Spatial scaling in ecology. Functional Ecology. **3**(4): 385-397. doi:10.2307/2389612.

Zwick, P. 1992. Stream habitat fragmentation — a threat to biodiversity. Biodiversity and Conservation. **1**(2): 80-97. doi:10.1007/BF00731036.

LIST OF FIGURES

individuals represented. Top left figure demonstrates that dispersal is occurring from upstream to downstream.

Figure 5. Haplotype network showing 4 unique haplotypes for *Hydropsyche colonica* (Trichoptera) when separated into upper (Sites 1A, 1B, 2A, 3A 4A) and lower stream sites (Sites 1C, 1D, 2B, 2C, 3B). Size of circles is proportional to the number of individuals represented. Top left figure represents locations for upper and lower sites on a topographical map of Mount Taranaki.

Figure 6. Haplotype networks for individuals of *Pyncocentrodes aeris* (Trichoptera) when separated into upper (Sites 1A, 1B, 2A, 3A 4A) and lower stream sites (Sites 1C, 1D, 2B, 2C, B). Size of circles is proportional to the number of individuals represented. Top left figure represents locations for upper and lower sites on a topographical map of Mount Taranaki.

Figure 7. Hypothetical dispersal of *Pyncocentrodes aeris* (Trichoptera) among sites based on the distribution of a unique haplotype.

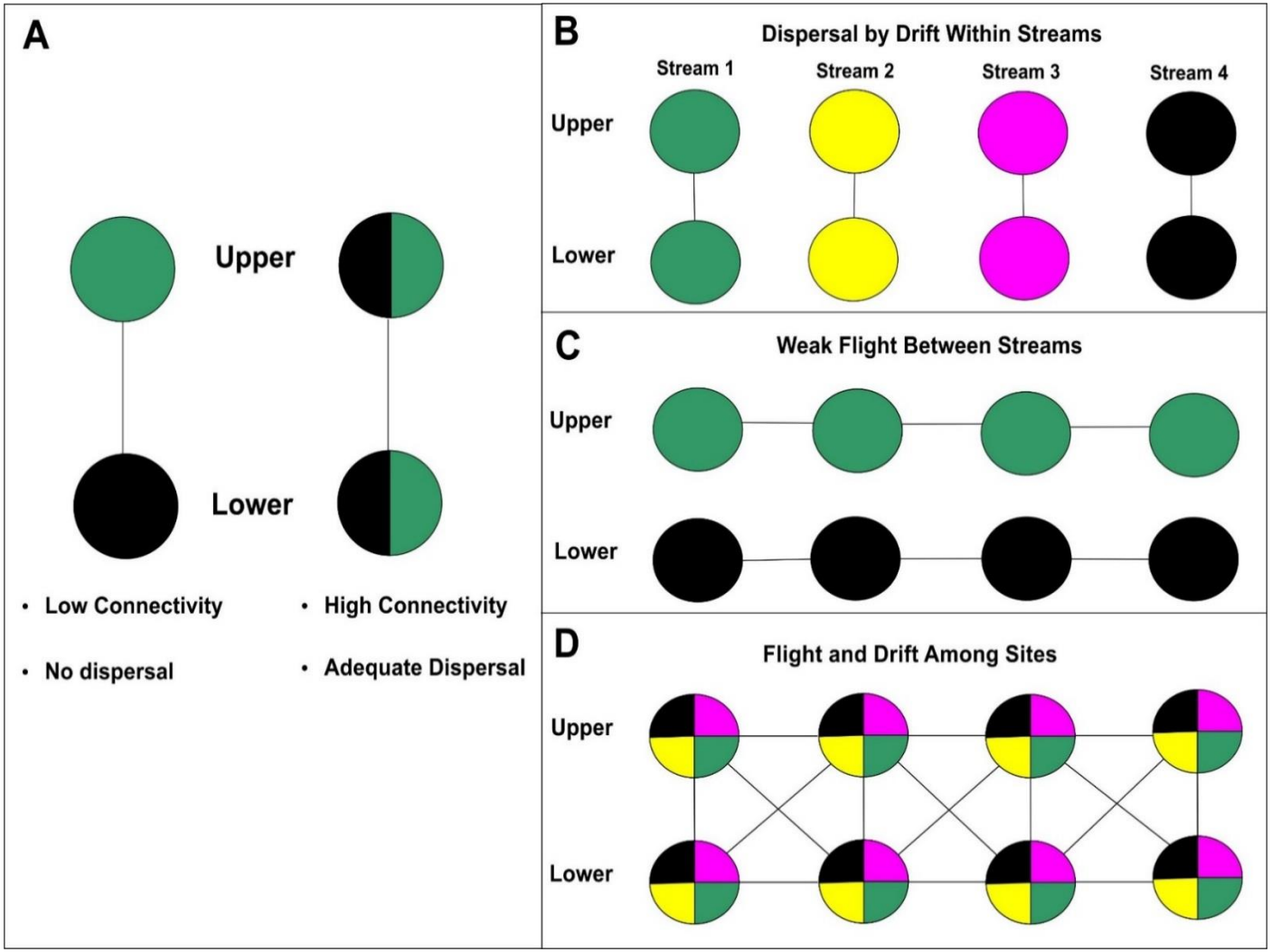

A
Upper
Lower
• Low Connectivity
• No dispersal
• High Connectivity
• Adequate Dispersal
B
Dispersal by Drift Within Streams
Stream 1
Stream 2
Stream 3
Stream 4
Upper
Lower
C
Weak Flight Between Streams
Upper
Lower
D
Flight and Drift Among Sites
Upper
Lower

Fig. 1

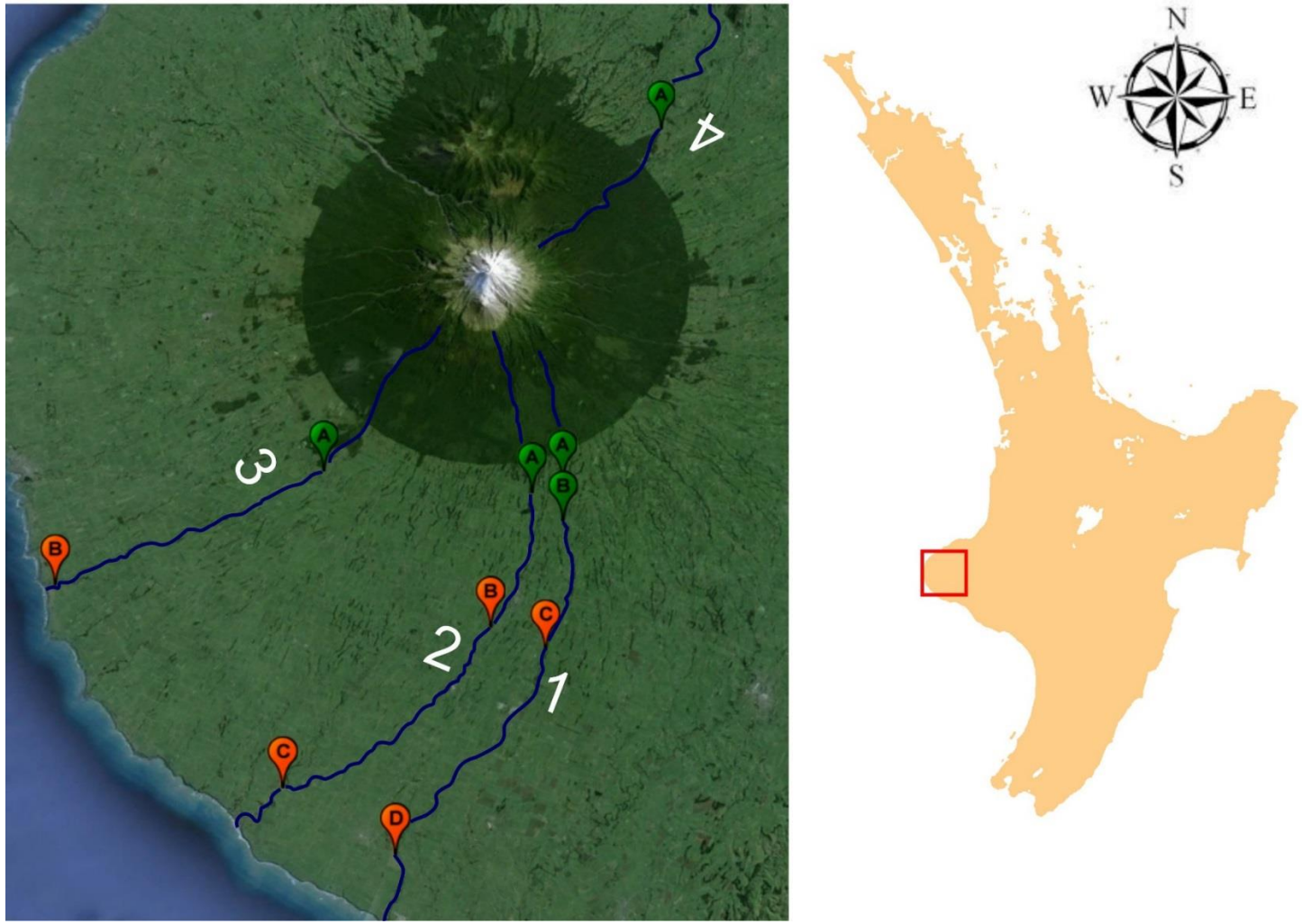


Fig. 2

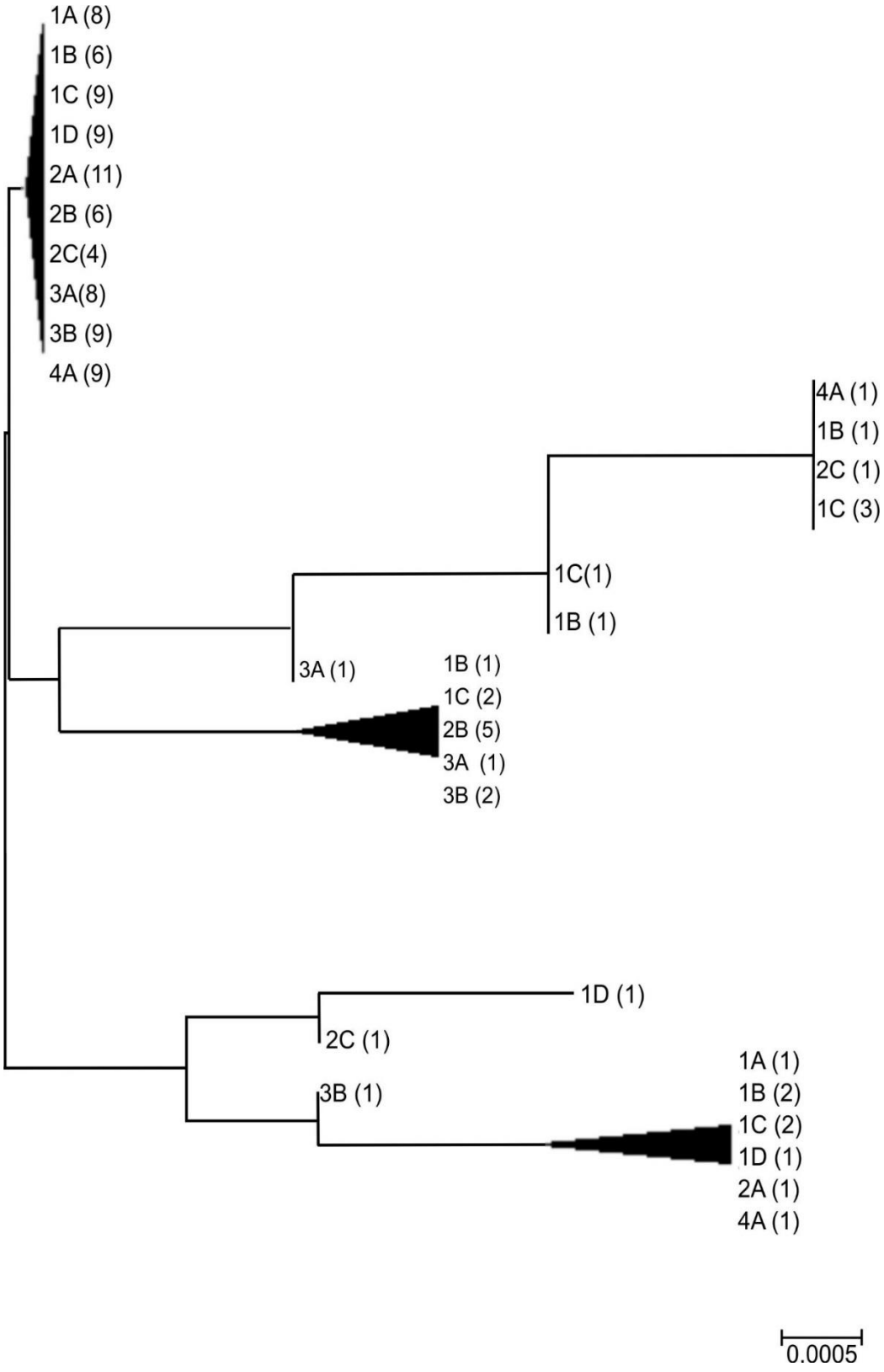

1A (8)
1B (6)
1C (9)
1D (9)
2A (11)
2B (6)
2C(4)
3A(8)
3B (9)
4A (9)
4A (1)
1B (1)
2C (1)
1C (3)
1C(1)
1B (1)
3A (1)
1B (1)
1C (2)
2B (5)
3A (1)
3B (2)
1D (1)
2C (1)
3B (1)
1A (1)
1B (2)
1C (2)
1D (1)
2A (1)
4A (1)
0.0005

Fig. 3A

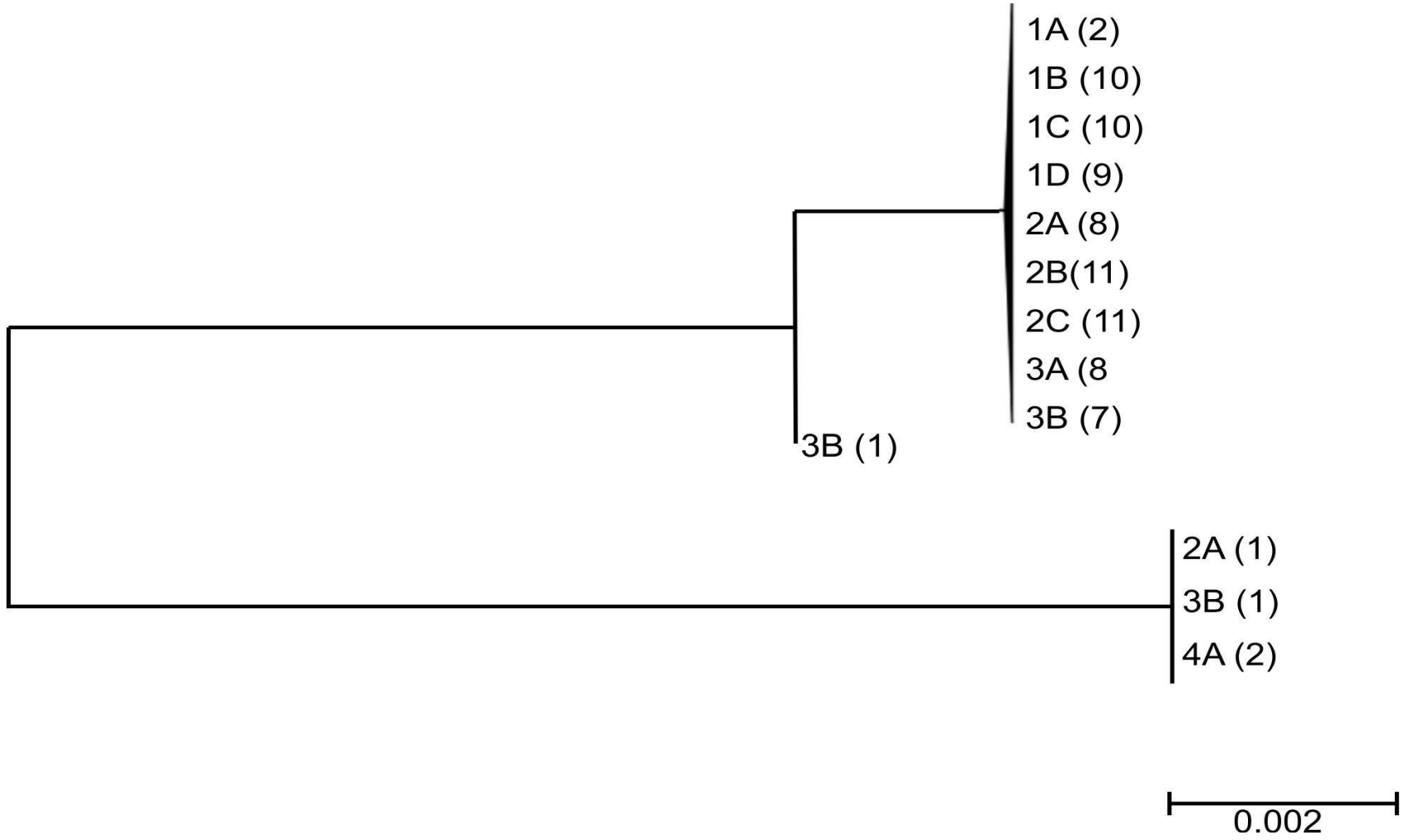


Fig. 3B

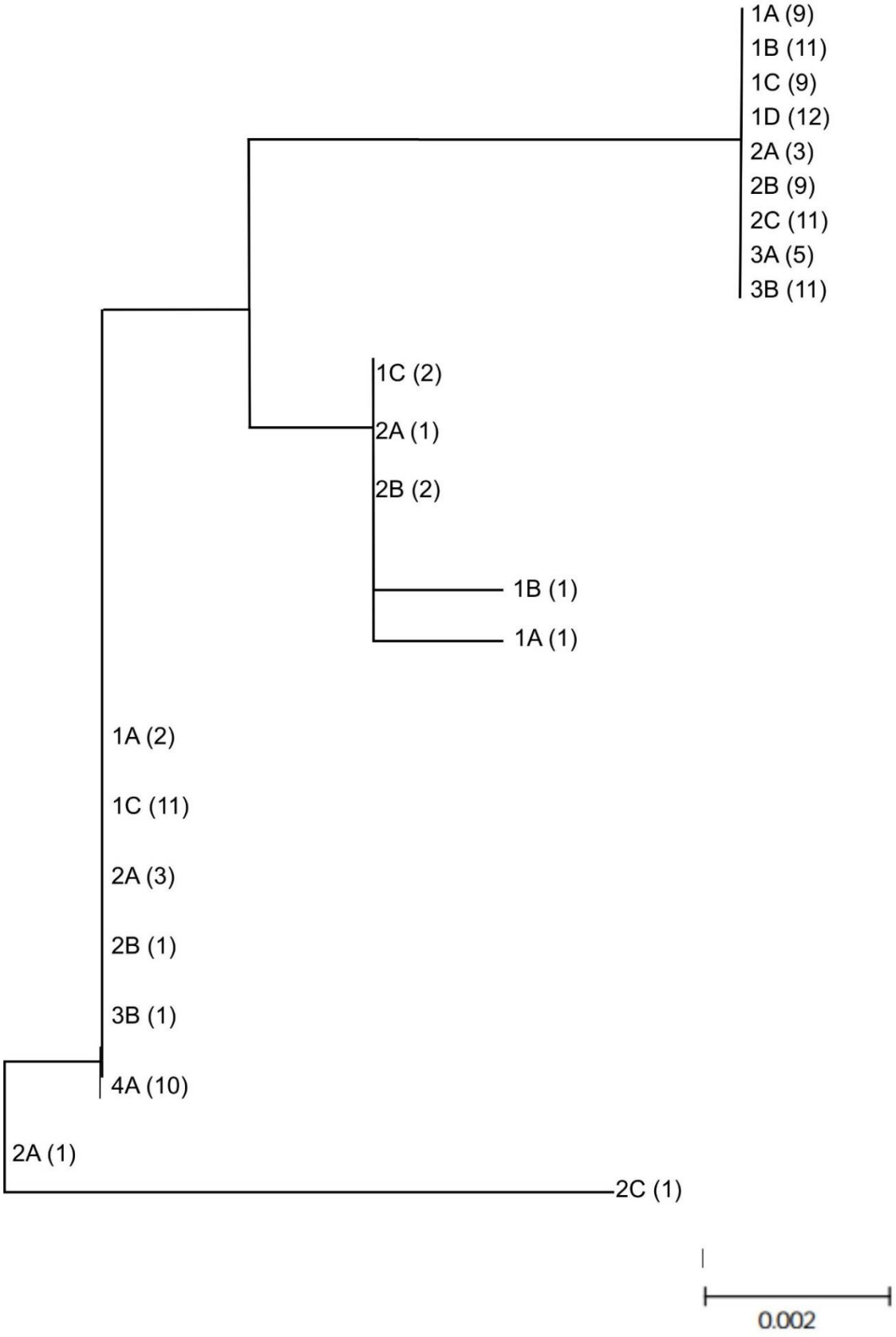

1A (9)
1B (11)
1C (9)
1D (12)
2A (3)
2B (9)
2C (11)
3A (5)
3B (11)
1C (2)
2A (1)
2B (2)
1B (1)
1A (1)
1A (2)
1C (11)
2A (3)
2B (1)
3B (1)
4A (10)
2A (1)
2C (1)
0.002

Fig. 3C

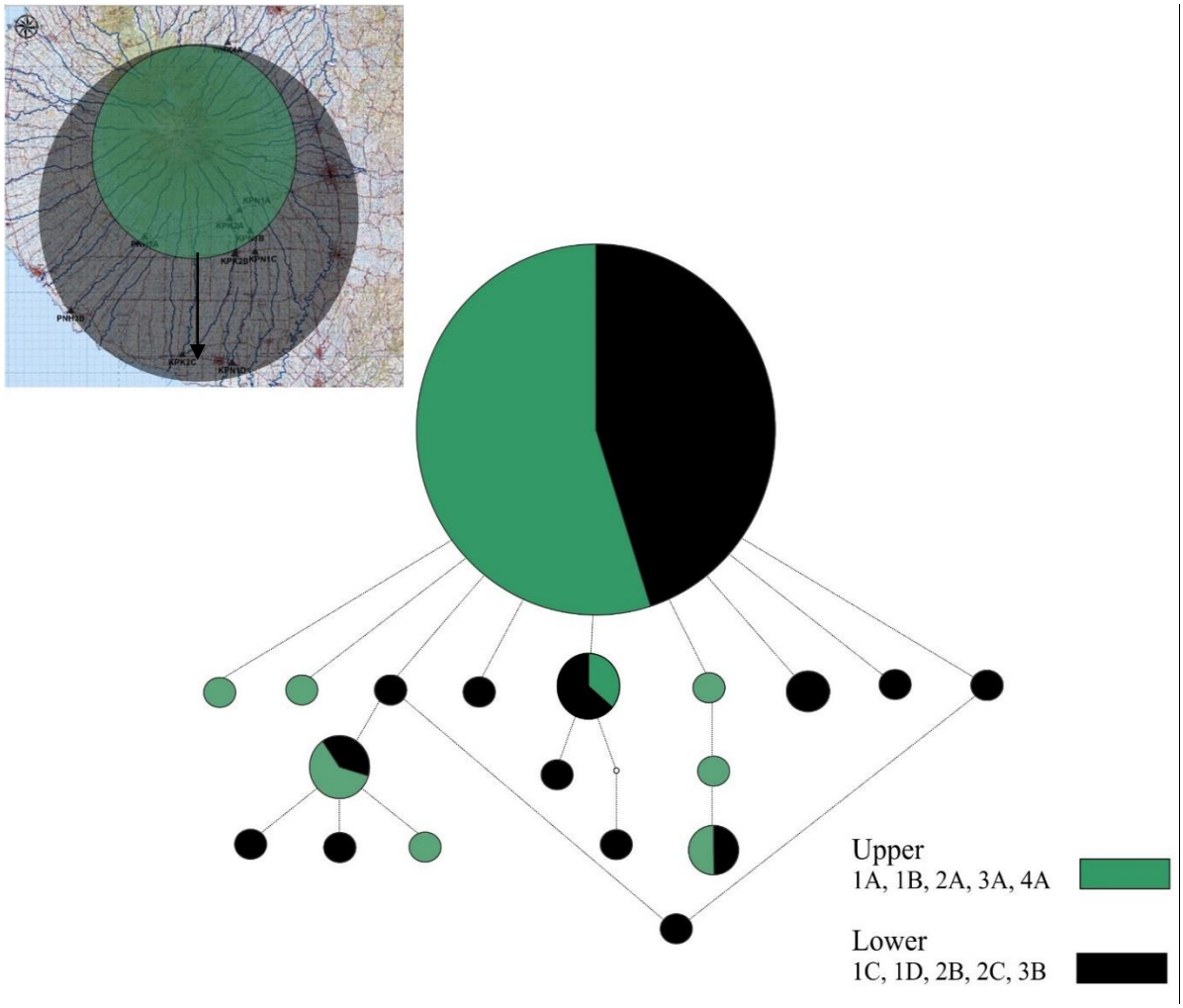

KPK2B
KPN1C
PNH1B
KPK1C
KPN1D
Upper
1A, 1B, 2A, 3A, 4A
Lower
1C, 1D, 2B, 2C, 3B

Fig. 4

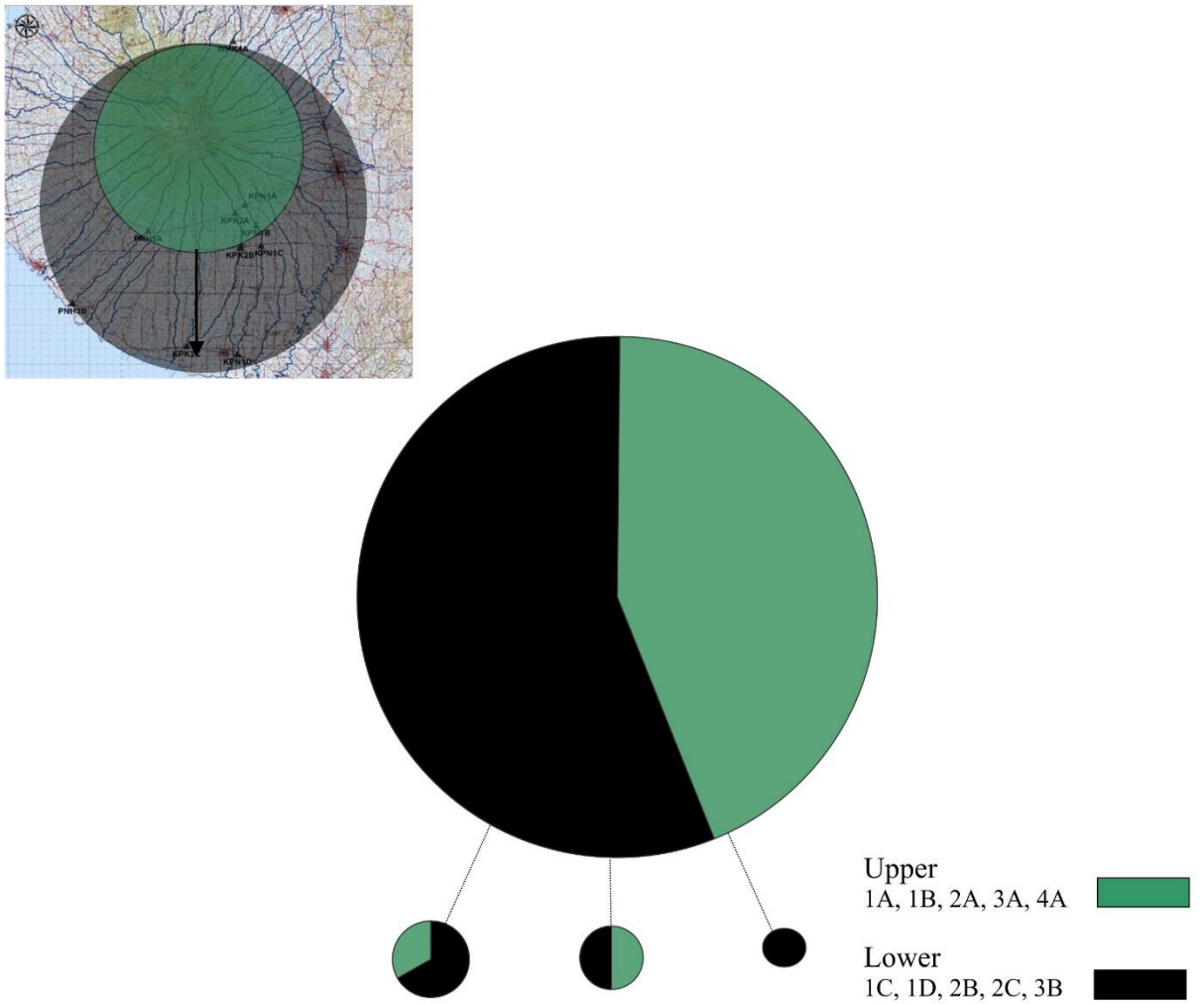

KPN1A
KPK2A
KPK2B
KPN1C
PNH3B
KPN1D
Upper
1A, 1B, 2A, 3A, 4A
Lower
1C, 1D, 2B, 2C, 3B

Fig. 5

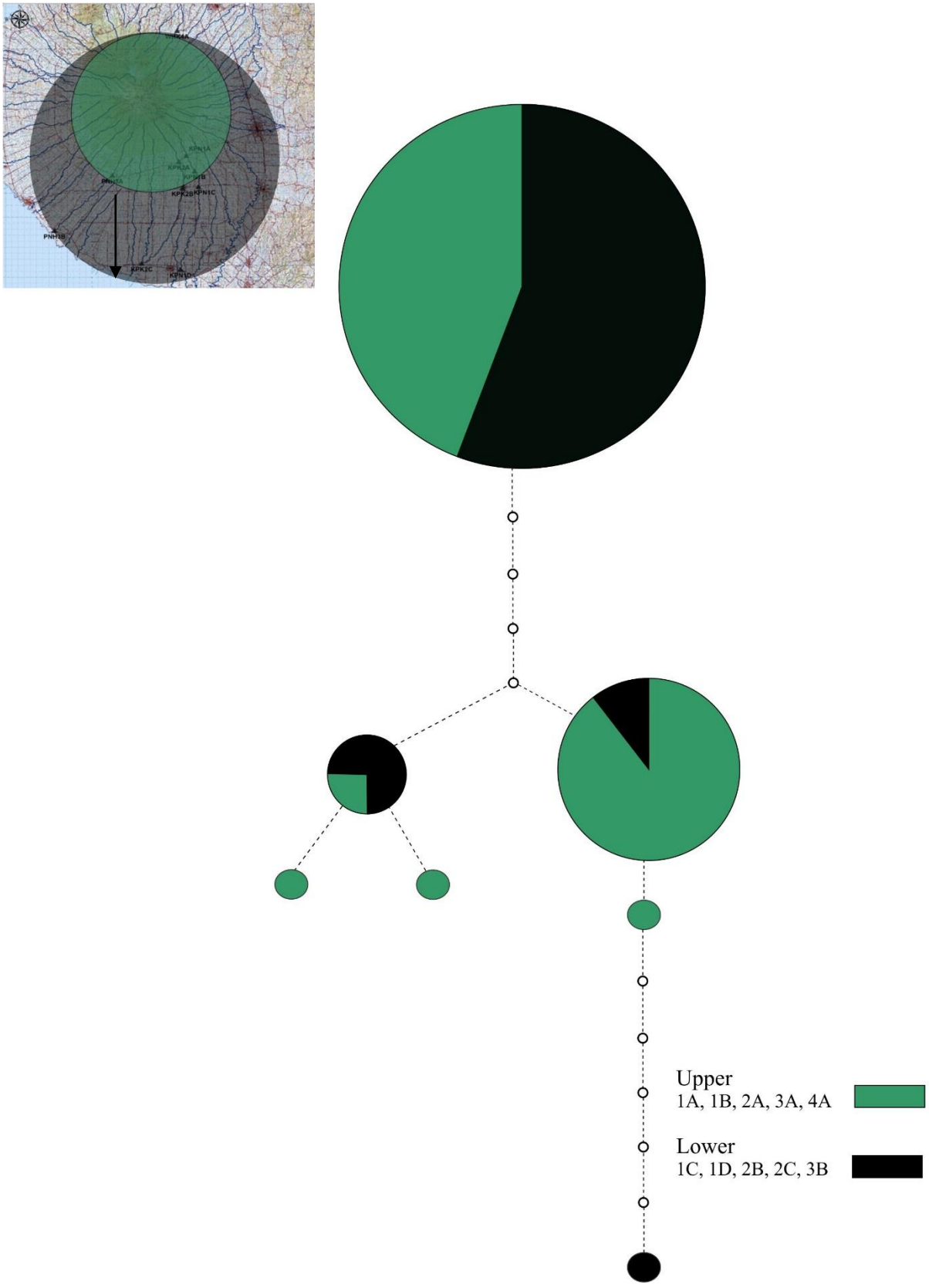

KPN1A
KPN1B
KPN2B
KPN1C
PNH1A
PNH1B
KPK2C
KPK1D
Upper
1A, 1B, 2A, 3A, 4A
Lower
1C, 1D, 2B, 2C, 3B

Fig. 6

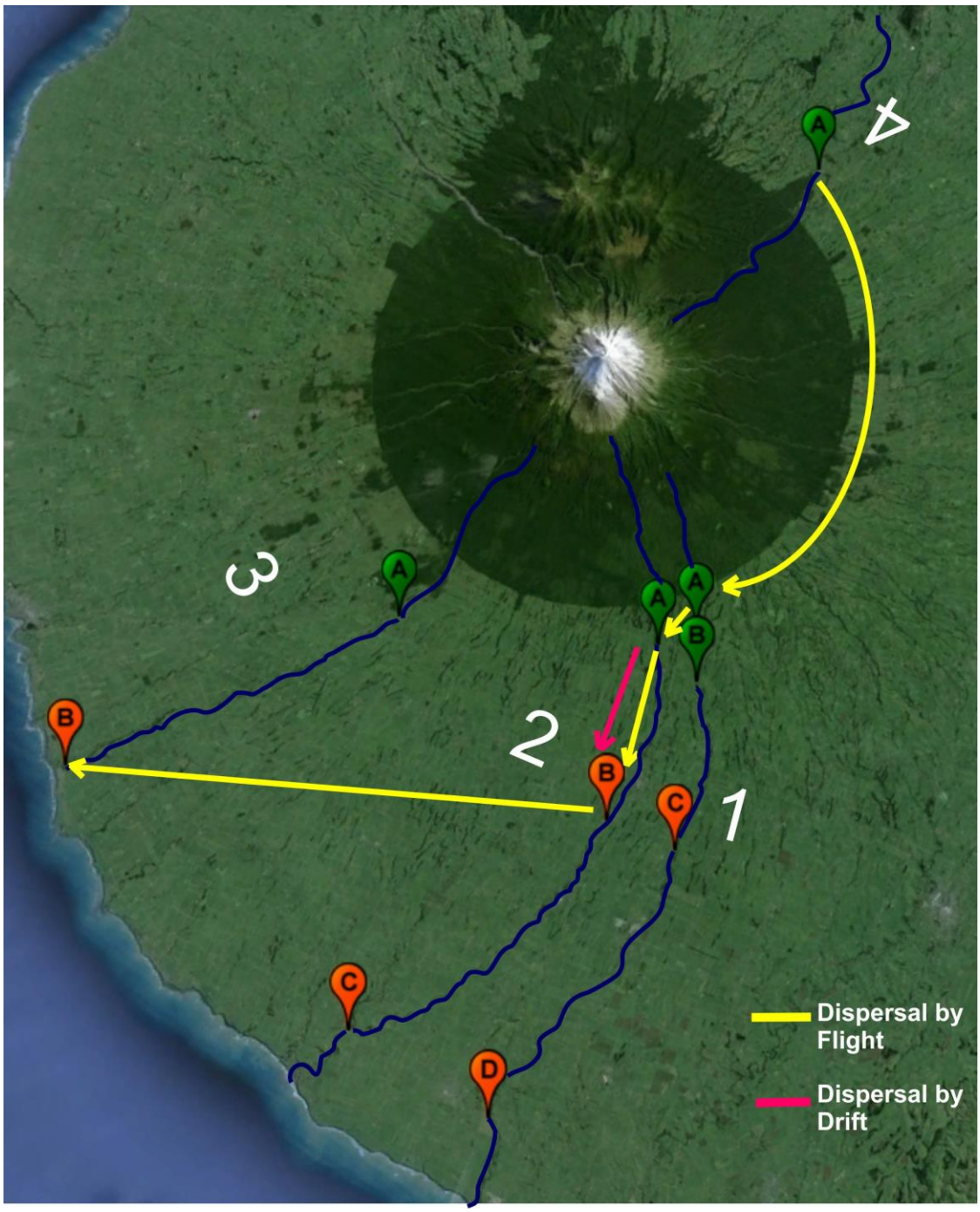


Fig. 7

SUPPLEMENTARY MATERIAL

Figure S1: Haplotype networks showing 19 haplotypes for *Archichauliodes diversus* (Megaloptera) collected from sites on and around Mt Taranaki (site codes correspond to Figure 1).

Figure S2: Haplotype network for *Hydropsyche colonica* (Trichoptera) collected from sites on and around Mt Taranaki (site codes correspond to Figure 1).

Figure S3: Haplotype network for *Pyncocentrodes aeris* (Trichoptera) collected from sites on and around Mt Taranaki (site codes correspond to Figure 1).

Figure S4. Multidimensional scaling plot for *Archichauliodes diversus* (Megaloptera) showing sequence divergences for upper (Sites 1A, 1B, 2A, 3A 4A; green) and lower stream sites (Sites 1C, 1D, 2B, 2C, 3B; Black).

Figure S5. Multidimensional scaling plot for *Hydropsyche colonica* (Trichoptera) showing sequence divergences for upper (Sites 1A, 1B, 2A, 3A 4A; green) and lower stream sites (Sites 1C, 1D, 2B, 2C, 3B; Black).

Figure S6 Multidimensional scaling plot for *Pyncocentrodes aeris* (Trichoptera) showing sequence divergences for upper (Sites 1A, 1B, 2A, 3A 4A; green) and lower stream sites (Sites 1C, 1D, 2B, 2C, 3B; Black).

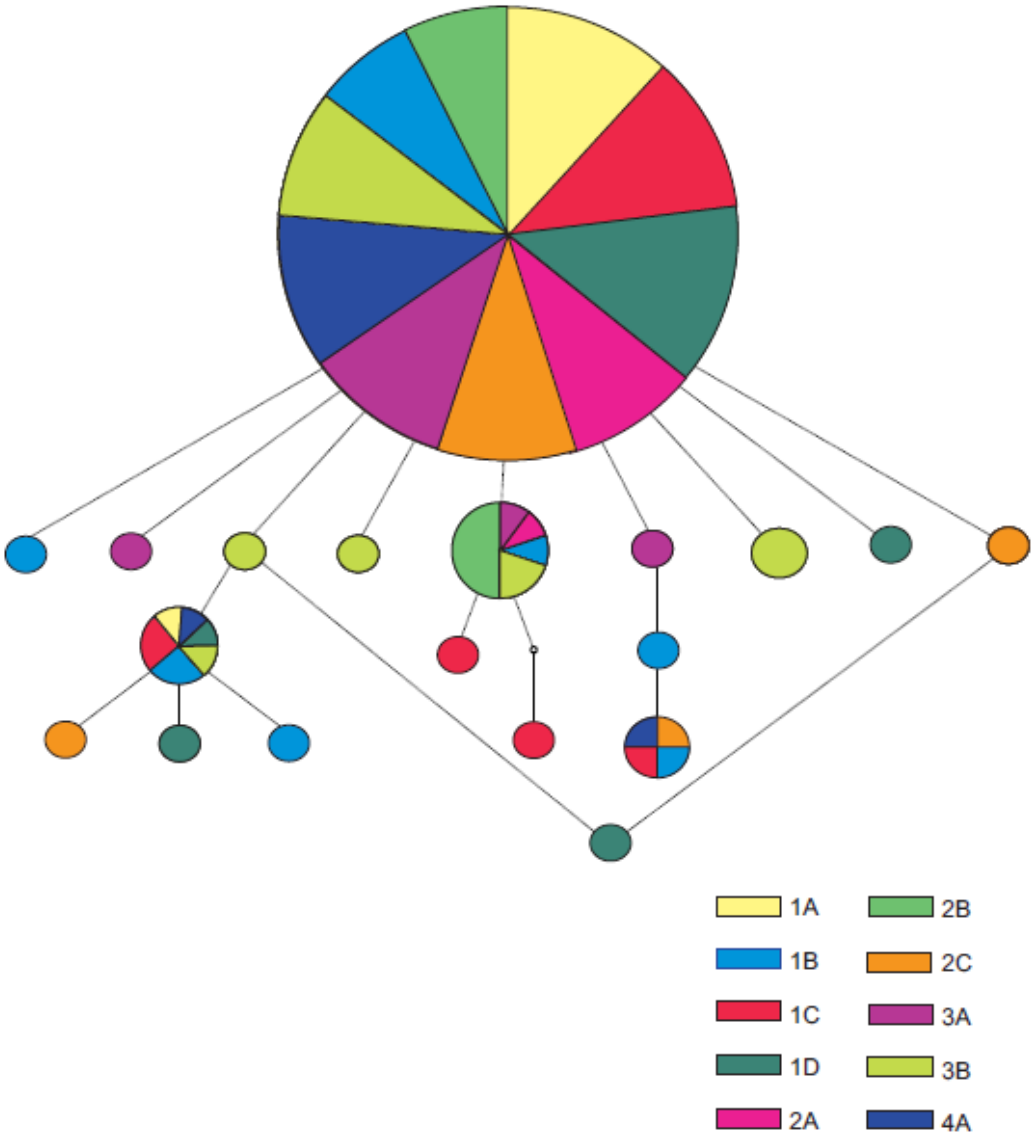


Fig. S1

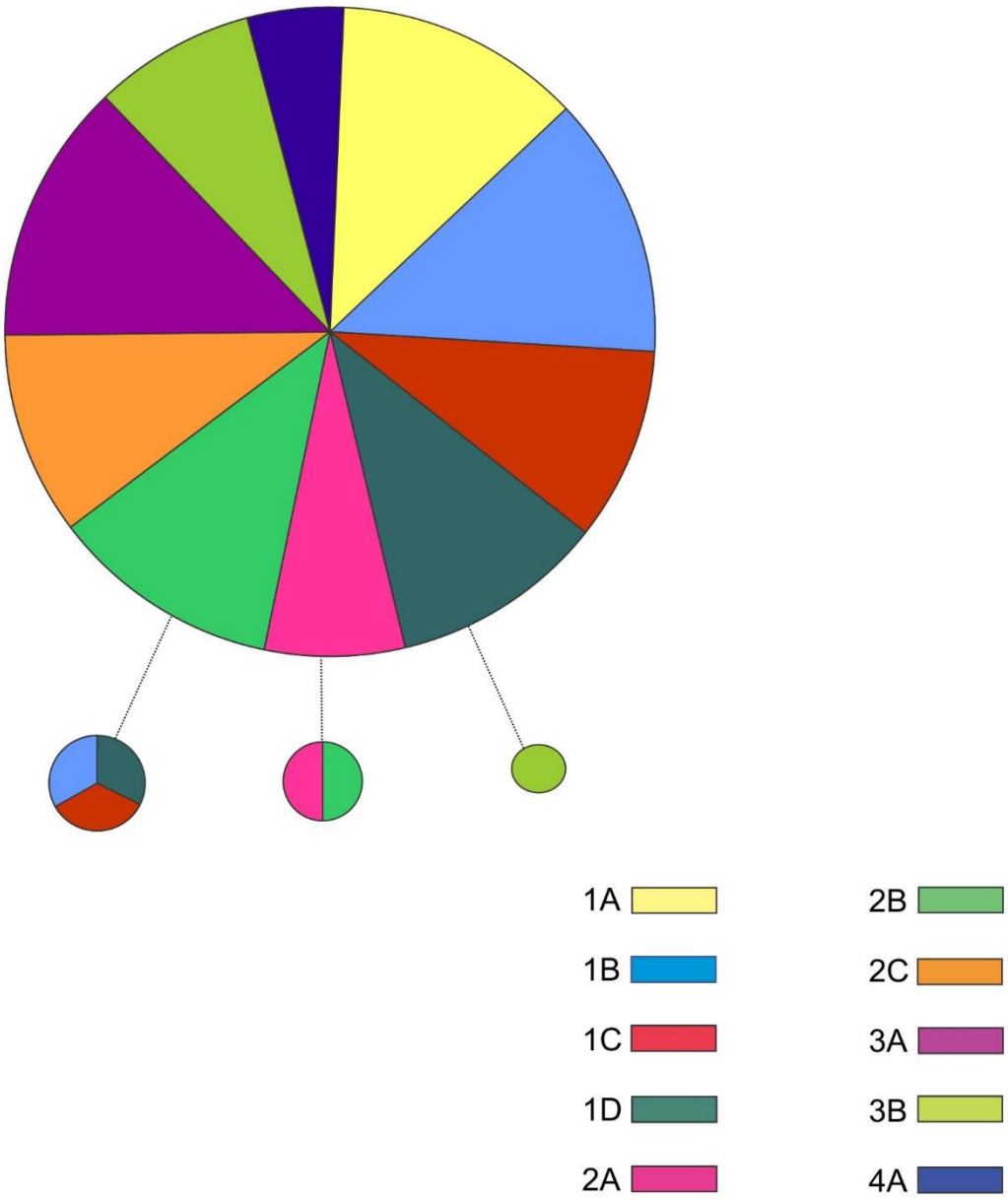


Fig. S2

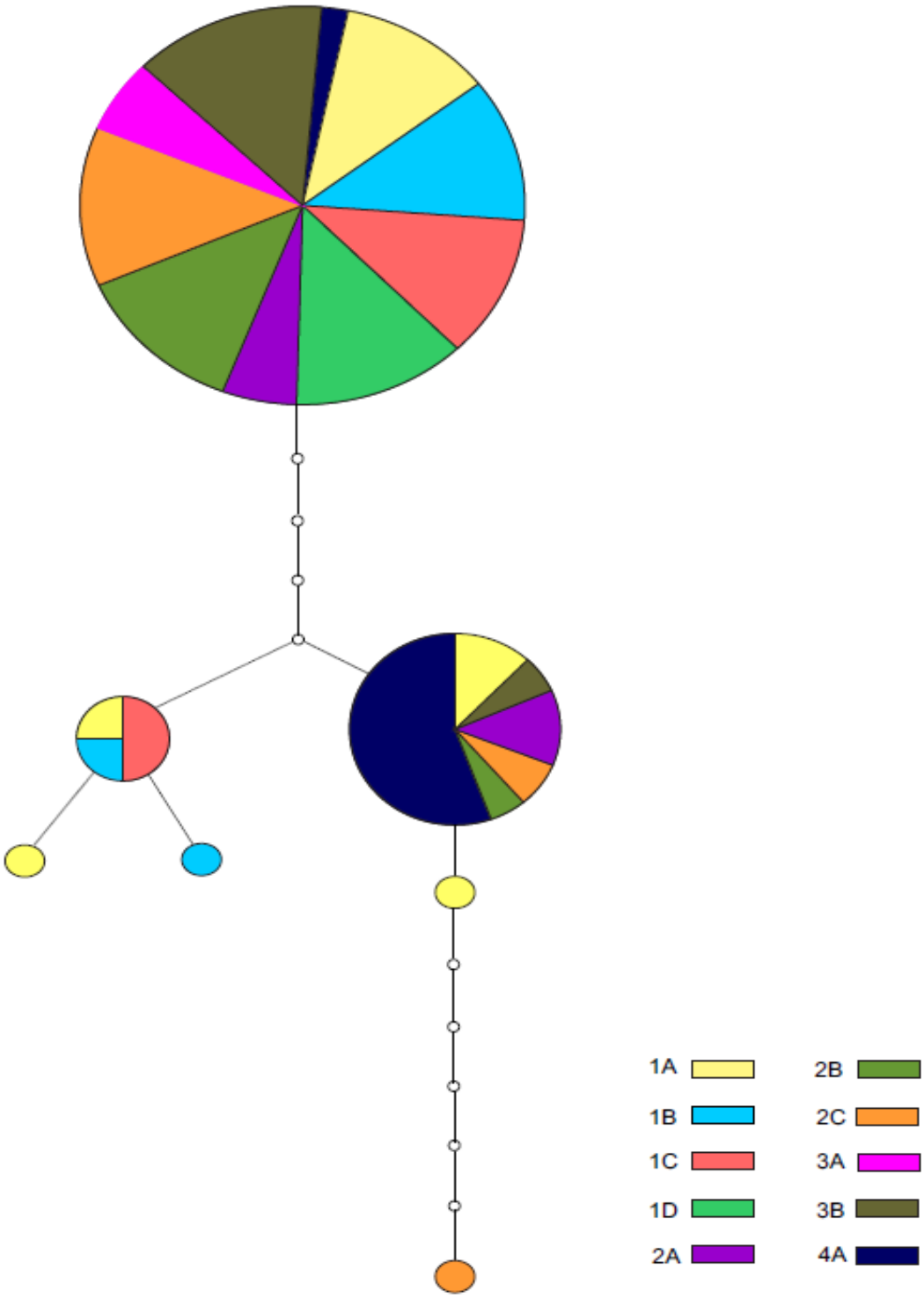


Fig. S3

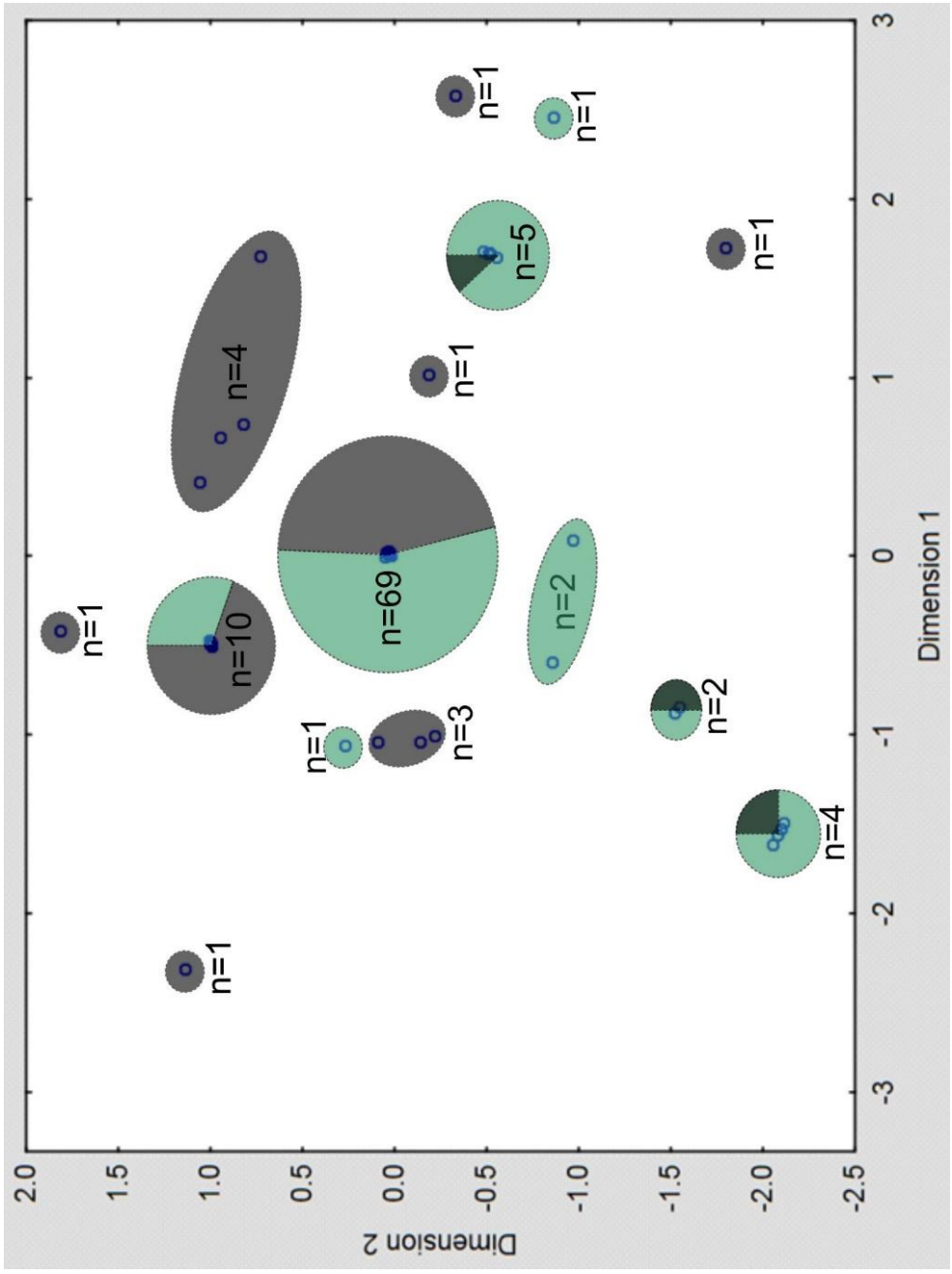

n=1
n=1
n=1
n=5
n=1
n=4
n=1
n=69
n=10
n=2
n=1
n=3
n=2
n=4
n=1
Dimension 1
Dimension 2
3
2
1
0
-1
-2
-3
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5

Fig. S4

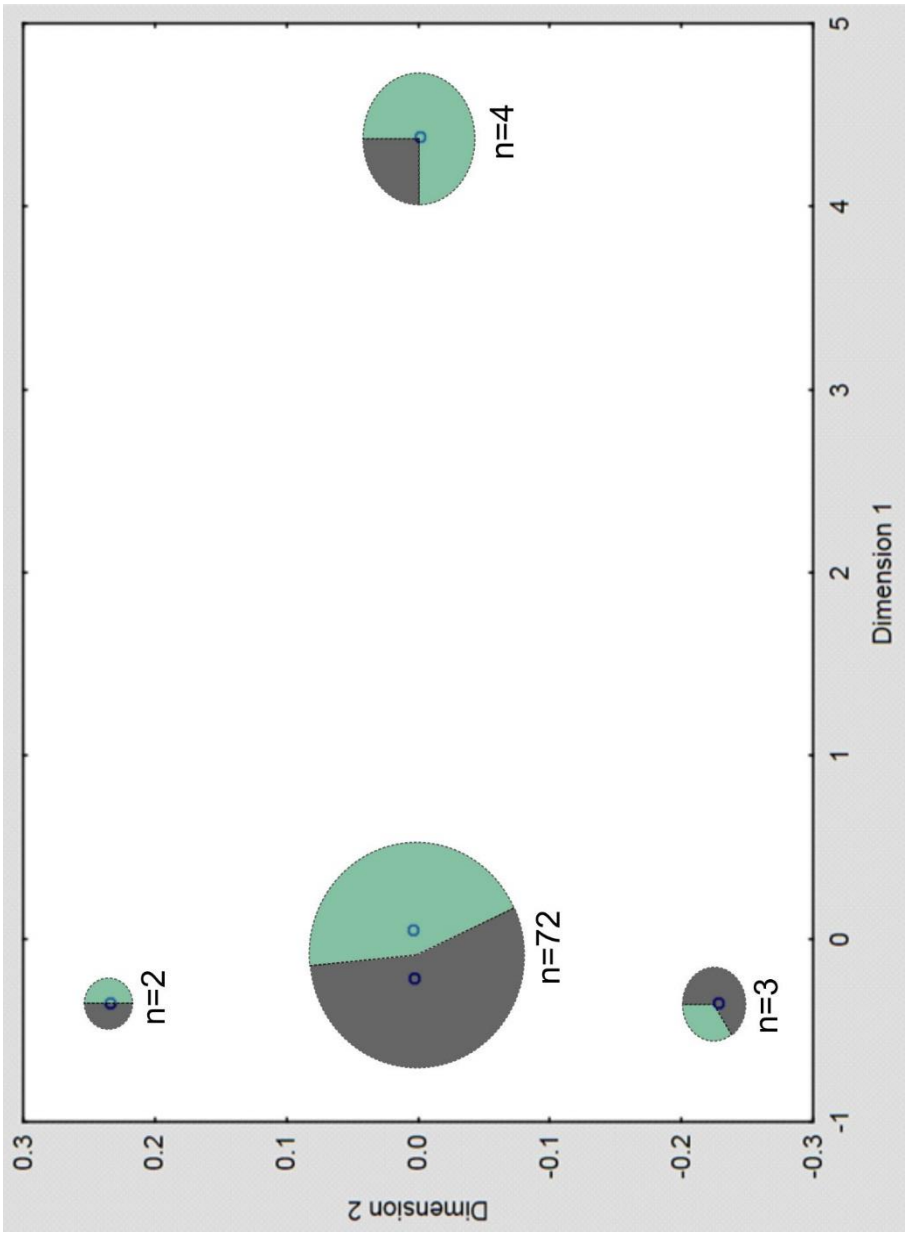

n=4
n=2
n=72
n=3
Dimension 1
Dimension 2
5
4
3
2
1
0
-1
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3

Fig.S5

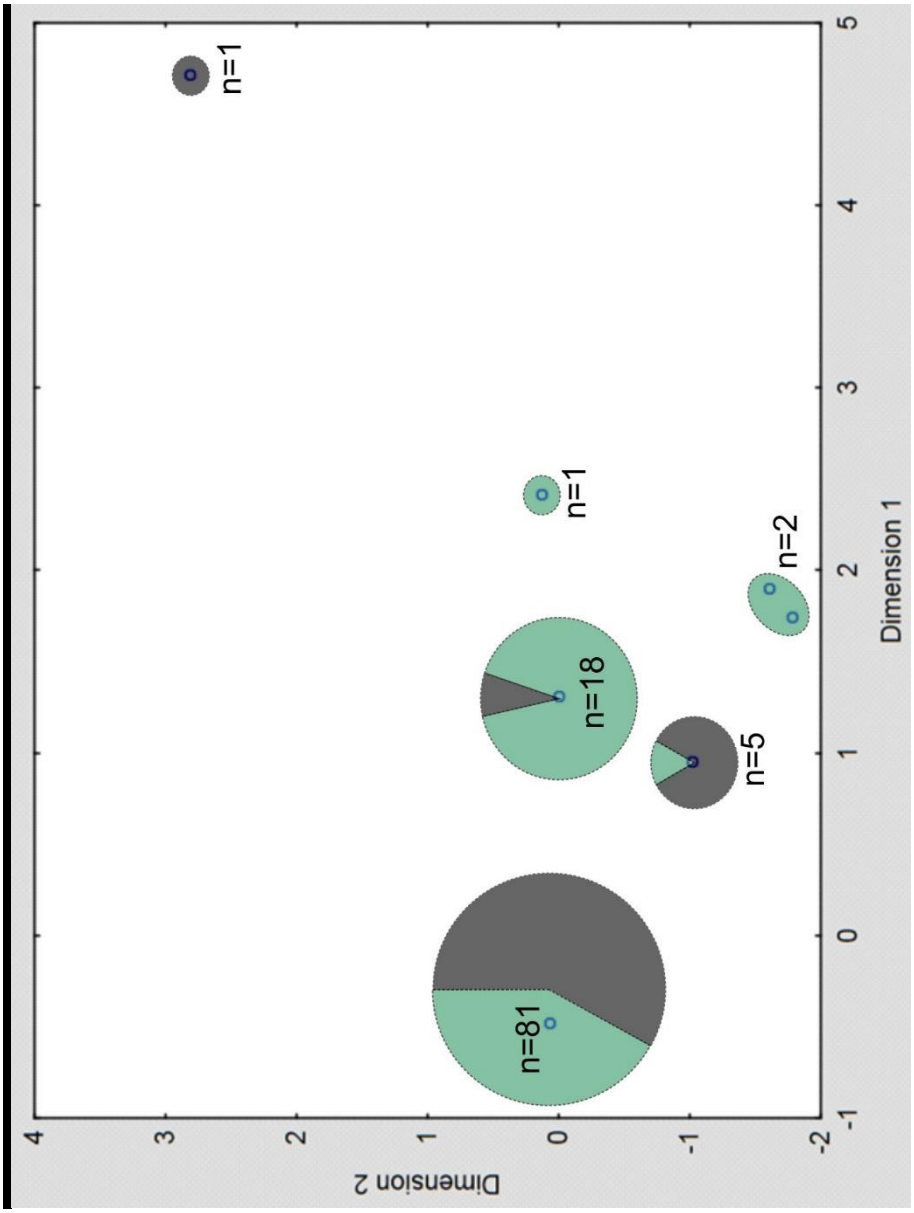


Fig. S6

CHAPTER III

Phylogeography of New Zealand *Archichauliodes diversus* (Insecta, Megaloptera) assessed using mitochondrial DNA (COI) sequences

*To be submitted under the same title as: Morgan A. Riding, Ian D. Hogg

ABSTRACT

We used mitochondrial DNA (COI) sequences to examine the levels of diversity within and among 159 individuals of *Archichauliodes* (Insecta; Megaloptera) from 14 sites in New Zealand's North Island, 10 sites in South Island and one site from Australia. We found high levels of sequence divergence between the New Zealand *A. diversus* and Australian *Archichauliodes* sp. (11.8%), and between New Zealand North Island and South Island *A. diversus* populations (3%). In the South Island, there were 12 haplotypes and 22 for the North Island. There were 12 missing mutational steps between haplotypes from the North Island and the South Islands. The Australian *Archichauliodes* sp. was separated by 70 missing mutational steps from the South Island of New Zealand. Haplotypes were shared by up to 150 km within the South Island, suggesting that *A. diversus* can disperse relatively well on small spatial scales across land. However, it appears limited by geographic barriers (e.g. Cook Strait) on larger spatial scales. Molecular clock estimates suggest that New Zealand and Australian *Archichauliodes* sp. were isolated around 5Mya and isolation between individuals from the North and South occurred within the last 2 Mya, corresponding to the Pliocene and Pleistocene, respectively.

Key Words: Megaloptera, biogeography, Pliocene, Pleistocene, population fragmentation

INTRODUCTION

New Zealand is an archipelago isolated by more than 1500km of ocean (Goldberg et al. 2008). The biogeographies of New Zealand's invertebrate taxa have been significantly influenced by a dynamic geological past which has resulted in isolation of populations over large spatial scales (Hogg et al. 2006; Trewick et al. 2011). As a result, New Zealand harbours a unique biota and has often been described as an endemism hot spot (Daugherty et al. 1993). Despite isolation of the New Zealand land mass from Australia dating back 80 million years, molecular dating estimates suggest that species assemblages only originated within the last 5 Myr (Suggate, 1990; Winkworth et al. 2005; Goldberg et al. 2008). During the Pliocene and Pleistocene (<5 Mya) New Zealand experienced rapid uplift (rates as high as 4.5m/Kyr), landmass changes and significant climatic fluctuations (Lewis et al. 1994; Trewick et al. 2011). Large climatic oscillations resulted in a progressive decrease in air temperatures and developed into the first major glaciation of the early Pleistocene (2.5 Mya to 2.0 Mya) (Suggate, 1990). The most recent glacial activity in New Zealand consisted of three major glaciations throughout the Pleistocene which resulted in eustatic changes in sea levels (34,000 Kya to 28,000 Kya; 24,000 Kya to 21,500 Kya and 20,500 Kya to 18,000 kya) (Proctor et al. 1989; Alloway et al. 2007). During glacial and interglacial periods of the Pleistocene, land extension of the Cook Straight provide new habitat and dispersal pathways for previously restricted flora and fauna (Fig. 1). The last glacial maximum (LGM) ended around 18 Kya resulting in sea level rise and a subsequent loss of the Cook Strait 'land bridge' (Proctor et al. 1989; Boyer et al. 2009). By around 15 Kya the land bridge was breached due to high tidal amplitudes and subduction-related basins migrating southwards, separating

the North Island and the South Island (Proctor et al. 1989; Lewis et al. 1994; Alloway et al. 2007; Marske et al. 2009).

By applying molecular techniques to assess the genetic diversity of natural populations, we now have the ability to assess patterns of genetic diversity relative to geological events. For example, Schmitt et al. (2001) used allozymes to access populations of the butterfly *Polyommatus coridon* in relation to post-glacial expansions in central Europe. Conn et al. (2007) used microsatellites to examine patterns of divergence and distribution for the sand fly (*Lutzomyia longipalpis*), mosquitoes (*Anopheles darlingi* and *A. albitarsis*) and assassin bugs (*Rhodnius prolixus* and *R. robustus*) in relation to the Pliocene and Pleistocene. Cytochrome c oxidase subunit I (COI) gene sequences also provide the opportunity to assess diversity on large spatial scales (Hebert et al., 2003). For example, the COI gene has been used to determine the effects of habitat isolation on large biogeographical scales to examine patterns of speciation (Macey et al, 1998; Vandergast et al. 2004). Understanding the effects of large-scale geographic isolation on natural populations may provide further insight into biological hotspots, determine species' dispersal capabilities and help define overall levels of biological diversity. The geographic isolation of populations is a well understood mechanism of speciation (Mayr, 1940) and a species' dispersal abilities can dictate genetic differentiation among populations and ultimately rates of speciation (Shafer et al. 2013). In this respect taxa with limited dispersal ability are particularly prone to speciation events (allopatric and peripatric speciation). The aquatic insect *Archichauliodes diversus* (Megaloptera) is considered to have poor flight capabilities as an adult (Hogg et al. 2002; Heilviel et al. 2004). Thus, geological events during the Pliocene and Pleistocene would have likely presented

major dispersal barriers. Previous research suggested that there are genetically distinct populations occurring on within the North Island and the within the South Island of New Zealand (Hogg et al. 2002). Here, we examined the present day diversity of *A. diversus* in relation to the geological history of New Zealand. We examined mitochondrial (COI) sequence divergences for *A. diversus* from the North Island and South Island of New Zealand and included available sequences from an *Archichauliodes* species in Australia. We tested the hypothesis that the North Island and South Island *A. diversus* populations are genetically distinct based on COI sequences. We predicted that timing of divergence, estimated using molecular clock dating would correspond with geological events within the last 5 Myr and particularly through the isolation of the North and South Islands.

MATERIALS AND METHODS

Individuals of *Archichauliodes diversus* (Insecta: Megaloptera) were collected over several months from March 2014 to January 2015 from the North Island and South Island of New Zealand (Table 1; Fig. 2). Samples were typically taken using a standard, 30 cm width, D- net from riffle habitats at each site and contents were placed into white 20 x 30 cm sorting trays with water from the same stream. Contents of each sample where then visually examined and individuals of *A. diversus* were removed with forceps and placed directly into 100% ethanol. Once in the lab, ethanol in the samples was replaced with fresh 100% ethanol and samples were stored at 4^{o}C until needed for genetic analyses.

A 4 mm sample of leg tissue was taken from each individual using sterilised (flamed) forceps and added to a single well of a 96-well plate. DNA sequencing was conducted at the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph, Canada following standard protocols (Ivanova et al. 2006). A 658 bp region of the mitochondrial cyctochrome c oxidase subunit I (COI) gene was PCR amplified using the primer pair LepF1 (5' ATTCAACCAATCATAAAGATATTGG-3') and LepR1 (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') (Hebert et al. 2004). Genomic DNA was extracted following the AcroPrepTM PALL Glass Fibre plate method using a total mix of 5 ml insect lysis buffer (0.5 ml of Proteinase K, 20 mg/ml per 96-well plate) (Ivanova et al. 2006). A total of 5 µl of the DNA extraction product was used for PCR. PCR thermal cycling conditions were: initial denaturation of samples at 94^{o}C for 1 minute, followed by five cycles of 94^{o}C for 30 seconds. Annealing occurred at 48°C for 1.5 minutes with the extension occurring at 72°C

for 1 minute, 35 cycles of 94°C at 30 seconds, 52°C for 1 minute with the final extension occurring at 72°C for 10 minutes. Successful PCR products were cleaned using Sephadex® and then sequenced using an ABI3730xl DNA analyser. All data have been added to the Barcode of Life Data systems (BOLD) database (Ratnasingham & Hebert, 2007) under project NZDIS (Dispersal of New Zealand invertebrates).

Sequences were aligned using MUSCLE implemented in Geneious 7.1.9 (Biomatters, Auckland) and trimmed to 634 bp to include sequences of *Archichauliodes* sp. from New South Wales, Australia sourced from the Barcode of Life Datasystems (BOLD; accession numbers MDFRC_MMN0001 to MDFRC_MMN0004). PAUP* 4.0 (Swofford, 2002) was used to identify parsimony-informative sites. Chi square tests implemented in PAUP* 4.0 were used to determine if base frequencies were equal among all sites and among first, second or third codon positions. A jModelTest (2.1.1) was used to determine the most appropriate model of evolution as GTR+I+G (-InL=1, 424.179) (Posada, 2008). Bayesian trees were generated using BEAST software v1.7.5 (Drummond et al. 2007). A relaxed clock model and speciation yule process with a Markov chain Monte Carlo (MCMC) was set at 100, 000 000 generations, sampling trees every 5,000 generations was employed in BEAUTI v1.7.5. The Bayesian analysis was run in BEAST v1.7.5 and the result quality was evaluated in TRACER v1.5. A burn of 1000 trees was entered into Tree Annotator v1.7.5 and the final tree was produced and visualized in FigTree v1.4.0. Neighbour Joining (NJ), Maximum Likelihood (ML) and Maximum Parsimony (MP) trees were constructed using MEGA v5.2.2 (Tamura et al. 2011). A Jukes Cantor model was used for NJ and a GTR+I+G was used for ML as models of evolution. All three trees NJ, ML and

MP were set to standard default settings with 1000 bootstrap replicates. Uncorrelated pair-wise genetic distances between COI sequences at different locations were calculated using MEGA v5.2.2 (Tamura et al. 2011; Srivathsan et al. 2012). Haplotype networks were produced using TCS v1.2 set to a correction limit of 20 steps at 95% (Clement et al. 2000). Comparisons of log likelihood sores using X^2 tests implemented in PAUP* were used to determine if sequences were evolving in a 'clock-like' manner. The likelihood ratio test did not detect evidence of significant rate heterogeneity (x^2=838.6 p=<0.001; df=324) and a molecular clock is appropriate. Subsequently, approximate geological ages between the species pairs were estimated through a molecular clock analyses in BEAST software v1.7.5 (Drummond et al. 2007). Files generated in BEAUTI used a GTR+I+G with speciation Yule Processes as the tree prior along with the same MCMC set up used for the Bayesian inference analysis. A strict clock model with a fixed rate of 0.0115 was used to simulate 2.3% divergence per million years, determined for invertebrate data (as per Roslin, 2001; Quak et al. 2004; Trewick et al. 2005; Wilke et al 2009). Estimated molecular dates were further manually calculated using $t_D=d_N/\lambda$ (Wilke et al 2009).

RESULTS

We sequenced 164 individuals and obtained sequences from 159 individuals for a 97% success rate. Of the 634 nucleotide positions 601 were constant with 6 variable although parsimony uninformative and 27 parsimony informative. There was an overall A-T base frequency bias of 64.9% (A=26.9%, C=17.6%, G= 16.2%, T=37.0%). Base frequencies were homogeneous across sequences at all sites (x^2=9.18, df=324, p=1) at variable sites (x2=182.35, df=324, p=1), informative sites (x2=202.9, df=324, p=0.99) and at first (x2=0.001, df=324, p=1), second (x2=0.00, df=324, p=1) and third (x2=67.8, df=324, p=1) codon positions.

There were 12 haplotypes for the South Island and 22 haplotypes for the North Island. Sequence divergences between the New Zealand *A. diversus* and Australian *Archichauliodes sp.* were 11.8% and divergences between the North and South Islands was 3%. The phylogenetic analyses showed three genetically distinct groups corresponding to individuals from Australia and the North and South Islands of New Zealand. The NJ, ML, MP and Bayesian analyses all produced concordant results, with each branch showing high support (>87%) (Figs 3-6), supporting genetic differentiation between the Australian and New Zealand taxa.

The haplotype network further demonstrated a significant genetic split between the North Island and South Island *A. diversus*. Overall, there are three links between the North Island and the South Island haplotypes each divided by 12 missing mutational steps. South Island haplotypes appear to be further divided by site. For example, some haplotypes were restricted to the upper South Island locations (Collins River, Wakapunaka, Wai-iti River, The Brook and Blue Duck

River) while other haplotypes were restricted to the lower South Island sites (Waikaia River, Caitlins River and Otamita Stream). Individuals sequenced from Opuha shared haplotypes with individuals from Blue Duck River, The Brook and Wai-iti River. Individuals from Harris Creek shared the same haplotype as that found on the west coast of the South Island (Figs. 7, 8). The Australian species showed 70 missing mutational steps between the New Zealand *A. diversus*. Moreover, the Australian *Archichauliodes* sp. showed its primary connection with the South Island *A. diversus* (Fig. 7).

Based on a sequence divergence rate of 2.3% per million years, isolation of the New Zealand *A. diversus* from the Australian *Archichauliodes* sp. was estimated to have occurred 5.2 Mya. Isolation of the North and South Island *A. diversus* was estimated at around 1.4 Mya (Fig. 7).

DISCUSSION

Our mitochondrial DNA (COI) analyses of the 113 *Archichauliodes diversus* showed measurable genetic divergences between the North and South Islands of New Zealand. These data are concordant with previous studies on New Zealand terrestrial insects such as cicadas and the ground weta (e.g. Marshall et al. 2008; Trewick et al. 2011; Trewick et al. 2012). A previous study based on allozyme analyses also showed genetically distinct populations of *A. diversus* on the North and South Islands of New Zealand. In this previous study it was suggested that genetic variation within *A. diversus* may have resulted from a combination of historic change as well as contemporary dispersal abilities. By using molecular clock estimates we were able to examine linkages between population divergences and possible geological events (Quek et al, 2004). Using a molecular clock calibration of 2.3% sequence divergence per million years we estimated that populations of *A. diversus* diverged within the last 2 Myr congruent with events during the Pleistocene (Proctor et al. 1989; Alloway et al. 2007; Marske et al. 2009). Therefore, population genetic structure shown for *A. diversus* may reflect allopatric isolation occurring during the Pleistocene glaciations. Trewick et al. (2011) suggested possible scenarios whereby populations would show an association with the LGM. For example, populations may be allopatric from North and South Islands during the Pliocene (or whenever), and having then moved between Islands by over-sea dispersal later (3Mya - 5Mya) before remaining isolated in each of the two Islands. A second scenario is that a population may have existed on one Island (either North Island or South Island), and expanding the range (e.g. via the Cook Strait land bridge) and then becoming separated as sea level rose (Fig. 9) (Trewick et al. 2011). Both these outcomes are

possible given the potential isolation of *A. diversus* (1.4 Mya). However, previous studies have suggested *A. diversus* has poor flight capabilities (Hogg et al. 2002; Heilviel et al. 2004). Given the first scenario these species would have had to disperse by over-sea flight leaving dispersal during the Pliocene less likely. We suggest that populations existed on one the Islands while expanding their range during the LGM following separation through sea level rise. The inclusion of the *Archichauliodes* sp individuals from the Murray Darling River, Australia showed 70 missing mutational steps from the South Island with no direct connection to the North Island. This provides some evidence that the most recent common ancestor for the New Zealand *A. diversus* was from Australia and arrived on the South Island before moving to the North Island during range expansion during the Pleistocene. The divergence of the Australian *Archichauliodes* sp. and New Zealand *A. diversus* was estimated around 5 Mya coinciding with the Pliocene (5 Mya - 2 Mya). This is congruent with previous research suggesting that species assemblages originated only within the last 5 Myr (Steven, 1980; McGlone et al. 2001; Goldberg et al. 2008). For example, Vink et al. (2003) used mitochondrial gene regions of the COI and NADH dehydrogenase subunit 1 (ND1) and determined that species of New Zealand wolf spider (*Lycosidae antoteropsis*) share close Australian relatives and suggested that divergence occurred within the last 5Mya. Brown et al. (1999) found the *Hepialid* moth species dispersed from Australian over the past 4Mya - 5Mya around the time of the uplift of the alpine fault. On this basis, we suggest that *Archichauliodes* sp. is likely to have arrived in New Zealand roughly the same time as these other species.

Isolation of populations over prolonged periods can result genetic divergences and ultimately speciation. Such populations may consist of morphologically similar yet genetically distinct individuals (Taylor et al. 1998; Bilton et al. 2001; Hughes

et al. 2011). Based on a preliminary morphological analysis of the New Zealand North and South Island *A. diversus*, we were unable to find any obvious morphological differences among individuals. However, the levels of divergence found between individuals from the North and South Islands may represent potential sibling species (sensu Bickford et al. 2007). Marshall et al. (2008) conducted studies on New Zealand cicadas (*Kikihia* spp.) and suggested that the required level of divergence for reproductive isolation between populations is at least 2 Myr. Accordingly, it is possible that *A. diversus* have not yet diverged to a level where they would be reproductively isolated. In the interim, the North Island *A. diversus* and South Island *A. diversus* should be considered genetically distinct 'sibling species' until further data are obtained. On a smaller scale, fragmentation of *A. diversus* is further apparent within the South Island. For example, haplotypes were restricted to and shared among the upper South Island sites while other haplotypes were restricted to and shared among sites in the lower South Island. This suggests an isolation-by-distance occurring between the upper and lower regions of the South Island. Similar results were obtained by Chinn et al. (2004) where COI sequence data was used to determine phylogeographical patterns of endemic South Island cockroaches. They suggested that divergence occurred in response to mountain building during the Pliocene-Pleistocene. These data may therefore be indicative of isolation during Plio-Pleistocene events such as the uplift and formation of the Southern Alps.

ACKNOWLEDGEMENTS

Special thanks to K. Shearer and J. Banks (Cawthron Institute) for providing samples of *Archichauliodes* from the Nelson region and, to B. Smith, M. de Wit, N. Groot and L. Laboyrie for assistance with sampling within Taranaki, and from Pirongia.

LITERATURE CITED

Alloway, B.V., Lowe, D.J., Barrell, D.J.A., Newnham, R.M., Almond, P.C., Augustinus, P.C., Bertler, N.A.N., Carter, L., Litchfield, N.J., McGlone, M.S., Shulmeister, J., Vandergoes, M.J and Williams, P. W. 2007. Towards a climate event stratigraphy for New Zealand over the past 30 000 years (NZ-INTIMATE project). Journal of Quaternary Science. **22**(1): 9-35. doi:10.1002/jqs.1079.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K and Das, I. 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution. **22**(3): 148-155. doi:10.1016/j.tree.2006.11.004.

Bilton, D.T., Freeland, J.R and Okamura, B. 2001. Dispersal in freshwater invertebrates Annual Review of Ecology and Systematics. **32**(1): 159-181. doi:10.1146/annurev.ecolsys.32.081501.114016.

Boyer, S.L and Giribet, G. 2009. Welcome back New Zealand: Regional biogeography and gondwanan origin of three endemic genera of mite harvestmen (Arachnida, Opiliones, Cyphophthalmi). Journal of Biogeography. **36**(6): 1084-1099. doi:10.1111/j.1365-2699.2009.02092.x.

Chinn, W.G and Gemmell, N.J. 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**(6): 1507-1518. doi:10.1111/j.1365-294X.2004.02160.x.

Daugherty, C.H., Gibbs, G.W. and Hitchmough, R.A. 1993. Mega-island or micro-continent? New Zealand and its fauna. Trends Ecol. Evolution. **8**: 437–442. doi:10.1016/0169- 5347(93)90006-B.

Estoup, A., Solingnac, M., Conrnuet, J.M., Goudet, J and Scholl A. 1996. Genetic differentiation of continental and Island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. Molecular Ecology. **5**(1): 19-31. Doi: 10.1111/j.1365-294X.1996.tb00288.x.

Goldberg, J., Trewick, S. A and Paterson, A.M. 2008. Evolution of New Zealand's terrestrial fauna: A review of molecular evidence. Philosophical Transactions of the Royal Society B: Biological Sciences. **363**(1508): 3319-3334. doi:10.1098/rstb.2008.0114.

Hebert, P.D.N., Penton, E.H., Burns, J., Janzen, D.H., and Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly, Astraptes fulgerator. Proceedings of the National Academy of Sciences. **101**(41). 14812-14817.

Heilviel, S.J. 2004. Mating Habits, Dispersal and Postglacial Range Expansion of Nigronia serricornis (Say) (Megaloptera; Corydalidae). Ph.D. Thesis, Department of Entomology, University of Illinois at Urbana-Champaign, Illinois, United States.

Hogg, I., Willmann-Huerner, P and Stevens, M. 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**(3): 491-501. doi:10.1080/00288330.2002.9517104.

Hogg I.D., Stevens M.I., Schnabel K.E., Chapman A.M., 2006. Deeply divergent lineages of the widespread New Zealand amphipod *Paracalliope fluviatilis*

revealed using allozyme and mitochondrial DNA analyses. Freshwater Biology. **51**: 236–248.

Hughes, J.M., Huey J.A., McLean A.J and Baggiano O. 2011. Aquatic insects in Eastern Australia: A Window on Ecology and Evolution of Dispersal in Streams. Insects. **2**(4): 447-461. doi: 10.3390/insects2040447.

Ivanova, N.V., Dewaard, J.R. and Hebert, P.D.N. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology. **6**(4): 998–1002.

Knowlton, N. 1986. Cryptic and sibling species among the decapod crustacea. Journal of Crustacean Biology. **6**(3): 356-363. doi:10.1163/193724086X00208.

Lewis, K.B., Carter, L., and Davey, F.J. 1994. The opening of Cook Strait: Interglacial tidal scour and aligning basins at a subduction to transform plate edge. Marine Geology. **116**(3): 293-312. doi:10.1016/0025-3227(94)90047-7.

McGlone, M.S., Duncan, R.P and Heenan, P.B. 2001. Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand.Journal of Biogeography. **28**(2): 199-216. doi:10.1046/j.1365-2699.2001.00525.x.

Marshall, D.C., Slon, K., Cooley, J R., Hill, K.B.R. and 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**(3): 1054-1066. doi:10.1016/j.ympev.2008.05.007.

Marske, K.A., Leschen, R.A.B., Barker, G.M and Buckley, T.R. 2009. Phylogeography and ecological niche modelling implicate coastal refugia

and trans-alpine dispersal of a New Zealand fungus beetle. Molecular Ecology. **18**(24): 5126. doi:10.1111/j.1365-294X.2009.04418.x.

Mayr, E. 1940. Speciation phenomena in birds. The American Naturalist. **74**(752): 249-278. doi:10.1086/280892.

Posada, D. 2008. jModelTest: phylogenetic model averaging. Molecular Biology. Evolution. **25**: 1253–1256.

Pratt, R. C., Morgan-Richards, M and Trewick, S. A. 2008. Diversification of New Zealand weta (orthoptera: Ensifera: Anostostomatidae) and their relationships in Australasia. Philosophical Transactions of the Royal Society B: Biological Sciences. **363**(1508): 3427-3437. doi:10.1098/rstb.2008.0112.

Proctor, R and Carter, L. 1989. Tidal and sedimentary response to the Late Quaternary closure and opening of Cook Strait, New Zealand: Results from numerical modelling. Paleoceanography. **4**(2): 167-180. doi: 10.1029/PA004i002p00167.

Quek, S., Davies, S., Ilino, T., Pierce, N. 2004. Codiversification in an ant-plant mutualism: stem testure and the evolution of host use in *Crematogaster* (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). Evolution. 58 (3): 554–570.

Ratnasingham, S. and Hebert, P. D. N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology. **7**: 355-364. DOI: 10.1111/j.1471-8286.2006.01678.x.

Roslin, T. 2001. Spatial population structure in a patchily distributed beetle. Molecular Ecology. **10**(4): 823-837. doi:10.1046/j.1365-294X.2001.01235.x.

Shafer, A.B.A., Wolf, J.B.W and Wiens, J. 2013. Widespread evidence for incipient ecological speciation: A meta-analysis of isolation-by-ecology. Ecology Letters. **16**(7): 940-950. doi:10.1111/ele.12120.

Stark, J., Boothroyd, I., Harding, J., Maxted, J., Scarsbrook, M. 2001. Protocols for sampling macroinvertebrates in wadeable streams. New Zealand Macroinvertebrate Working Group Report no.1 ISSN 1175-7791.

Srivathsan, A., Meier, R. 2012. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA barcoding literature. Cladistics. **28**: 190-194.

Suggate R.P. 1990. Late Pliocene and Quaternary glaciations of New Zealand. Quaternary Science Reviews. **9**: 175-197.

Swofford, D. 2002. PAUP*: phylogenetic analysis using parsimony, version 4.0b10 for Macintosh. Sinauer Associates Inc Publishers, Sunderland.

Taylor, D.J., Finston, T.L and Hebert PDN. 1998. Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. Evolution. **52**: 1648-70.

Trewick, S. A and Morgan-Richards, M. 2005. After the deluge: Mitochondrial DNA indicates Miocene radiation and Pliocene adaptation of tree and Giant Weta (Orthoptera: Anostostomatidae): Molecular phylogenetics of weta. Journal of Biogeography. **32**(2): 295-309. doi:10.1111/j.1365-2699.2004.01179.x.

Trewick, S.A., Wallis, G.P and Morgan-Richards, M. 2011. The invertebrate life of New Zealand: A Phylogeographic Approach. Insects. **2**: 297-325. doi:10.3390/insects2030297.

Trewick, S.A and Bland, K. 2012. Fire and slice: Palaeogeography for biogeography at New Zealand's north Island/South island juncture. Journal

of the Royal Society of New Zealand. **42**(3): 153-31. doi:10.1080/03036758.2010.549493.

Vink C.J and Paterson A.M. 2003. Combined molecular and morphological phylogenetic analyses of the New Zealand wolf spider genus *Anoteropsis* (Araneae: Lycosidae). Molecular Phylogenetics and Evolution. **28**(2): 576–587. doi:10.1016/S1055-7903(03)00219-7.

Wilke, T., Schultheiß. R and Albrecht, C. 2009. As time goes by: A simple fool's guide to molecular clock approaches in invertebrates. American Malacological Bulletin. **27**: 25-45.

Williams, B.L., Brawn, J.D. and Paige, K.N. 2003. Landscape scale genetic effects of habitat fragmentation on a high gene flow species: Speyeria idalia (nymphalidae): Genetics of fragmentation in *Speyeria Idalia*. Molecular Ecology. **12**(1): 11-20. doi:10.1046/j.1365-294X.2003.01700.x.

Winkworth R., Wagstaff S., Glenny D and Lockhart P. 2005. Evolution of the New Zealand mountain flora: Origins, diversification and dispersal. Organisms Diversity and Evolution. **5**(3): 237–247.

LIST OF TABLES

Table 1: Individuals of *Archichualiodes diversus* collected from the North and the South Islands of New Zealand

Collection Date	Site	Region	Island
18-Sep-15	Mount Pirongia	Waikato	North Island
21-Jan-2015	Mount Taranaki	Waikato	North Island
11-May-2015	Collins River	Nelson	South Island
15-Apr-2015	Wakapunaka	Nelson	South Island
04-Nov-2015	The Brook	Nelson	South Island
29-Jan-2015	Wai-iti River	Tasman	South Island
03-Dec-2015	Blue Duck River	Kiakoura	South Island
19-Mar-2015	Harris Creek	West Coast	South Island
22-Jan-2015	Opunha River	Canturbury	South Island
22-Jan-2015	Waikaia River	Southland	South Island
22-Mar-2015	Caitlins River	Otago	South Island
10-Apr-2015	Otamita Stream	Southland	South Island

LIST OF FIGURES

and the Australian *Archichauliodes* sp. Sizes of circles are proportional to the number of individuals of each haplotype. The numbers between haplotype groups are the number of mutational steps missing between the respective haplotypes.

Figure 7: Phylogeographic distribution of haplotypes from sites on the North and South Island New Zealand

Figure 8: Maximum likelihood phylogeny showing the distribution of unique haplotypes at the North and the South Island sites.

Figure 9: Hypothetical dispersal of the Cook Strait during the Pliocene (5Mya) and Pleistocene glaciations (2Mya). **A**) Suggests *A. diversus* populations might be allopatric on older Islands before moving between Island through oversea dispersal during the Pliocene, before remaining specific to the two Islands (North Island and South Island) following the LGM. **B**) Suggests that one population of *A. diversus* exists on one Island, expanding the range (Cook Strait land bridge) during the LGM and then becoming separated as sea level rose. Figures adapted from Trewick et al. (2011).

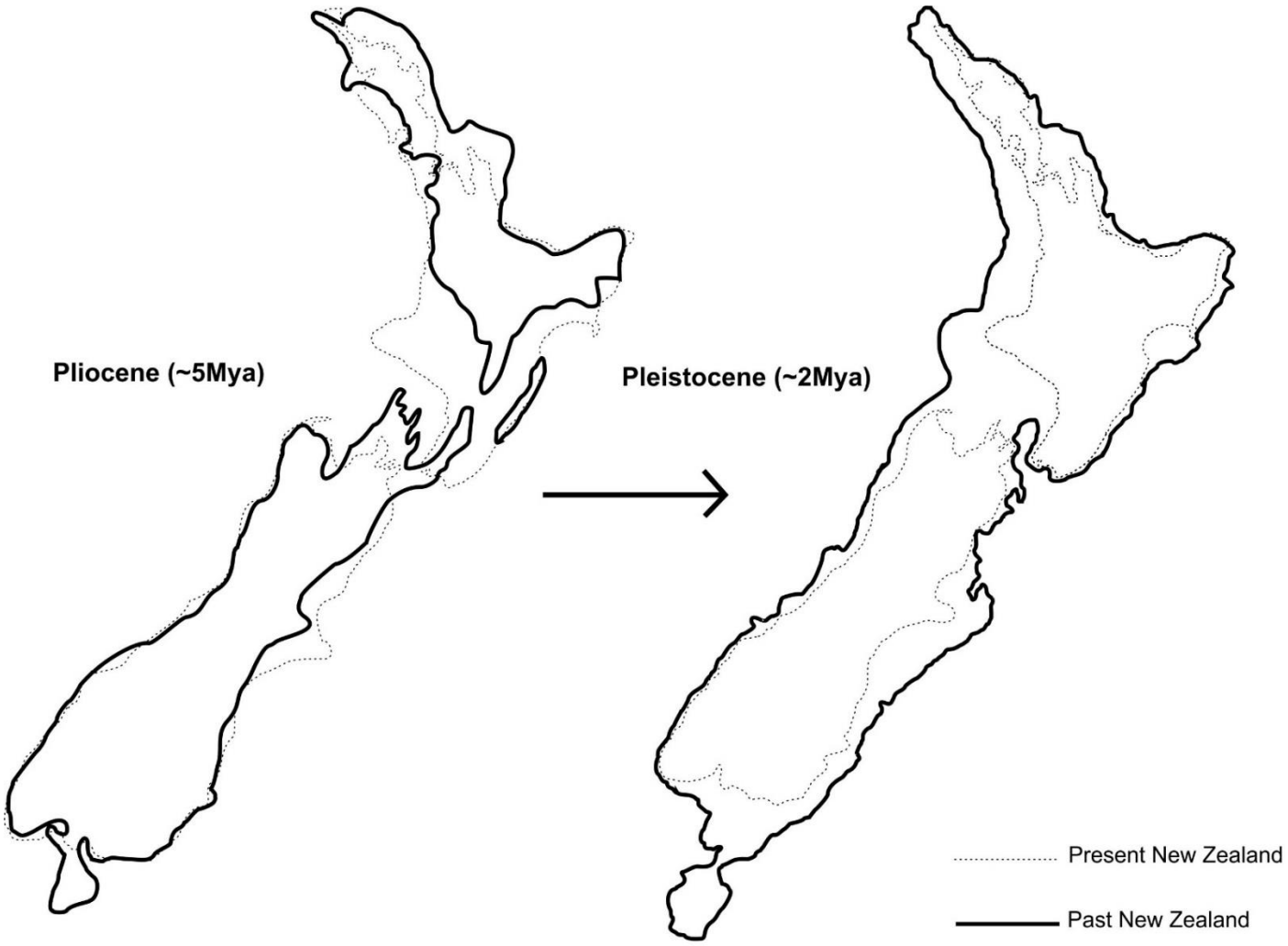

Pliocene (~5Mya)
Pleistocene (~2Mya)
Present New Zealand
Past New Zealand

Fig. 1

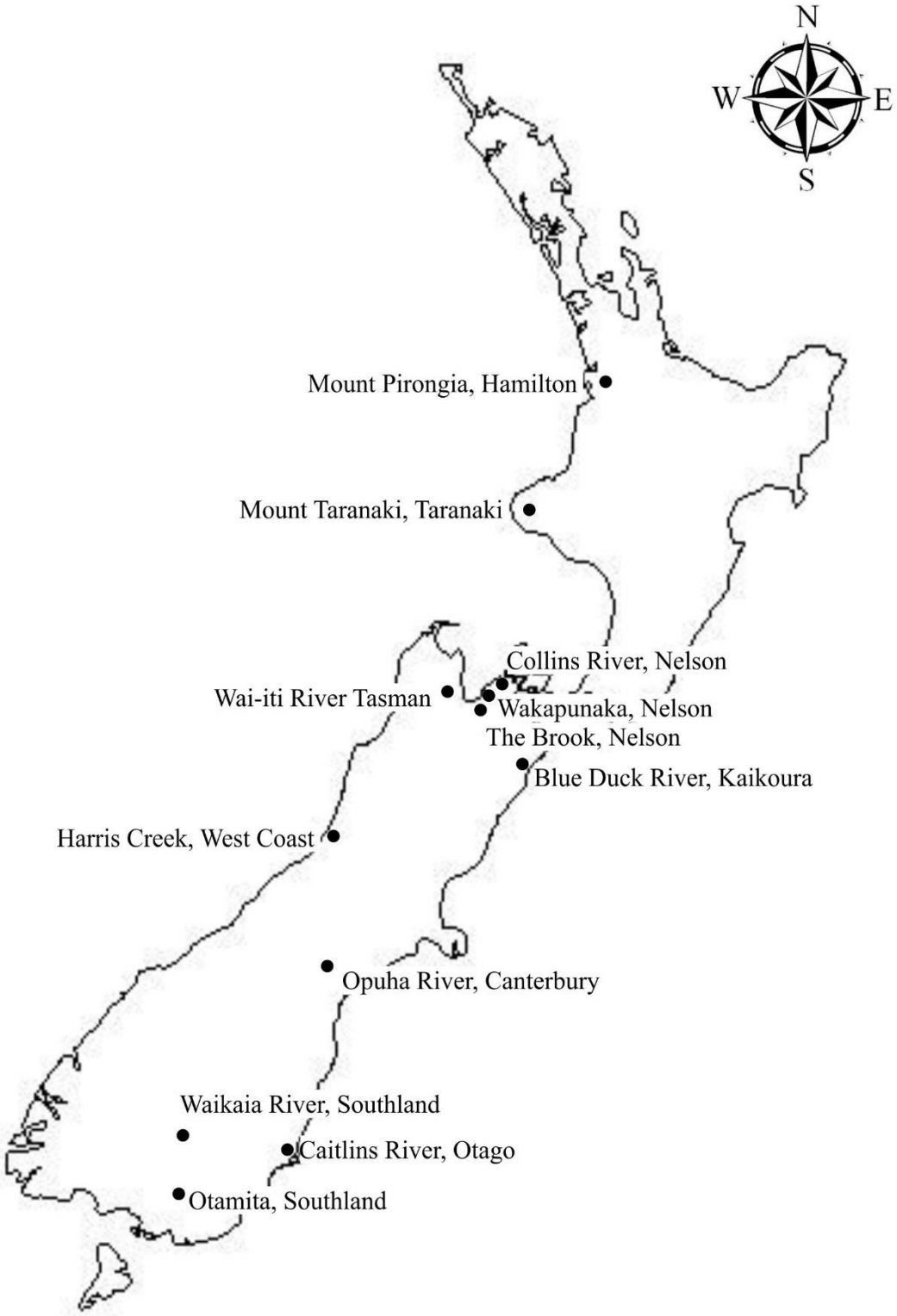

N
W
E
S
Mount Pirongia, Hamilton
Mount Taranaki, Taranaki
Collins River, Nelson
Wai-iti River Tasman
Wakapunaka, Nelson
The Brook, Nelson
Blue Duck River, Kaikoura
Harris Creek, West Coast
Opuha River, Canterbury
Waikaia River, Southland
Caitlins River, Otago
Otamita, Southland

Fig. 2

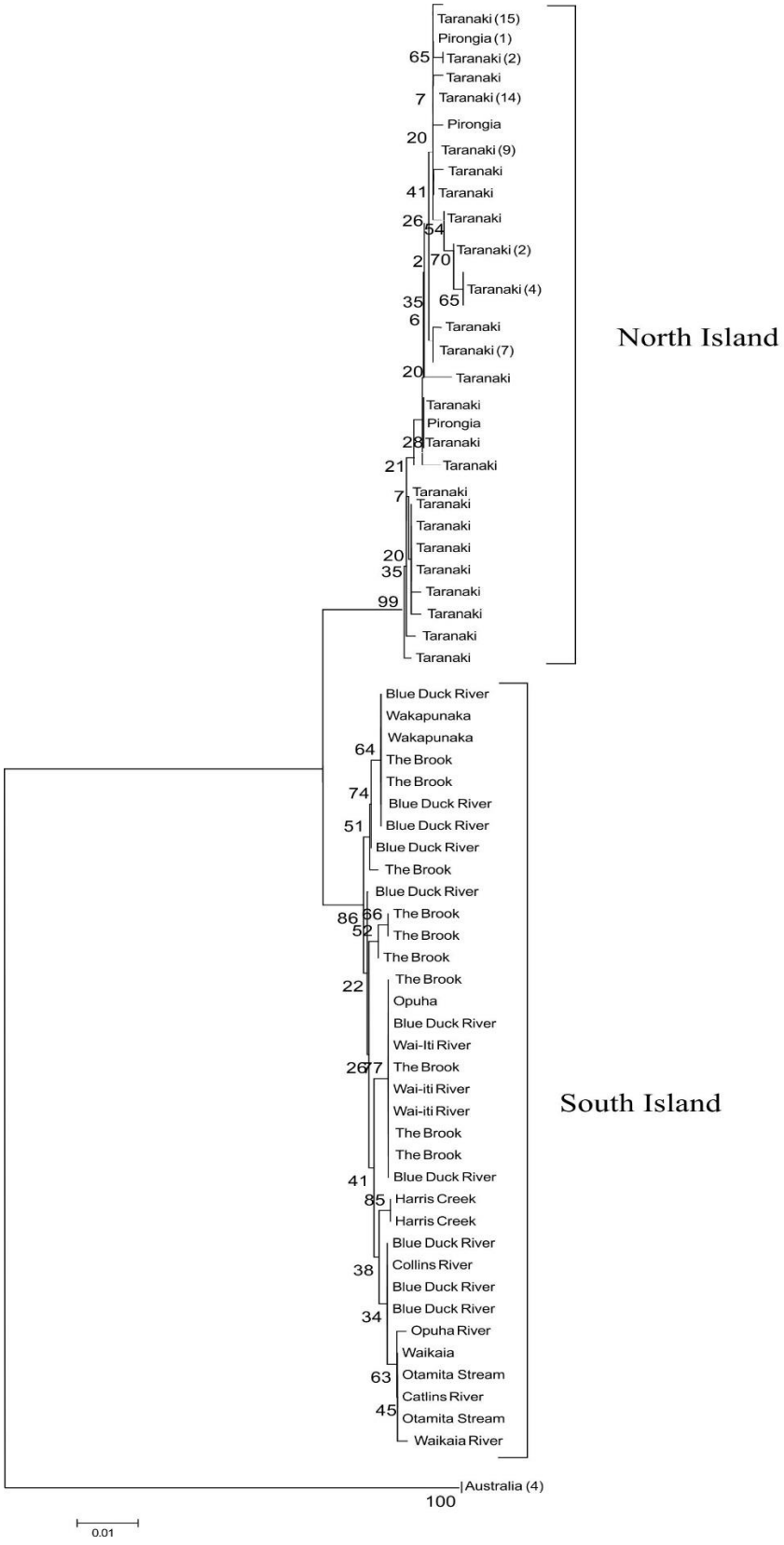

Taranaki (15)
Pirongia (1)
65
Taranaki (2)
Taranaki
7
Taranaki (14)
Pirongia
20
Taranaki (9)
Taranaki
41
Taranaki
26
Taranaki
54
Taranaki (2)
2
70
Taranaki (4)
35
65
6
Taranaki
Taranaki (7)
20
Taranaki
Taranaki
Pirongia
28
Taranaki
21
Taranaki
7
Taranaki
Taranaki
Taranaki
20
Taranaki
35
Taranaki
99
Taranaki
Taranaki
Taranaki
North Island
Blue Duck River
Wakapunaka
Wakapunaka
64
The Brook
The Brook
74
Blue Duck River
51
Blue Duck River
Blue Duck River
The Brook
Blue Duck River
86
66
The Brook
52
The Brook
The Brook
22
The Brook
Opuha
Blue Duck River
Wai-Iti River
26
77
The Brook
Wai-iti River
Wai-iti River
The Brook
The Brook
Blue Duck River
41
85
Harris Creek
Harris Creek
Blue Duck River
38
Collins River
Blue Duck River
Blue Duck River
34
Opuha River
Waikaia
63
Otamita Stream
Catlins River
45
Otamita Stream
Waikaia River
South Island
Australia (4)
100
0.01

Fig. 3

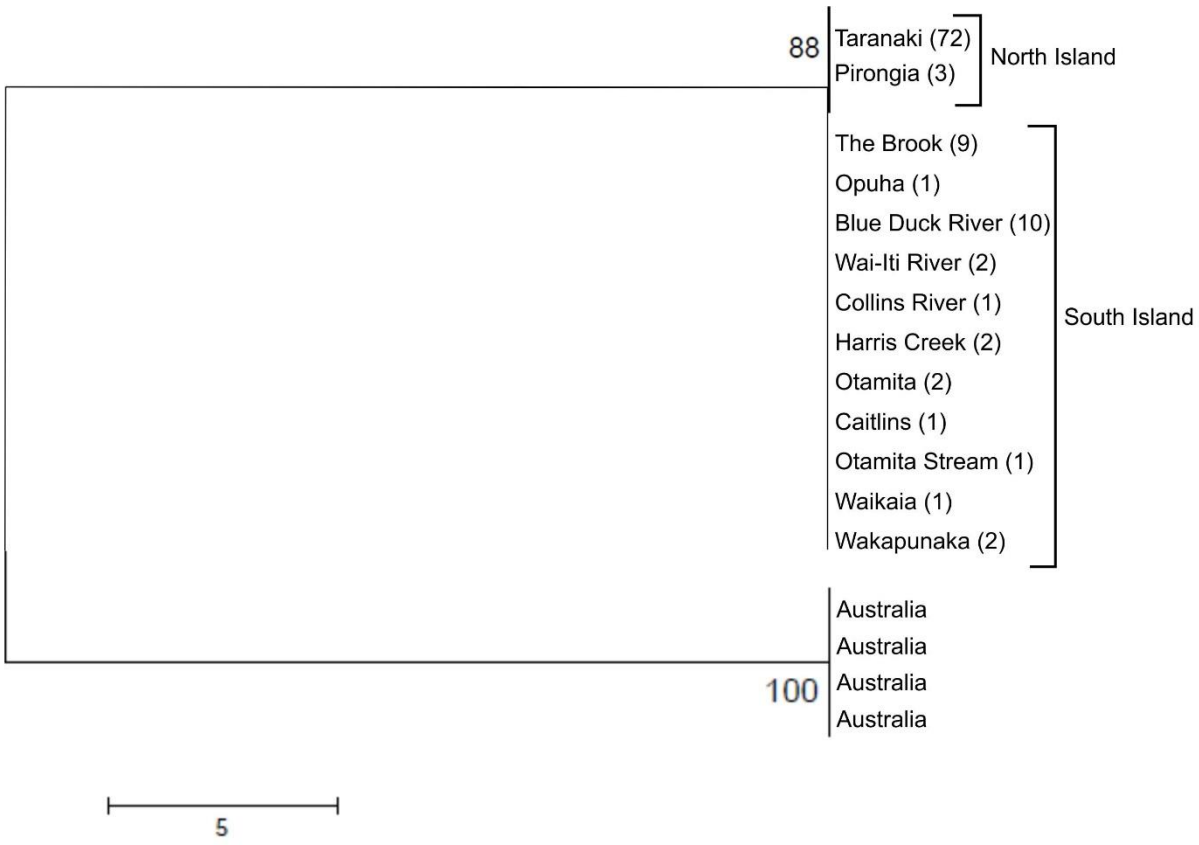

88
Taranaki (72)
Pirongia (3)
North Island
The Brook (9)
Opuha (1)
Blue Duck River (10)
Wai-Iti River (2)
Collins River (1)
Harris Creek (2)
Otamita (2)
Caitlins (1)
Otamita Stream (1)
Waikaia (1)
Wakapunaka (2)
South Island
Australia
Australia
100
Australia
Australia
5

Fig. 4


Australia
Australia
Australia
Australia
Taranaki
Taranaki
Taranaki
Taranaki
Taranaki
Taranaki
Taranaki
Taranaki
North Island
The Brook
Wakapunaka
The Brook
Blue Duck
Blue Duck
Wakapunaka
Blue Duck
Blue Duck
The Brook
The Brook
The Brook
The Brook
Blue Duck
The Brook
Wai-Iti River
Blue Duck
The Brook
The Brook
Opunha
Wai-Iti River
Blue Duck
The Brook
Wai-ITi River
Otamita
Opuha
Waikaia
Waikaia
Caitlins River
Otamita
Blue Duck
Harris Creek
Harris Creek
Blue Duck
Collins River
Blue Duck
South Island

Fig. 5

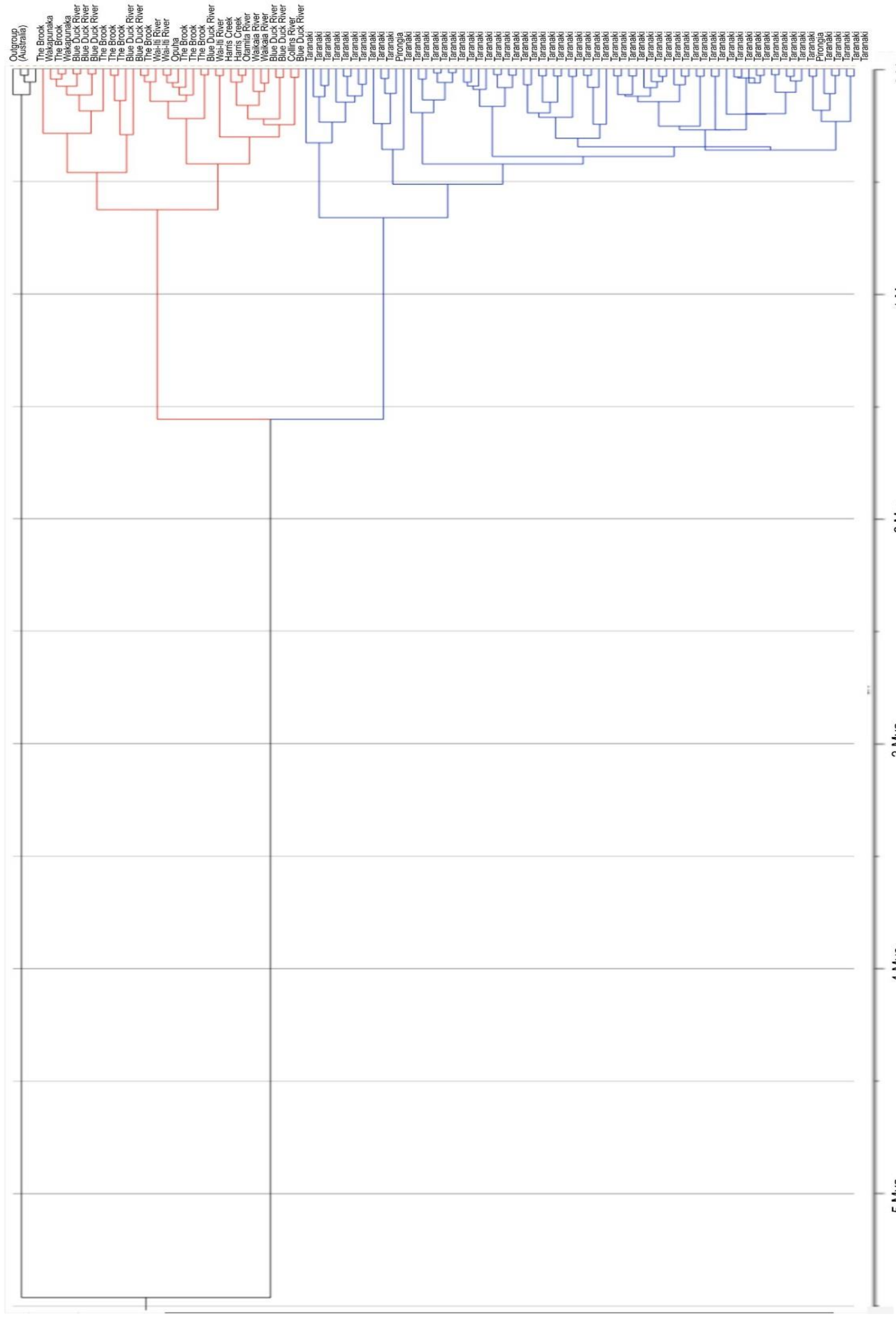


Fig. 6

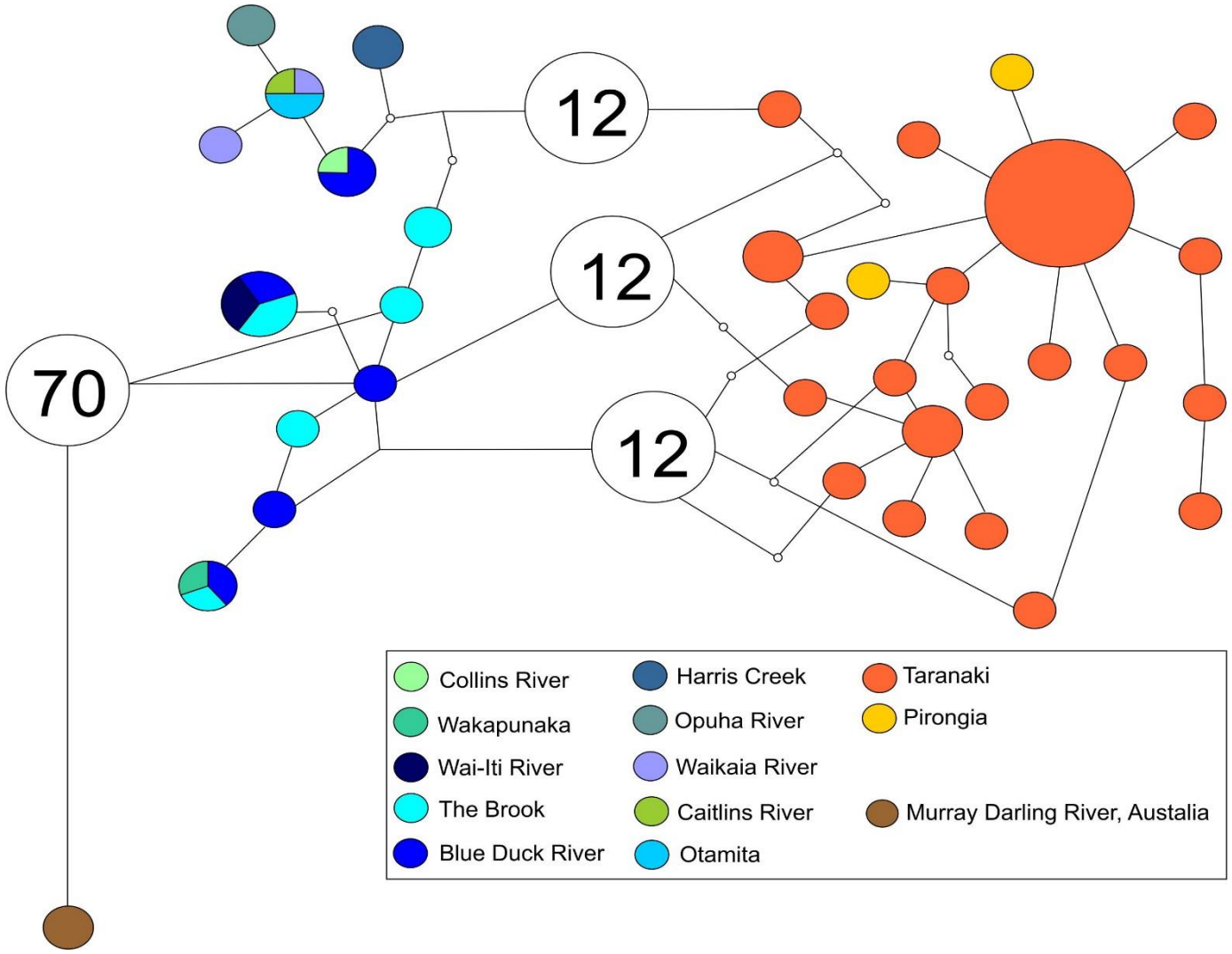

12
12
12
70
Collins River
Harris Creek
Taranaki
Wakapunaka
Opuha River
Pirongia
Wai-Iti River
Waikaia River
The Brook
Caitlins River
Murray Darling River, Austalia
Blue Duck River
Otamita

Fig. 7

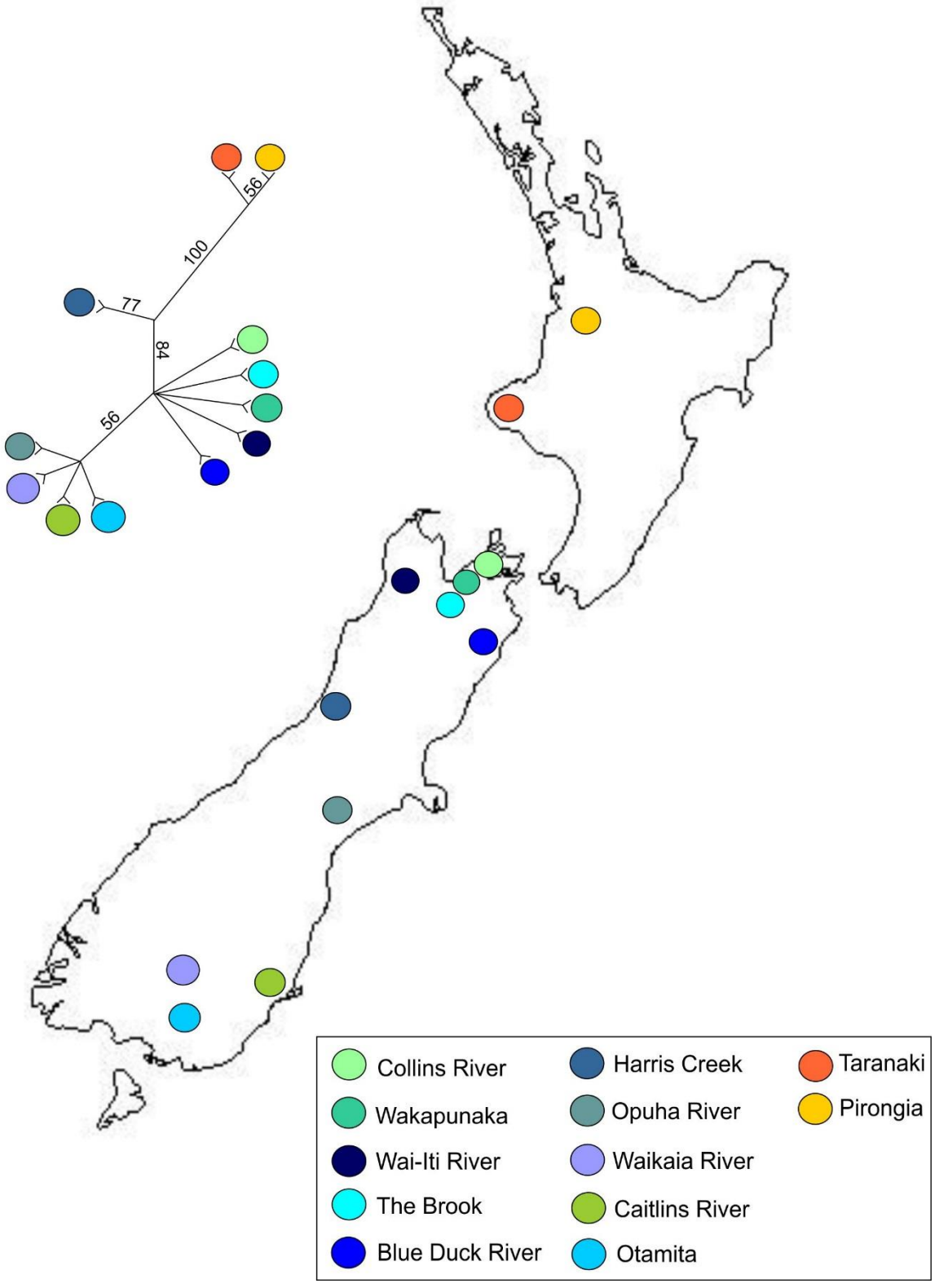

56
100
77
84
56
Collins River
Harris Creek
Taranaki
Wakapunaka
Opuha River
Pirongia
Wai-Iti River
Waikaia River
The Brook
Caitlins River
Blue Duck River
Otamita

Fig. 8

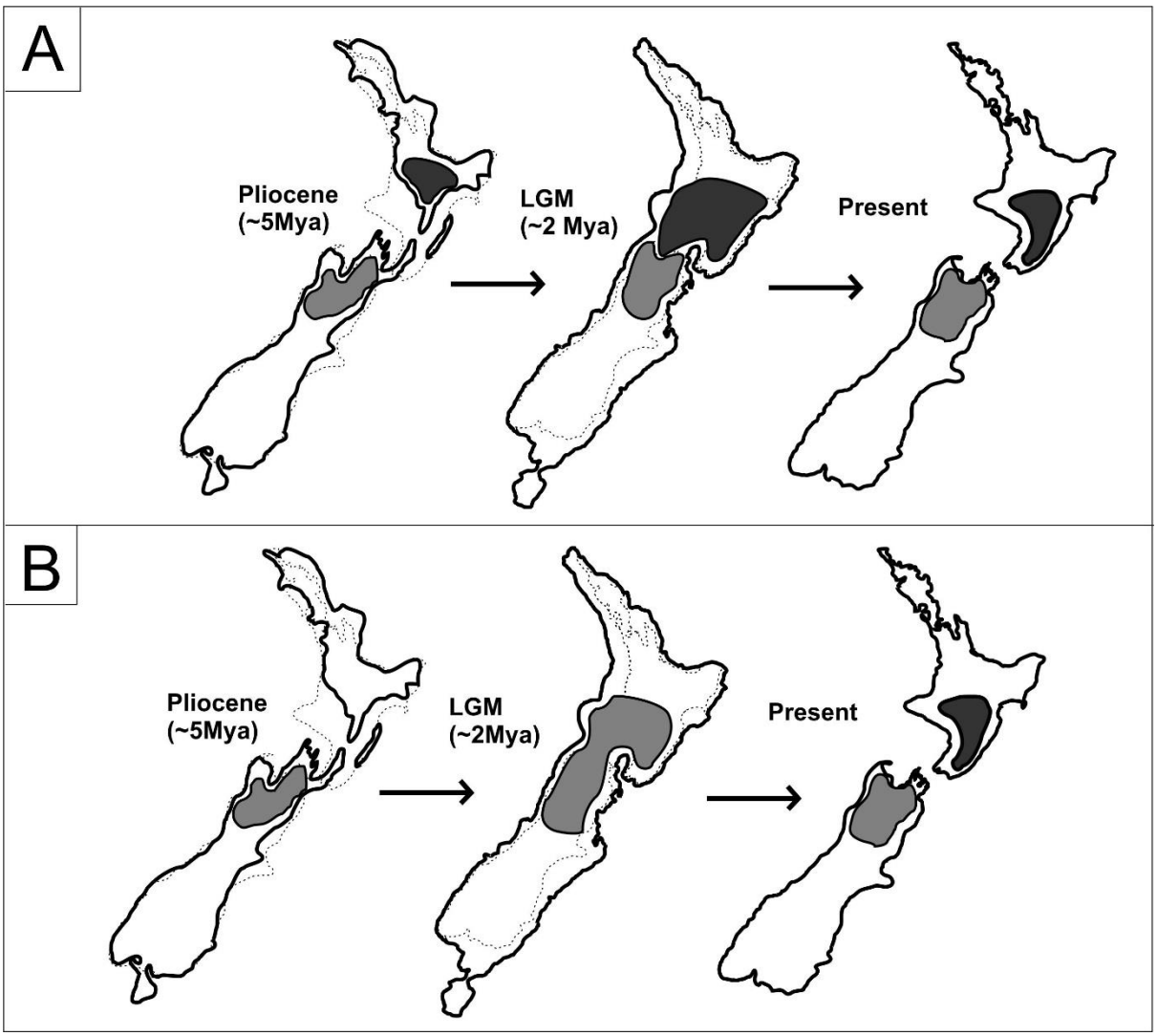

A
Pliocene
(~5Mya)
LGM
(~2 Mya)
Present
B
Pliocene
(~5Mya)
LGM
(~2Mya)
Present

Fig. 9

SUPPLIMENTARY MATRIAL

Fig. S1: Bayesian analysis using a GTR+G+I relaxed clock model showing distinct genetic differences between individuals from the North Island (blue) and the South Island (red) of New Zealand. Individuals of *Archichauliodes* sp. (n=4) from New South Wales, Australia were also included in the analysis as an outgroup.

Fig. S1

CHAPTER IV

Thesis Conclusion

Prior to beginning my thesis research, previous studies had indicated that mitochondrial DNA (COI) gene sequences could provide a useful proxy for overall genomic differences occurring among individuals (Macey et al. 1998; Vandergast, et al. 2004; Trewick et al. 2011). Here, I found that COI sequences were also useful for identifying genetic variation on both small (<50km) and large scales (>1000km). Specifically, I analysed COI sequences for three common aquatic insects *Archichauliodes diversus* (Megaloptera), *Hydropsyche colonica* and *Pycnocentrodes aeris* (Trichoptera) to examine the effects of habitat fragmentation on levels of intraspecific genetic diversity.

In my first research chapter (Chapter II), I used genetic variability across a relatively small scale as a proxy for dispersal. These data were then applied to assess connectivity among source and restored habitats on Mount Taranaki. Of the three species analysed, *Archichauliodes diversus* showed the greatest level of haplotype diversity (19 haplotypes). There were no obvious patterns of differentiation among sites for any of the three species. I suggested that limited differentiation was due to relatively recent gene flow from previously isolated populations and that dispersal was occurring adequately among source and restored habitats. However, for *Pycnocentrodes aeris* one of the less common haplotypes was more prevalent in the source verses the restored habitats. Using the distribution of this haplotype as an example, I hypothesised potential dispersal pathways for *P. aeris* individuals and suggested the boundary of the forested national park had facilitated dispersal at the upper sites. Overall, I concluded that COI sequences can provide a useful indicator for tracking restoration efforts within relatively small geographic scales.

In Chapter III, I determined genetic divergences between populations of the North Island and South Island *Archichaulidoes diversus* and sequences from an

Archichauliodes species from Australia. New Zealand *A. diversus* populations and the Australian *Archichauliodes* sp. showed significant divergence with 70 missing mutational steps based on a haplotype network. Using a molecular clock calibration of 2.3% sequence divergence per million years (Roslin, 2001; Quak et al. 2004; Trewick et al. 2005; Wilke et al. 2009), I was able to estimate that the Australian *Archichauliodes sp.* and New Zealand *A. diversus* diverged around 5 Mya, and coinciding with previous research suggesting species assemblages in New Zealand have originated within the last 5 Myr (McGlone et al. 2001; Vink et al. 2003; Goldberg et al. 2008

Phylogenetic analyses of *Archichauliodes diversus* within New Zealand showed distinct genetic differentiation between the North Island and South Island. Here, isolation was estimated to have occurred around 1.4 Mya during the Pleistocene. It was during this time that New Zealand experienced significant interglacial periods where new habitat allowed range expansion for previously restricted biota (Boyer et al. 2009; Proctor et al. 1989; Alloway et al. 2007). Thus, I concluded that the population genetic structure shown in *A. diversus* could be attributed to allopatric isolation occurring during the Pleistocene glaciations. Isolation of *A. diversus* populations was further apparent within the South Island. Haplotypes were shared between the upper South Island sites whereas others were shared more closely between lower South Island sites suggesting an isolation-by–distance pattern of divergence. Despite the relatively poor flight capabilities of *A. diversus*, individuals of shared haplotypes at sites up to 150km apart. On this basis, I suggested that flight capabilities of *A. diversus* flight may be greater than previously thought, but are limited by dispersal barriers (e.g. Cook Strait between the North and South Islands). Overall, the preliminary morphological analyses showed no obvious morphological differences between individuals from the North

and South Islands. However, given the levels of genetic divergence and present-day isolation of individuals, I concluded that the North and South Island *A. diversus* merit further taxonomic attention as potentially sibling species of relatively recent ancestry.

In summary, my research has investigated genetic similarity as a measure of gene flow (and hence dispersal) among populations of three New Zealand stream insects and examined the effects of small scale fragmentation and large scale historical fragmentation. This work has also broadened our knowledge of the possible applications of using COI sequences to address ecologically-based questions.

FUTURE RESEARCH

The COI gene has previously been used to determine large-scale genetic diversity linking populations on geological scales (Chinn et al. 2004; Pratt et al. 2008; Boyer et al. 2009). The evidence presented in Chapter II suggested that COI can provide useful insight into dispersal occurring on small scales (<50km). However, the more widespread application of using COI gene sequences to assess recolonization of aquatic insects following restoration efforts is still relatively unknown. Further research in this area would be profitable. I selected insects that possessed sufficient sequence diversity (>4 haplotypes in all cases) to facilitate comparisons among locations. However, if genetic diversity is not sufficient enough to infer dispersal, results may suggest adequate dispersal among sites when in fact populations are isolated. Further, given that COI is a maternally inherited mitochondrial gene, results could be biased if there was any strong male-biased dispersal. In this regard, the use of additional nuclear markers may deliver the additional detail necessary to determine dispersal of aquatic insects. Multi-gene locus approaches may provide an opportunity for higher levels of resolution. For example, Yaegashi et al. (2014) used microsatellite data and sequenced individuals of caddisfly and concluded that fine scale estimates of dispersal matched direct observations of flight. Research at this finer scale may provide insight into recovery rates and the recolonization of aquatic insects. Such approaches will allow the basic knowledge for planning corridors or 'stepping stones' for reconnecting local populations of aquatic insects (Baquette et al. 2013). Given that the Taranaki ring plain consists of restored fragmented forested patches, it may be possible to examine dispersal from one forested patch to another both within and among streams using a multi-method approach. Thus, I

suggest using a multiple genetic marker approach for inferring dispersal following restoration which could provide insight into recovery of aquatic insects upon Mount Taranaki.

Chapter III has enhanced our knowledge of the divergence of New Zealand's iconic stream "toebiter" (*Archichauliodes diversus*). Many studies have used phylogenetic techniques to determine the effects of large scale isolation events through genetic diversity of taxa. Specifically, studies suggest the biogeographies of New Zealand have been highly influenced by a dynamic geological past, with species assemblages only originating relative to the Pliocene and Pleistocene (5 Mya) (Trewick et al. 2005; Marshall et al. 2008; Trewick, 2011; Hogg et al. 2006). Future research using next generation sequencing (NGS) through multiple genetic markers holds promise for studies of phylogeography and phylogeny (McCormack et al. 2013). For example, the analysis of multiple markers could provide estimates of population and species history and account for random variation in patterns of gene inheritance (McCormack et al. 2013). Jones et al. (2016) highlights the evolutionary and ecological applications of next generation sequencing (NGS) and suggests minimising reliance on a single approach for all research questions. In this regard, the COI gene data available in the Barcode of Life Datasystems (BOLD) database will be critical to link these data to the biota under study. This will allow for the determining genetic diversity of aquatic insects on greater spatial scales at a relatively low cost.

LITERATURE CITED

Baguette, M., Blanchet, S., Legrand, D., Stevens, V. M and Turlure, C. 2013. Individual dispersal, landscape connectivity and ecological networks. Biological Reviews. **88**(2): 310-326. doi:10.1111/brv.12000.

Behjati, S and Tarpey, P.S. 2013. What is next generation sequencing? Archives of Disease in Childhood. Education and Practice Edition. **98**(6): 236–238. http://doi.org/10.1136/archdischild-2013-304340.

Bilton, D.T., Freeland, J.R and Okamura, B. 2001. Dispersal in freshwater invertebrates Annual Review of Ecology and Systematics. **32**(1): 159-181. doi:10.1146/annurev.ecolsys.32.081501.114016.

Bond N.R. and Lake P.S. 2003. Local habitat restoration in streams: Constraints on the effectiveness of restoration for stream biota. Ecological Management and Restoration. **4**(3): 193–198. doi: 10.1046/j.1442-8903.2003.00156.x.

Boyer, S and Wratten, S.D. 2010. The potential of earthworms to restore ecosystem services after opencast mining – A review. Basic and Applied Ecology. **11**(3): 196-203. doi:10.1016/j.baae.2009.12.005.

Brown B, Emberson R.M, Paterson A.M. 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**(3): 463-473. doi:10.1006/mpev.1999.0662.

Chinn, W. G and Gemmell, N.J. 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: Adaptive radiation within NZ endemic species of the

cockroach. Molecular Ecology. **13**(6): 1507-1518. doi:10.1111/j.1365-294X.2004.02160.x.

Goldberg, J., Trewick, S.A and Paterson, A.M. 2008. Evolution of New Zealand's terrestrial fauna: A review of molecular evidence. Philosophical Transactions of the Royal Society B: Biological Sciences. **363**(1508): 3319-3334. doi:10.1098/rstb.2008.0114.

Hogg I.D., Stevens M.I., Schnabel K.E., Chapman A.M., 2006. Deeply divergent lineages of the widespread New Zealand amphipod *Paracalliope fluviatilis* revealed using allozyme and mitochondrial DNA analyses. Freshwater Biology. **51**: 236–248.

Jones, M. R and Good, J. M. 2016. Targeted capture in evolutionary and ecological genomics. Molecular Ecology. **25**(1): 185-202. doi:10.1111/mec.13304.

McCormack, J.E., Hird, S.M., Zellmer, A.J., Carstens, B.C and Brumfield, R.T. 2013. Applications of next generation sequencing to phylogeopraphy and phylogenetics. Molecular Phylogenetics and Evolution. **66**(2): 526-538. doi:10.1016/j.ympev.2011.12.007.

Marshall, D.C., Slon, K., Cooley, J R., Hill, K.B.R. and 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**(3): 1054-1066. doi:10.1016/j.ympev.2008.05.007.

Parkyn, S.M., Davies-Colley, R.J., Halliday, N.J., Costley, K.J and Croker, G. F. 2003. Planted riparian buffer zones in New Zealand: Do they live up to expectations? Restoration Ecology. **11**(4): 436-447. doi:10.1046/j.1526-100X.2003.rec0260.x.

Pratt, R. C., Morgan-Richards, M and Trewick, S. A. 2008. Diversification of New Zealand weta (orthoptera: Ensifera: Anostostomatidae) and their relationships in Australasia .Philosophical Transactions of the Royal Society B: Biological Sciences. **363**(1508). 3427-3437. doi:10.1098/rstb.2008.0112.

Ratnasingham, S and Hebert, P. D. N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology. **7**: 355-364. doi: 10.1111/j.1471-8286.2006.01678.x.

Trewick, S. A and Morgan-Richards, M. 2005. After the deluge: Mitochondrial DNA indicates miocene radiation and pliocene adaptation of tree and giant weta (Orthoptera: Anostostomatidae): Molecular phylogenetics of weta. Journal of Biogeography. **32**(2): 295-309. doi:10.1111/j.1365-2699.2004.01179.x.

Trewick, S.A., Wallis, G.P and Morgan-Richards, M. 2011. The invertebrate life of New Zealand: A Phylogeographic Approach. Insects. **2**: 297-325; doi:10.3390/insects2030297.

Yaegashi, S., Watanabe, K., Monaghan, M. T and Omura, T. 2014. Fine-scale dispersal in a stream caddisfly inferred from spatial autocorrelation of microsatellite markers. Freshwater Science. **33**(1): 172-180. doi:10.1086/675076.

APPENDICES

Appendix 1: Photos taken from PBRL laboratory microscope under 10x magnification.

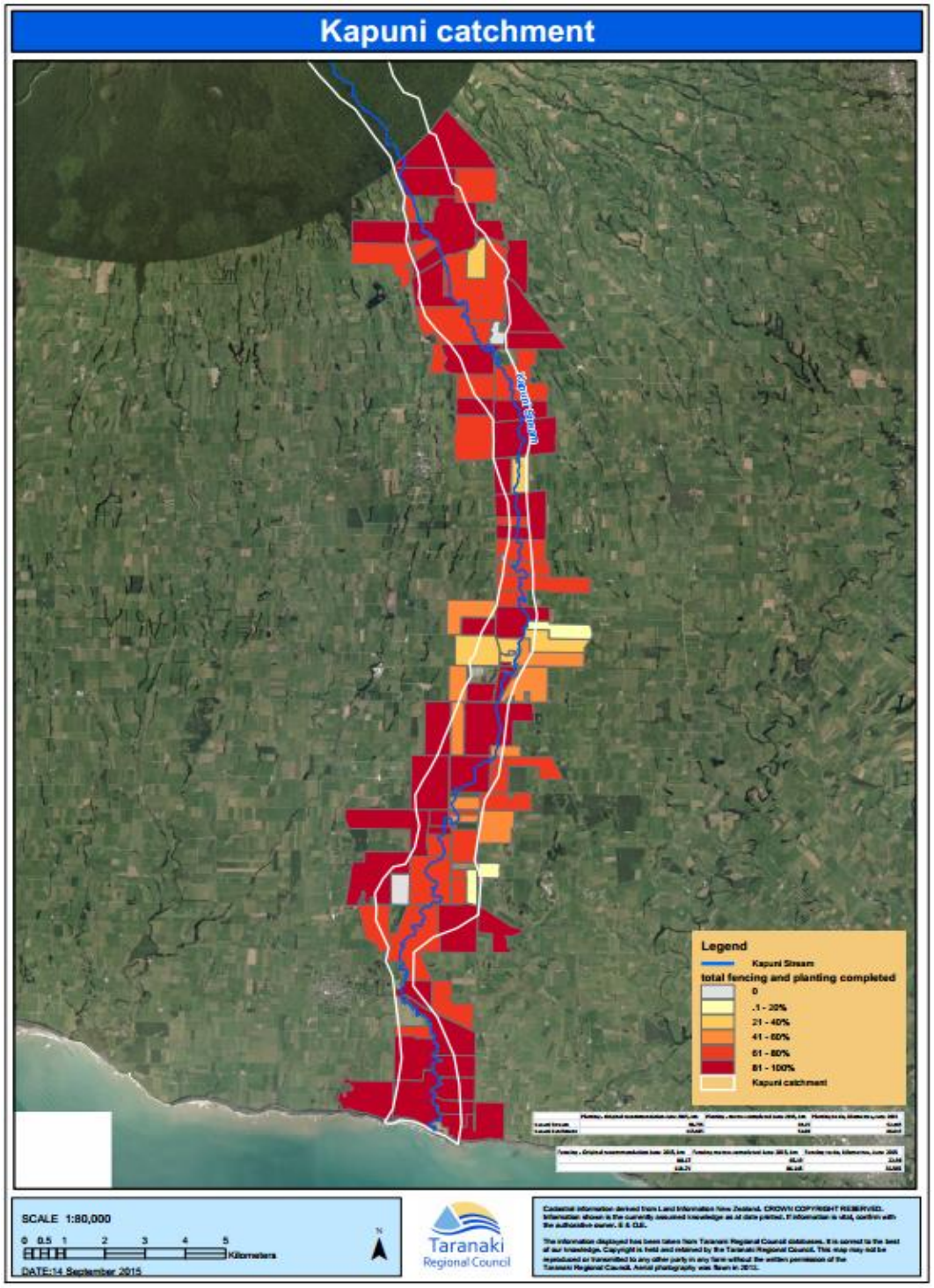
A) *Archichauliodes diversus* **B)** *Hydropsyche colonica* **C)** *Pycnocentrodes aeris*

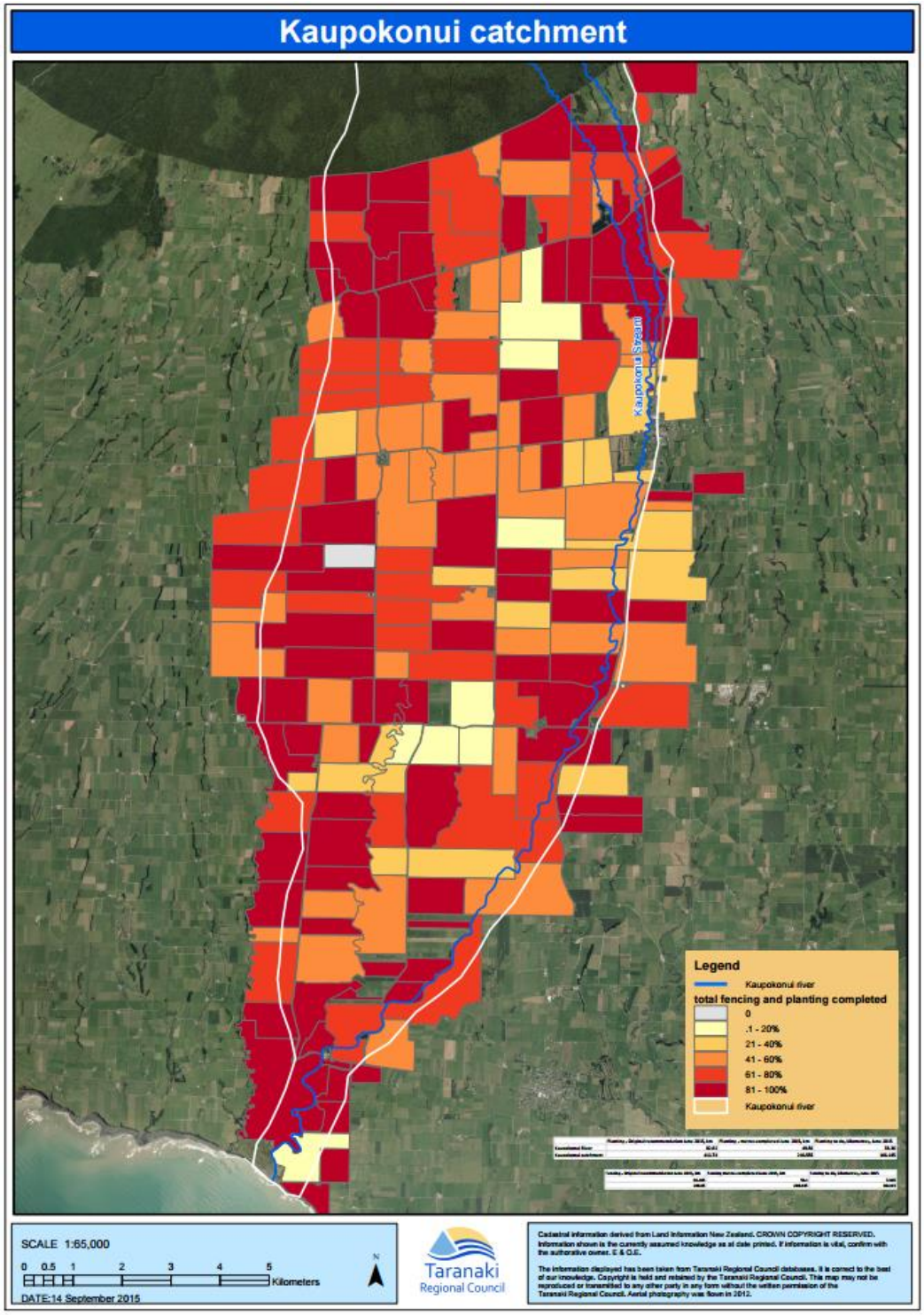
A)

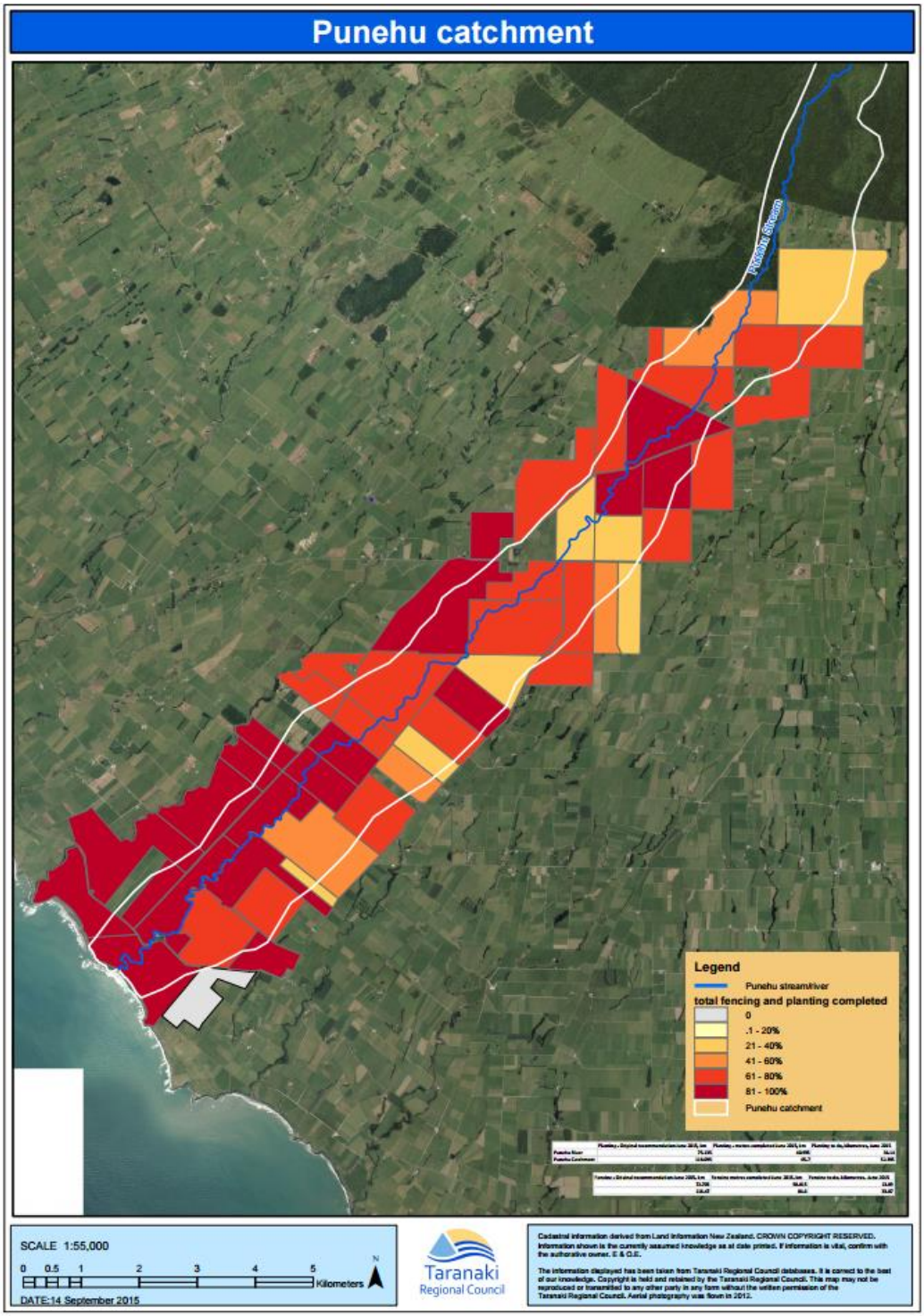
B)

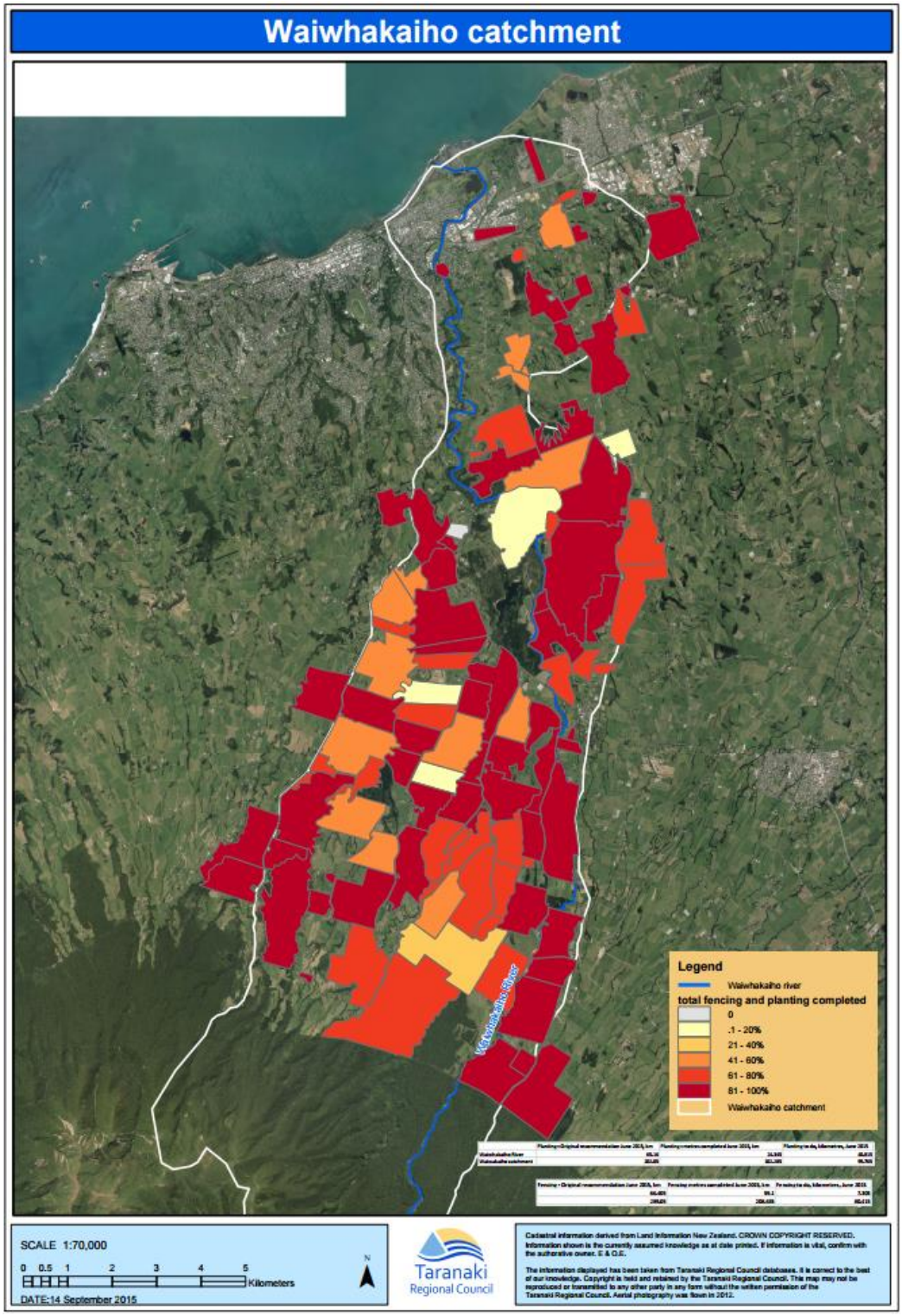
C)

Appendix 2. Restoration (planting and fencing) completion in the Kapuni, Kaupokanui, Punehu and Waiwhakaiho catchments showing patchy conditions that vary on both temporal and spatial scales (Provided by the Taranaki Regional Council, 2015).




Kaupokonui catchment
Kaupokonui Stream
Legend
Kaupokonui river
total fencing and planting completed
0
.1 - 20%
21 - 40%
41 - 60%
61 - 80%
81 - 100%
Kaupokonui river
SCALE 1:65,000
0 0.5 1 2 3 4 5
Kilometers
N
DATE:14 September 2015
Taranaki
Regional Council


Punehu catchment
Punehu Stream
Legend
Punehu stream/river
total fencing and planting completed
0
.1 - 20%
21 - 40%
41 - 60%
61 - 80%
81 - 100%
Punehu catchment
SCALE 1:55,000
0 0.5 1 2 3 4 5
Kilometers
N
DATE:14 September 2015
Taranaki
Regional Council
Cadastral information derived from Land Information New Zealand. CROWN COPYRIGHT RESERVED.
Information shown is the currently assumed knowledge as at date printed. If information is vital, confirm with the authoritive owner. E & O.E.
The information displayed has been taken from Taranaki Regional Council databases. It is correct to the best of our knowledge. Copyright is held and retained by the Taranaki Regional Council. This map may not be reproduced or transmitted to any other party in any form without the written permission of the Taranaki Regional Council. Aerial photography was flown in 2012.


Waiwhakaiho catchment
Legend
Waiwhakaiho river
total fencing and planting completed
0
.1 - 20%
21 - 40%
41 - 60%
61 - 80%
81 - 100%
Waiwhakaiho catchment
SCALE 1:70,000
0 0.5 1 2 3 4 5
Kilometers
N
DATE:14 September 2015
Taranaki
Regional Council