Investigating a Northland Native

Resolving the phylogeographic structure of Northland, New Zealand's *Paryphanta busbyi* land snails

by

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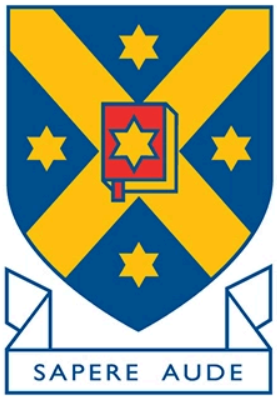

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Abstract

Ever-changing landscapes throughout geological history have resulted in high levels of endemism throughout Northland, New Zealand, with one endemic genus being *Paryphanta* — a threatened and culturally significant land snail. Two morphotypes exist within the genus that have historically been considered distinct species. The larger, green morphotype, *P. busbyi*, ranges from Auckland to Kaitaia, whereas the smaller, black morphotype, *P. watti*, occurs only in the northernmost region of Northland. The clear geographic split between the two morphotypes corresponds with Northland's geological history, therefore, it was originally assumed that the phylogeographic split of genus *Paryphanta* would also follow a north-south pattern. Contrary to this assumption, previous analysis of the COI gene inferred that the two morphotypes were conspecific. However, genetic differences were identified between eastern and western *P. busbyi* populations, with *P. watti* being grouped with *P. busbyi* east. The discrepancies between molecular data and morphological data, that corresponded to Northland's geological history, generated uncertainties surrounding the true phylogeographic pattern of the genus. This project aimed to test, through genotyping by sequencing (GBS) of nuclear DNA, the phylogeographic pattern of *P. busbyi*, and determine if the east-west split of *P. busbyi,* revealed by COI data, is correct. In doing so, it was aimed that an in-depth understanding of the population structure within *P. busbyi* would be achieved, translocated populations would be identified, and inferences about the origins of translocations would be made. Analyses revealed three distinct clades (northern, southern, and Waitākere) and an additional intermediate form. A clear north-south phylogeographic pattern was observed, although this pattern did not include the Waitākere clade, presumably due to divergence following its translocative origins. Throughout the project, science was communicated and recommendations were made to increase the accessibility of the knowledge gained, thereby enhancing opportunities for further research and conservation of the genus. This project was the first to successfully identify thousands of SNPs in *Paryphanta* using GBS, although still highlights the importance of comprehensive assessments, including, where possible, multiple genetic assessments, consideration of morphology, and assessment of phylogeography, regardless of whether multiple species have been identified. It is recommended that these assessments are considered in future management plans to ensure both genetic and morphological variation, as well as phylogeographic patterns, are conserved.

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Chapter One

General Introduction

Abstract

Uncertainties surround the true phylogeographic pattern of *Paryphanta* spp. due to discrepancies between prior genetic analysis and the morphological patterns that correspond to the geological history of Northland. Previous phylogeographic research on *Paryphanta* spp. has only been achieved using COI data, which inferred an east-west phylogeographic split across Northland. This chapter begins by discussing the history of phylogeographic research and the differences between using morphological versus molecular data, as well as the differences between using nuclear versus mitochondrial data. The chapter then discusses the geological history and corresponding phylogeographic trends across Northland before introducing the study genus, *Paryphanta*. Science communication, which is a prominent theme throughout this thesis, is then introduced. Discussions outline the history of science communication, approaches towards science communication and how they have changed over time, as well as effective ways of communicating science to different target audiences. Finally, the justifications for the research conducted for this thesis will be outlined, as will the thesis aims and chapter summaries.

1.1 History of Phylogeographic Research

1.1.1 Evolution and Phylogenetics

Many researchers have credited Jean-Bapstiste Lamarck with being the first to thoroughly consider the theory of evolution in 1809 and others still consider him a highly significant contributor to the theory alongside household names, including Charles Darwin (Packard, 1901; Humphreys, 1996; Koonin and Wolf, 2009; Burkhardt, 2013). In 1837, the first recorded evolutionary tree was sketched by Darwin in his early notes for the publication “On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life” (Darwin, 1859). Both Darwin and Alfred Russel Wallace independently conceived the theory of evolution by natural selection and published their theories in 1858 (Darwin and Wallace, 1858), although the most notable publication is considered to be Charles Darwin’s “On the Origin of Species” (Darwin, 1859).

Phylogenetics is the study of an organism's evolutionary history, inferred by comparing heritable traits such as deoxyribonucleic acid (DNA) sequences or morphology (Honeycutt, 2019). Understanding the phylogeny of an organism, or group of organisms, can aid in taxonomy, which is the classification of organisms from the kingdom level down to the species and subspecies level (Mora et al., 2011). One way to visually depict the relationships between the taxa of interest is to produce a phylogenetic tree, which hypothesises an evolutionary relationship and can be statistically tested to include levels of confidence for each hypothesised tree (Yang and Rannala, 2012). The first comprehensive phylogenetic tree was published by Ernst Haeckel in 1866 and was based on palaeontological, embryological and systemic data (Haeckel, 1866; Hossfeld and Levit, 2016). Haeckel coined the term "phylogeny" in the same publication (Haeckel, 1866; Hossfeld and Levit, 2016), undoubtedly making a considerable impact on the development of phylogenetic studies.

The advancement of phylogenetic studies has resulted in it becoming one of the leading methods used by taxonomists to identify and classify species (de Queiroz and Gauthier, 1990). Typically, the heritable traits, which may be either DNA or morphological characteristics, are compared between taxa, i.e. the ingroup, and against an outgroup. The outgroup must be a separate taxon known to have diverged from all ingroup taxa before the ingroup taxa diverged from one another (Schneider and Cannarozzi, 2009). An outgroup roots the tree and acts as a reference from which the polarity of character changes can be determined (Nixon and Carpenter, 1993; Lyons-Weiler et al., 1998). A shorter evolutionary distance is typically preferred between the outgroup and ingroup, as a closer relationship leaves less room for statistical error (Schneider and Cannarozzi, 2009).

Haplotype networks are another resource used to visualise relationships by comparing DNA sequences, specifically haplotypes. A haplotype is a set of variations at the same genomic locus that are linked and often inherited together (Crawford and Nickerson, 2005). One circumstance in which haplotype networks may be chosen over phylogenetic trees is when studies are looking at intraspecific datasets, particularly when restricted by missing data. Haplotypes may also be beneficial when visual representation of evolution does not appear "tree-like," such as in biological turnover events (e.g. Rawlence et al., 2017). However, since levels of divergence

decrease at lower taxonomic levels, the resolution of the haplotype network should be taken with caution. Multiple sequences may appear identical and potential differences may be neglected that otherwise would have been represented by the missing data (Joly et al., 2007).

1.1.2 Morphological vs Molecular Data

For species lineages to evolve, genetic mutations occur and over time, different nucleotides become fixed or lost within a population, resulting in the divergence of lineages (Levasseur and Pontarotti, 2011). The frequencies of alleles, which are alternative variations of the same gene, may change throughout time through random genetic drift (Masel, 2011). Certain alleles may also be selected for among individuals, which drives evolution on the population level (Nowak et al., 2010). Phenotypes, which are the observable characteristics of an organism, are associated with alleles (Yang et al., 2017). While morphology often reflects molecular changes, morphologically diverse populations may instead represent an ecotype that has evolved due to the organisms' surrounding environment selecting for a particular phenotype, rather than the occurrence of speciation (Foote and Nystuen, 2008; Caizergues et al., 2018). The missed identification of an ecotype may lead to inaccurate conclusions regarding phylogeny where it is assumed that different populations' unique morphology is indicative of a significant molecular divergence. It is possible, however, that their same genes are simply expressed differently as a result of their environment. The misidentification of causative effects can lead to overestimates of diversity and total species richness.

An example of an ecotypic difference that has occurred in nature is the peppered moth (*Biston betularia*). In the mid-nineteenth century, industrial areas in England darkened the landscape with smoke. Peppered moth individuals with a black phenotype were selected for in these regions due to their ability to camouflage, while those in less polluted areas maintained a higher frequency of white phenotypes. Despite the two populations being morphologically distinct, the black phenotype was associated with a series of dominant alleles at a single locus rather than molecular divergence leading to speciation (Cook and Saccheri, 2012).

Molecular data in this case can more accurately reveal the relationships between clades and allow for estimates of additional information, including divergence dates, evolutionary rates, occurrence of translocations, and their origins (Rambaut and Bromham, 1998; Arbogast et al., 2002; Bromham and Penny, 2003; Suchentrunk et al., 2006). However, it is often true that only one or the other of the molecular and morphological data may be available for scientific use. For example, the DNA in fossils may be degraded to a point in which DNA extraction is not possible. Alternatively, too few physical remains of an organism may be available to accurately compare morphology, and thus, in these circumstances, molecular data may be obtained. While it may be optimal to conduct comparisons using both morphological and molecular data, either can be sufficient alone given the researcher's financial or time constraints and the project aims.

1.1.3 Nuclear vs Mitochondrial DNA

DNA in animals is present in cells' nuclei and mitochondria. Depending on whether nuclear (nDNA) or mitochondrial (mtDNA) DNA is sequenced, slight to sometimes significant differences in results can occur, leading to different inferences of lineage timelines or evolutionary patterns (Avise, 2000; Shaw, 2002).

nDNA is passed down lineages following Mendellian biparental inheritance in sexually reproducing animals (Hartl and Clark, 1997). With this form of inheritance, nDNA can provide information from both parental lineages, unlike mtDNA which only provides information about the maternal lineage. Although nDNA has a slower rate of divergence than mtDNA, this rate of divergence can allow researchers to assess deeper divergence within a lineage than is typically capable of mtDNA (Ballard and Whitlock, 2004).

The majority of an organism's genome comprises nDNA due to its larger size in comparison with mtDNA (Doležel and Greilhuber, 2010). With each animal nuclear genome consisting of billions of base pairs, versus thousands of base pairs within the mitogenome (Gregory, 2005; Dufresne and Jeffery, 2011), the nuclear genome provides many more loci for analysis (Roderick, 1996). When more markers are available for sequencing, confidence in the results increases, should the markers indicate the same answer. These markers may be whole genes or single nucleotide

polymorphisms (SNPs) that are present across the entire genome and can be identified using methods such as genotyping by sequencing (GBS; Narum et al., 2013). Many of these loci, including the ITS-2 and other nuclear ribosomal genes, are highly conserved between species while still displaying variable alleles and are therefore considered particularly useful in phylogenetic research (Marcilla et al., 2001; Yao et al., 2010).

With larger and more fragments often being sequenced, it is understandable that sequencing nDNA usually comes at a greater cost. Furthermore, nuclear markers may be more difficult to sequence than mtDNA due to only two copies of any nuclear gene being available versus hundreds to thousands of copies for mitochondrial genes (Bendich, 1987; Birky Jr. et al., 1995; Wai et al, 2010). Another drawback of nDNA in genomic research is that it typically depends on reference genomes (Lander and Waterman, 1988; Howe et al., 2013). When reference genomes are unavailable, such as when studying non-model organisms, nDNA may still be analysed using methods such as those described in Section 2.2.5 *Bioinformatics*, although mtDNA may provide an alternate and more convenient option (Pääbo, 1989). Although, options to analyse nuclear data without a reference genome are also available, for example genotyping by sequencing, which will be discussed in Chapter 2.

mtDNA is commonly used for phylogenetic and biodiversity studies (Avise et al., 1987; Roderick, 1996; Spencer et al., 2006; Nabholz et al., 2009), and early phylogeographic studies saw mtDNA used 80% of the time (Martin and McKay, 2004). Due to its smaller size and higher copy number, the mtDNA is much easier to effectively sequence than nDNA. MtDNA is, with rare exceptions (exhibited by several molluscan genera, e.g. Breton et al., 2007; Rawlings et al., 2010; Van Wormhoudt et al., 2011), maternally inherited and does not undergo recombination (Birky Jr. et al., 1995; Irwin, 2002; Smith, 2016). A much faster mutation rate is exhibited in mtDNA than nDNA, meaning many conspecific populations or closely related species exhibit deep, geographically structured mtDNA patterns (Avise et al., 1987). These patterns mean inferences can be made regarding the evolutionary history of a species or population much earlier within the evolutionary process than when nDNA is used (Unruh and Woolley, 1999; Ballard and Whitlock, 2004). Although, mtDNA provides only a limited representation of the overall evolutionary history (Godinho et al., 2008).

With many more techniques currently available, it may be up to the researchers as to which technique to use. Both nDNA and mtDNA have their advantages and limitations, and so the type of DNA analysed must depend on cost limitations, sequencing technology available, and questions to be answered. Where possible, it can be beneficial to sequence and compare both nDNA and mtDNA, especially in unstudied taxa, as inconsistencies between DNA types may be underestimated otherwise. As the field of genetics progresses, research can be carried out at a more affordable cost, meaning different techniques can be used alongside one another for a multiproxy approach, increasing confidence in answering research questions. However, most times, one option or the other is sufficient. While different techniques may indicate slightly varied results, any type of genetic approach is still considered more reliable than approaches using only morphological data.

1.2 Northland Phylogeography

1.2.1 Formation of New Zealand

Aotearoa New Zealand is an island nation located in the southwestern Pacific Ocean, comprising three main islands (North Island, South Island and Stewart Island) and several hundred smaller islands (Neall and Trewick, 2008; Wallis and Trewick, 2009). Millions of years of geographical isolation have resulted in high endemism of New Zealand's biota (Graham, 2008; Wallis and Trewick, 2009), particularly of plants and invertebrates (McGlone et al., 2001; Buckley et al., 2015). New Zealand was once part of the ancient supercontinent Gondwana, which also included the modern landmasses of Australia, South America, Africa, Madagascar, India, and Antarctica (Graham, 2008). Multiple lines of geological and geophysical evidence have recently identified New Zealand as a part of its own continent, known as Zealandia, despite being considered as part of Oceania for hundreds of years (Mortimer et al., 2017). Here a continent is defined as a large area of continental crust, including both dry and submerged land.

Zealandia was one of the final continents to split from what once was Gondwana, becoming separated from Antarctica and Sahul (the continent comprising mainland Australia, Tasmania,

New Guinea, and surrounding islands) with the formation of the Tasman Sea (Neall and Trewick, 2008). This split began around 82 MYA (Cooper and Millener, 1993; Trewick et al., 2007; Neall and Trewick, 2008) and was fully completed by around 50 MYA (Strogen et al., 2017).

1.2.2 Changing of Northland's Physical Landscape

New Zealand lies on the boundary of the Australian and Pacific tectonic plates (King, 2000), which explains the high levels of volcanism in New Zealand's recorded geological history. Shifting tectonic plates with geological subsidence and uplift, volcanism, Pleistocene glacial-interglacial cycles, and fluctuating sea levels have moulded New Zealand's physical landscape (Carter, 2005; Wallis and Trewick, 2009). This process has been fluid, occurring throughout the Oligocene (33.9 to 23 MYA); Miocene (23 to 5.3 MYA); Pliocene (5.3 to 2.58 MYA); and Quaternary period, which is further divided into the Pleistocene (2.58 MYA to 11.7 KYA) and Holocene (11.7 KYA to present). These geological processes have caused New Zealand's geographic barriers, such as mountain ranges, glaciers, sea levels, and straits, to be continually modified. Geographic barriers will typically have differing permeability for different taxa, isolating and connecting populations from one another over time. With certain taxa being affected to a greater degree, speciation occurred at different rates for different taxa. For example, the introduction of additional oceanic barriers from rising sea levels would have been close to impermeable to terrestrial invertebrates with strong intolerances to salt water. In comparison, marine organisms would have been more tolerant to these rising sea levels.

Varying proportions of Zealandia's continental crust have been submerged throughout history. Higher sea levels throughout the Pliocene meant many of Northland's present day coastal headlands were isolated as islands, including Tohoraha/Mount Camel, parts of the Karikari Peninsula and the northern tip of the Aupōuri Peninsula/Te Hiku o Te Ika (Figure 1.1; McLintock, 1966; Hare et al., 2008). New Zealand underwent repeated cycles of glaciation in the Pleistocene (Carter, 2005) and although Northland was devoid of glaciers (Wallis and Trewick, 2009), the global impact of the Last Glacial Maximum (LGM; occurring in New Zealand between 29-19 KYA) caused sea levels to fall globally to an average of 120 m below that of today (Peltier and Fairbanks, 2006), once again changing the coastline of New Zealand (Beu and

Edwards, 1984; Verry et al., 2022). With this sea level change, New Zealand's three major islands and most of the current offshore islands became connected (McLintock, 1966), allowing many previously isolated taxa to once again interact. However, due to the radiation of animal and plant species throughout the long-term isolation of the islands, many animals would have remained restricted to their original territories long after the formation of land bridges. It is likely that this high level of endemism would have driven allopatric speciation that reflected the original territories of the Pliocene islands, despite these territories no longer being physically isolated.

One exemplary region where high levels of allopatric speciation have occurred is Northland, particularly the northern region of the Aupōuri Peninsula. At present, this finger of land is a tombolo connecting the former island at the northern tip to mainland Northland (Powell, 1947; Neall and Trewick, 2008). With this area's distinct coastal environment, many forest species that existed in the northern region during the Pliocene have remained isolated from the mainland ever since (Powell, 1947). The borders of present-day forested areas act as boundaries to constrain population distribution for forest-dwelling species. Therefore, despite the Pliocene islands being physically connected to the rest of Northland, many species may have been unable to escape these forested areas to migrate and establish further south, which explains Northland's continued high levels of endemism.

New Zealand's, including Northland's, physical landscape has remained relatively stable throughout the Holocene. Presently, around 94% of Zealandia's continental crust is submerged, with only New Zealand and New Caledonia above sea level (Wallis and Trewick, 2009; Mortimer et al., 2017). Present-day pressures on population distribution can be attributed largely to anthropogenic factors rather than active geological changes. Upon East Polynesian arrival in New Zealand in the late 13th century, fires were used to clear forested areas (McWethy et al., 2014), resulting in New Zealand's forested areas declining from around 80% of land cover to 55% by the 19th century (Taylor and Smith, 1997). The advent of European settlers in the 19th century led to increased use of fire for forest clearance for the development of farming and urban areas, and hence further deforestation (McWethy et al., 2010). Reports by the Ministry for Primary Industries show that in 2010, as little as 24% of land cover was native forest and a

further 6% was plantation forest (MPI, 2012). Environmentalism has grown in popularity in New Zealand in recent years, as seen by the constant rise in forested areas. Updated reports by MPI indicate native forest has grown to make up 30% of land cover, with an additional 8% of land cover from plantation forest (MPI, 2020).

While trends show promising growth in New Zealand's forested areas, human-started fires for forest clearance are not the exclusive cause of bushfires, and there is still a large risk of forest fragmentation and habitat loss from naturally caused fires. Present-day Northland has a warm, temperate climate with drought-prone northern areas (Chappell, 2013). Introduced mammals threaten endemic species by occupying primary forests where they predate on native animals and degrade undergrowth. These factors, in combination with the Northland climate, exacerbate the risk of uncontrolled forest fires today (Guild and Dudfield, 2009; Whitlock et al., 2010; McWethy et al., 2013, 2014).

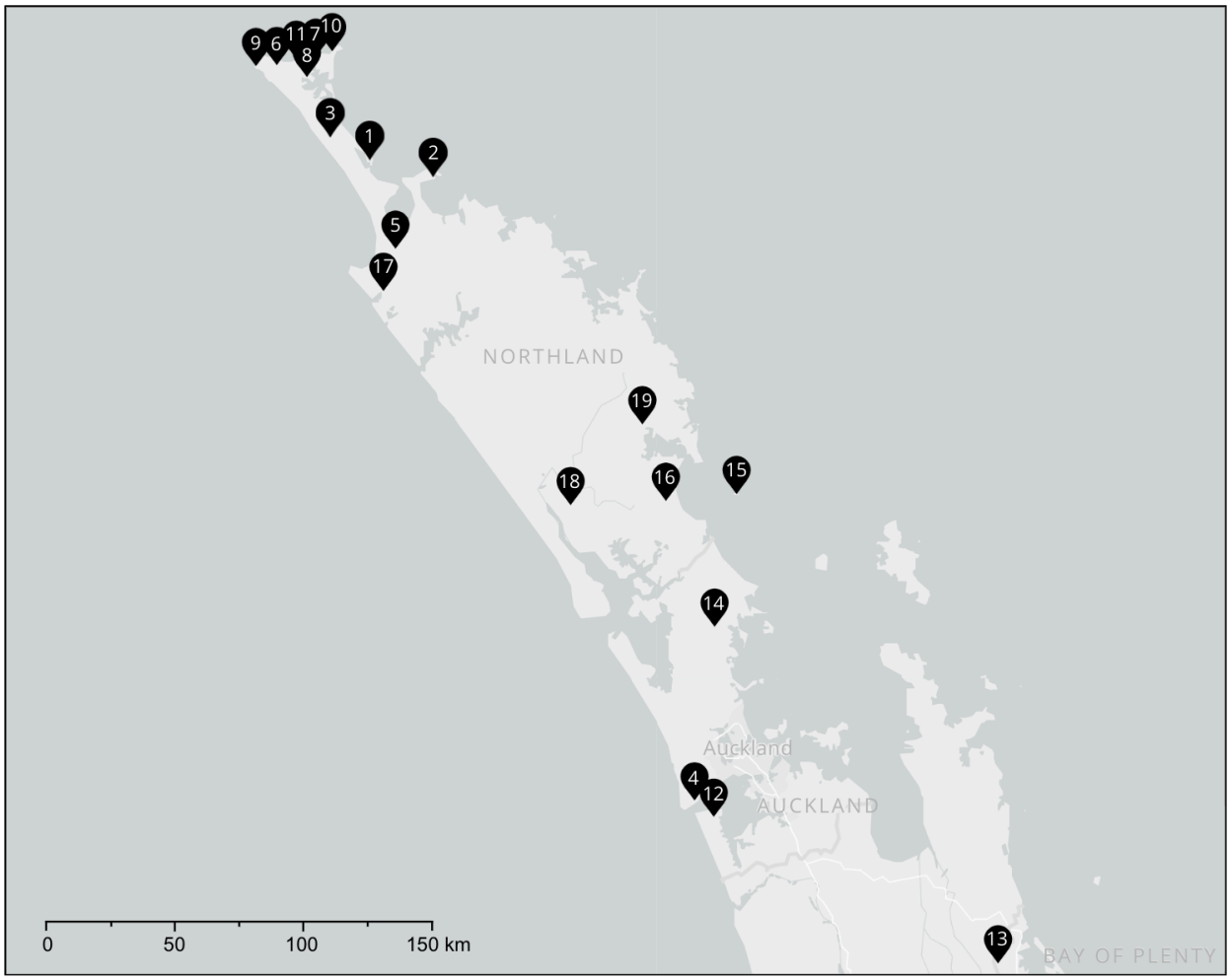


Figure 1.1 Relevant locations for the genus *Paryphanta* across the Northland, Auckland, and Bay of Plenty regions in New Zealand, in order of mention: 1) Tohoraha/Mount Camel, 2) Karikari Peninsula, 3) Aupōuri Peninsula/Te Hiku o Te Ika, 4) Waitākere Ranges, 5) Kaitaia, 6) Te Paki, 7) Unuwhao, 8) Kohuronaki, 9) Herangi Hill, 10) Tom Bowling Bay, 11) Piwhane/Spirits Bay, 12) Āwhitu Peninsula, 13) Kaimai Ranges, 14) Warkworth, 15) Taranga/Hen Island, 16) Waipu, 17) Herekino, 18) Kaipara, 19) Whangārei.

1.2.3 Phylogeography

From the above discussion, it is clear that geological and geographical changes across time can influence the speciation and radiation of species. Phylogeography, a term coined by Avise et al. (1987), refers to the distribution and frequency of genotypes across geographic space and which

factors, such as climate changes, glaciation cycles, sea level changes, river capture, or other geological events, may be responsible for the observed patterns (Marske, 2016). This field of study can be described as a sub-discipline of biogeography, which is the study of species distribution and density, as well as the factors responsible for the observed patterns (King et al., 2006; Leduc, 2009). Phylogeography can also be considered multidisciplinary, requiring input from molecular genetics, population genetics, ethology, demography, phylogenetic biology, palaeontology, geology, and historical geography (Avise, 2000).

Applications of Phylogeographic Research

Phylogeographic studies can be used to address questions covering the full spectrum of ecology and evolution (Marske, 2016). The origin and maintenance of regional and global biodiversity patterns can be inferred from phylogeographic research, including the locations, rates, dates, and modes of diversification (Bowen et al., 1991; Martin and McKay, 2004; Pyron and Burbrink, 2010; Marske, 2016).

The location of origin of a species can be deciphered by comparing the genetic variation of current populations. Typical trends show greater genetic variation within older populations; thus, populations with the highest genomic variation may be considered to be closer to the location of origin (Templeton, 1997; Martin and McKay, 2004). This effect can be explained by bottlenecks occurring within founding populations following a migration or translocation. Bottlenecks decrease genetic variation within the population and following the occurrence of a bottleneck, time is necessary for additional mutations to become fixed.

Evolutionary rates can be determined to provide temporal context to evolutionary history by using equations that compare the genetic divergence across time. While early evolutionary rates were calculated using a strict molecular clock, current standards account for differences in evolutionary rates across lineages (Ho et al., 2015; Dos Reis et al., 2016; Kumar and Hedges, 2016). Once the rate of evolution is determined, an estimated date of divergence can be calculated (Lee and Palci, 2015; Sagulenko et al., 2018).

Phylogeographic patterns can also reveal modes of speciation, with the assumption that the location of origin of each species being compared is accurate (Losos and Glor, 2003). Three common phylogeographic patterns each relate to either physical (hard) or ecological (soft) allopatric speciation, or a combination of both (Pyron and Burbrink, 2010). Speciation may also occur through alternative modes, for example, sympatric, peripatric, and parapatric speciation (Pyron and Burbrink, 2010). However, while these alternate modes of speciation would have occurred throughout parts of Northland, looking at the big-picture alone can infer much about a taxon's mode of speciation. Considering the assumption regarding location of origin combined with the high biodiversity in Northland and its many regions known to be previously isolated as islands, it is likely that much of the present-day taxa in these areas, including the study species of this project, diversified by means of allopatric speciation.

Once the location of origin, divergence dates, evolutionary rates, and mode of speciation are deciphered, patterns of genetic variation can be observed across time to assess other aspects of species' lineage. Inferences can be made regarding restricted gene flow, long-range colonisation, fragmentation, or range expansion (Templeton et al., 1995; Garrick et al., 2009), thus providing more context to a species' history (Irwin, 2002). This knowledge can then be used for informed decision-making around the conservation and prioritisation of phylogeographically distinct populations.

Biogeographic Regions

Ecologically and geographically distinct regions are considered biogeographic regions, which is a rankless term used to describe the biodiversity in any area, although often an area that is ecologically and geographically distinct (Udvardy, 1975; Vilhena and Antonelli, 2015). Since biogeographic regions are a subjective categorisation based on ecological and geographical similarities, not all researchers will agree where the borders to these regions lie (Udvardy, 1975; World Wildlife Fund, 2022). Biogeographic regions are often used as a reference point to describe regions of interest for conservation purposes; therefore, it is often in the researcher's best interest to expand the geographical boundaries as much as possible in order to conserve as much of the region as possible.

The geographical relationships of New Zealand's taxa have long been studied (Hutton, 1884) and researchers have proposed a range of biogeographic regions to categorise New Zealand's organisms into their ecological communities. New Zealand and its offshore islands have been recognised as a single biogeographic region on the world scale (Myers et al., 2000), or as multiple biogeographic regions, with the precise number alternating between researchers (Barker, 2005; Shears et al., 2008). In the case of land snails, there have been as many as 36 proposed biogeographic regions across New Zealand and its offshore islands (Barker, 2005). Other non-species-specific estimates are different again and are often made on the macro-scale (Barker, 2005), which incorrectly assumes all regions are equal in species richness and conservation status of their species.

Due to the geological history of Northland and the proportionately high level of endemism that has resulted (Wallis and Trewick, 2009), there are many parts of this region that deserve to be considered distinct biogeographic hotspots. This categorisation means that for conservation purposes, the area of a biogeographic region is not proportionate to its importance. While the Northland region has a smaller area in comparison to other New Zealand regions, Northland and many of its specific locations have proportionately larger species richness and therefore may receive increased attention for conservation.

Northland Phylogeographic Trends

While an east-to-west phylogeographic split is typical across the North Island (Wallis and Trewick, 2009), a north-south disjunction across Northland is commonly observed, with the northern tip of the Aupōuri Peninsula distinct from the rest of Northland (Spencer et al., 2006; Marshall and Barker, 2007, 2008).

The present-day Aupōuri Peninsula features farmland and occasional pine forest covering a low-lying tombolo. With the constant movement of sand and its lack of natural forested areas, the Aupōuri Peninsula continues to act as a barrier, separating forest-dwelling species that were once isolated to either the northern tip or the rest of Northland to the south. This barrier has contributed hugely to the north-to-south phylogeographic structure in the region, which is

consistent for terrestrial species such as the Auckland tree wētā (*Hemideina thoracica*; Morgan-Richards et al., 2001), the flax snail (*Placostylus* sp.; Triggs and Sherley, 1993; Ponder et al., 2003), and land snails within genus *Amborhytida* (Spencer et al., 2006).

An intermediate location is represented by Tohoraha/Mount Camel on the Aupōuri Peninsula and features species that are typically found either only north (e.g. *Placostylus ambagiosus*; Stringer and Grant, 2007), or only south (e.g. *Amborhytida* sp. "Aupouri"; Spencer et al., 2006), as well as featuring its own endemic species (e.g. *Cytora houhora*; Marshall and Barker, 2007; *Allodiscus camelinus*; Marshall and Barker, 2008). This uniqueness can be explained by its past as a Pliocene island, as well as its geographic location between Northland's northern tip and the rest of Northland.

While patterns may be consistent across multiple taxa, they do not necessarily reflect the phylogeographic history of all taxa in the region. An alternate east-west phylogeographic split has also occurred across Northland, demonstrated again by multiple plant and animal taxa, including the whau tree (*Entelea arborescens*; Shepherd et al., 2019), and shore skink (*Oligosoma smithi*; Hare et al., 2008).

In general, phylogeographic patterns do not need to be uniform across regions or taxa. While the phylogeographic history of one species may be used to infer information about another, genetic analysis is usually necessary to determine exactly where true phylogenetic splits lie for the taxa in question.

1.3 Rhytididae and the Genus *Paryphanta*

1.3.1 Phylum Mollusca

Invertebrates are a paraphyletic animal taxon, characterised by a lack of vertebral column (Britannica, 2022). Although invertebrates represent as many as 97% of all animal species (May,

1988), this taxon is proportionately underrepresented in published literature compared to vertebrates (Titley et al., 2017).

Molluscs (Phylum Mollusca) are a clade of soft-bodied bilateral invertebrates. While their body plans vary, molluscs typically have a soft and unsegmented body, a muscular foot, and a mantle that can secrete a calcareous shell (Brown and Lydeard, 2009). Members of Phylum Mollusca are classified based on morphological differences. These classes are not strictly defined, though the groupings of Gastropoda, Bivalvia, Cephalopoda, Scaphopoda, Aplacophora, Polyplacophora, and Monoplacophora are widely supported (Moretzsohn et al., 2009; Purchon, 2013).

Molluscs first appeared in the fossil record during the early Cambrian period around 550 MYA (Moretzsohn et al., 2009; Runnegar, 2020). The exact point of their emergence, however, is not clearly agreed upon as there are debates surrounding whether certain ancient Cambrian animals, such as genera *Kimberella* (Fedonkin and Waggoner, 1997; Fedonkin et al., 2007; Ivantsov, 2010; Parkhaev, 2017) and *Wiwaxia* (Butterfield, 2006; Caron et al., 2006), are ancestral molluscs or belonging to another phylum.

Molluscs make up the second most species-rich phylum in the kingdom Animalia (Moretzsohn et al., 2009; Prié, 2019; Cowie et al., 2022). However, scientists have not reached a consensus on the total number of extant mollusc species due to a number of inconsistencies in taxonomic classification and biodiversity estimates that have altered where species boundaries are considered (Wesselingh et al., 2019). Firstly, unresolved cohesion between taxonomists and changes in taxonomic approaches over time have led to inconsistencies in species estimates. Previously, taxonomy relied on morphological differences (Lydeard et al., 2004), though with advances in genetic approaches, molecular analysis is increasingly utilised in determining taxonomic classifications. A large proportion of mollusc species have been described by shell collectors without scientific backgrounds, potentially creating a bias towards aesthetically appealing morphs and those with greater value in shell collections. Secondly, as a result of vicariance and long-distance dispersal, molluscs are geographically distributed across all seven continents as endemic and invasive species, existing in marine, freshwater, and terrestrial habitats (Herbert, 2010; Gittenberger, 2012; Kappes and Haase, 2012). Considering Mollusca's

immensely diverse range is largely unexplored by humans, many further species remain undescribed or undiscovered (Lydeard et al., 2004). Biodiversity can be estimated using different approaches, though estimates obtained from different methods are subject to error and may not align (Cameron and Pokryszko, 2005). Current estimates of total extant species circulate around 85,000 (Joseph, 2016), 100,000 (Strong et al., 2007), or up to 120,000 (Rosenberg, 2014; Prié, 2019).

Some of the more recognisable molluscs include bivalves (e.g. mussels, clams, and oysters) and cephalopods (e.g. octopuses and squid) (Moretzsohn et al., 2009; Purchon, 2013). The most diverse molluscs, and the only class that includes several terrestrial clades, are gastropods (i.e. snails and slugs), making up three quarters of known molluscs (Brown and Lydeard, 2009). The term "gastropod" is derived from the Greek language and is a reference to the positioning of the stomach and foot that is unique to this class (Online Etymology Dictionary, 2017). Many gastropods breathe air, though this characteristic is not exclusive to a terrestrial lifestyle (Dayrat et al., 2011). Snails and slugs that breathe air are referred to as pulmonates and make up roughly 20,000 gastropod species (Brown and Lydeard, 2009), including the subject of this thesis.

Land snails are slow-moving and live in a vast range of climates, from tropical to desert to temperate (Bloch and Willig, 2006; Greve et al., 2017; Nicolai and Ansart, 2017). With fluctuating temperatures and weather conditions, land snails go through seasonal bottlenecks (Harvell et al., 2002; Barker et al., 2013) and have had to adapt to live and reproduce in these environments. Pulmonate gills were made redundant during their transition to terrestrial life, with land snail evolution leading them to breathe with a vascularized sac in the mantle, which allows for gas exchange (Brown and Lydeard, 2009).

The prevalence of hermaphroditism varies between molluscan taxa, though it occurs in 100% of pulmonates, which includes many land snails (Heller, 1993). Hermaphroditism does not necessarily mean self-fertilisation is possible, although it does provide additional opportunities to mate with other individuals without the constraints of each individual requiring complimentary sexual organs. In rare situations, parthenogenesis, which is the ability to self-fertilise, has also been recorded in some land snails (Selander and Kauffman, 1973; Nicklas and Hoffman, 1981;

Hoffmann, 1983). The act of reproducing asexually, while not considered typical in land snails, can become more frequent given certain environmental conditions. A well-studied freshwater example is *Potamopyrgus antipodarum,* which may either self-fertilise in environments with a low parasitic load or may reproduce sexually given an environment with a high parasitic load in order to increase genetic variation and parasite resistance (Lively et al., 2004). Although this freshwater snail is not closely related to land snails, its ability to adapt its reproduction method demonstrates the fitness advantages of being able to adapt to a changing environment.

There has been no definitive explanation to why land snails and other pulmonates are hermaphroditic or able to self-fertilise in select cases. Although, it may be that finding a reproductive partner of the opposite sex is more difficult terrestrially due to fluctuating and unpredictable conditions, combined with the fact that land snails are slow moving and often live in solitary rather than in colonies. Being able to reproduce both sexually and asexually can be advantageous, although both scenarios will include a trade-off. While self-fertilisation does not require finding a reproductive partner, the genetic blueprint passed on to offspring will be an exact copy of the parent's DNA. Self-fertilisation will in turn decrease genetic variation and allow for recessive lethal alleles to become more prominent in a population, thus ultimately producing an inbred population with low sexual fitness. This decrease in genetic variation occurs across multiple generations due to the populations' standing variation being less able to be positively selected for, which reduces the likelihood of advantageous alleles becoming fixed (Noël et al., 2017). Furthermore, self-fertilisation increases the chance of deleterious mutations becoming fixed in the population (Noël et al., 2017). This reduced ability to adapt ultimately puts the genetic variation and the entire population at risk of extinction in a changing environment. Pulmonates that self-fertilise have also been observed to mature later and have lower fecundity (Brown and Lydeard, 2009). Therefore, although some land snails are able to reproduce asexually, sexual reproduction will typically occur where possible (Brown and Lydeard, 2009). Therefore, hermaphroditism is an adaptation that will allow for genetic crossover while also providing more opportunities for reproduction without being constrained by requiring a mating partner of the opposite sex.

1.3.2 Introduction to Genus *Paryphanta*

The family Rhytididae (Order: Stylommatophora) is a group of carnivorous terrestrial pulmonate gastropods that occur across the Southern Hemisphere, with genera endemic to New Zealand, Australia, southern Africa, and Madagascar (Spencer et al., 2006). Rhytidids have also been discovered on other islands, including the islands of Indonesia, Seychelles, and the western Pacific (Efford, 1998; Spencer et al., 2006). Their distribution has often been described as "Gondwanan" (Spencer et al., 2006), though their location of origin, divergence date, and mode of lineage dispersal are unknown. Therefore, it may be inaccurate to describe their distribution patterns in this way (Goldberg et al., 2008).

Rhytidid diversity is highest in the island nation of New Zealand, with this country holding the largest number and percentage of described rhytidid species (van Bruggen, 1980; Efford, 1998). Current estimates suggest approximately 43 species and 31 further subspecies across 10 endemic rhytidid genera (Spencer et al., 2016). With taxonomic classifications under review for this family, the exact numbers are expected to change in the near future.

Rhytidid species are grouped into two subfamilies based on reproductive anatomy – Rhytidinae and Paryphantinae (Climo, 1977). Kauri snails, also known as pūpūrangi *Paryphanta* spp. Albers, 1850, belong to the subfamily Paryphantinae alongside several of New Zealand's other endemic genera: *Schizoglossa, Amborhytida,* and *Rhytidarex* (Climo 1977; Spencer et al., 2006).

It is unclear whether the sister genus of *Paryphanta* is *Schizoglossa* or a clade comprising *Schizoglossa* plus *Amborhytida* (Spencer et al., 2006). Although *Amborhytida* habitat and distribution overlap with those of *Paryphanta,* they are much smaller in size, as is the shell of *Schizoglossa*. Instead, the ecological analog of *Paryphanta* is the rhytidid *Powelliphanta* (Subfamily: Rhytidinae), which is comparable in both size and diet (Waterhouse et al., 2014).

As their common name, kauri snail, suggests, *Paryphanta* populations are found in kauri forests. However, rather than being in close proximity to kauri trees, *Paryphanta* individuals prefer areas with rich, moist soil. This environment is preferable for its abundance of earthworms, which

comprise the majority of *Paryphanta* diet, as well as insects, insect larvae, and smaller snails (Parrish et al., 1995; Stringer et al., 2003; Orwin, 2007; Department of Conservation, 2021).

Paryphanta have been reported to move up to 10 m in two weeks (Department of Conservation, 2021) and are more active nocturnally (Montefiore, 1995; Stringer et al., 2003; Ward, pers. obs. 2022). This nocturnal behaviour allows hunting of prey at night and avoidance of predators and warmer temperatures during the day. While many land snails can produce an epiphragm of dried mucus to protect from desiccation (Paul, 1991), *Paryphanta* lack this ability (Stringer et al., 2003). Therefore, individuals spend most of the day hidden under leaf litter or in dense vegetation with their apertures in contact with the ground. This behaviour undoubtedly assists individuals in avoiding predation by birds and protecting themselves from desiccation (Stringer et al., 2003).

The precise lifespan of genus *Paryphanta* is unknown, but considering the 20 year lifespan of genus *Powelliphanta* (Walker, 2003), it is assumed to be similar for genus *Paryphanta*. Reports indicate that growth time to reach the adult shell stage takes 3 to 4.5 years (Stringer et al., 2002), although it is unclear whether this stage reflects sexual maturity. In comparison, sexual maturity in captive *Powelliphanta* populations appears to be around 5 to 6 years old (Walker, 2003), so somewhere within this 5 to 6 year range is likely for genus *Paryphanta* as well.

Paryphanta specimens tend to mate during New Zealand's wettest months between April and July (Stringer et al., 2003). Previous reports have indicated that mating may actually be triggered by rainfall, as moist soil allows for easier digging of holes for oviposition (Stringer et al., 2003; Department of Conservation, 2021). Estimates suggest oviposition of around 17 eggs per year (Stringer et al., 2003), with a hatching rate of 83% (Stringer et al., 2002), resulting in around 14 young per mating pair annually. Once oviposited, eggs take between 5 and 7.5 months to hatch, then remain underground for a minimum of 2.8 months, or as long as six months, during their juvenile stage before the juveniles emerge above ground (Stringer et al., 2002).

1.3.3 *Paryphanta* Morphology

Paryphanta have two different morphotypes (Figure 1.2), found in geographically distinct populations separated by more than 50 km (Figure 1.3). The southern morphotype, which ranges from the Waitākere Ranges in Auckland to Kaitaia (Figure 1.1), is commonly referred to as *Paryphanta busbyi* (Gray, 1840). Its greenish shell grows up to 79 mm in diameter and it is found primarily in broadleaf forests (Stringer et al., 2003; Department of Conservation, 2021). In the most northern region of Northland, specifically at the three peaks of Te Paki, Unuwhao, and Kohuronaki (Figure 1.1), occurs the rarer morphotype, commonly referred to as *Paryphanta watti* Powell, 1946 (Montefiore, 1995). The shell of this morphotype is darker in colour and slightly smaller in size than *P. busbyi*, attaining a maximum diameter of 62 mm at maturity (Stringer et al., 2003; Department of Conservation, 2021).

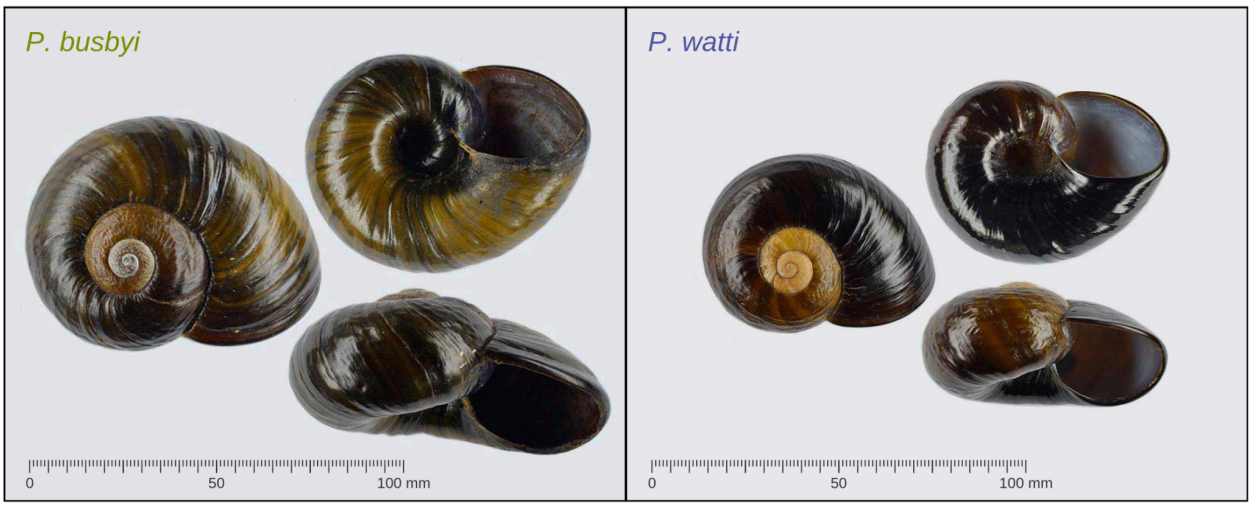
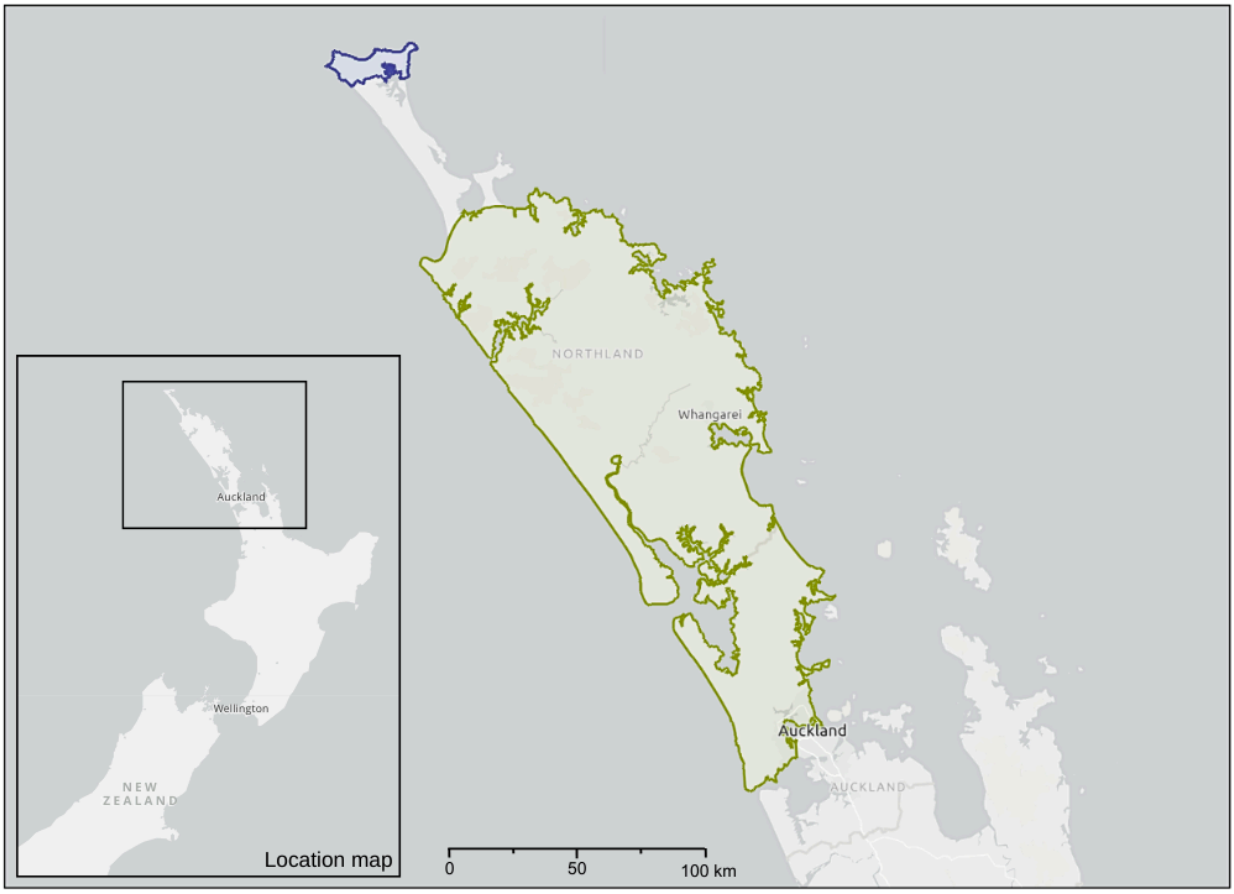


Figure 1.2 The range of the two morphotypes observed within the genus *Paryphanta,* these being the larger green morph, *Paryphanta busbyi* (left), and the smaller black morph, *Paryphanta watti* (right). Sizes to scale. Images were adapted from NZ Mollusca, Andrew Spurgeon 2021©.


NORTHLAND
Whangarei
Auckland
AUCKLAND
Auckland
Wellington
NEW ZEALAND
Location map
0
50
100 km

Inferences on the past distribution of *P. watti* can be made based on the fossil collection of the flax snail, *Placostylus* spp., which shows a north-south phylogeographic split similar to that assumed for the genus *Paryphanta* based on morphological and geographical distribution (as described in Section 1.3.4 *Paryphanta Morphology*). Due to the similar current distribution and phylogeographic split of *Pl.* spp. and *P.* spp., both genera may also have been impacted by similar historical events and therefore *Pl. spp.* are considered the most reliable genus to make inferences from regarding *Paryphanta* distribution and phylogeography.

Radiocarbon dating of *Placostylus* subfossils from the same sand deposits from which *P. watti* subfossils were found, suggests widespread forest and land snail populations were present in the area during the mid-Holocene (present until 1900 years BP then re-established between about 1800 and 800 years BP), though researchers also contend that some fossils might be of Pleistocene age (Brook, 1999). Although *Placostylus ambagiosus* is syntopic throughout the distribution of *P. watti*, *P. watti* does not occur throughout the distribution of *Pl. ambagiosus*. *Pl. ambagiosus* are known to live within 3 km of New Zealand's coast (Powell, 1938; Powell, 1947), but with New Zealand's coastline changing throughout glacial and interglacial cycles, the coastline has not always been consistent. The LGM, which lasted until 19 KYA, saw sea levels around 100 m below that of today (Rother et al., 2014; McSaveney, 2021). While *P. watti* populations are found in coastal regions, the forested areas in which they inhabit do not support fossilisation of shells as well as sand deposits, and if inland, fossil sites would unlikely include *Placostylus* remains. Therefore, much of the understanding we have about the past distribution of *P. watti* is inferred from limited information available from *Pl. ambagiosus* fossils, although these fossil sites do not represent the complete *P. watti* distribution.

P. watti are presently found mostly in drier coastal areas, although a population does still exist in the minute broadleaf forest remnants at the flank of Te Paki (Stringer et al., 2003; Mahlfeld et al., 2012). The presence of *P. watti* in these forested areas suggests that their preferred natural habitat would likely have been broadleaf forest, as is that of *P. busbyi*. However, this habitat is now limited in northern Northland due to burnoffs in the 1870s that destroyed most of the broadleaf forests in that area (Goulstone et al., 1993; Stringer et al., 2003). Unfortunately, the remaining

forest in the area is fragmented and pressures from introduced predators pose a threat to *P. watti* populations (Goulstone et al., 1993; Parrish et al., 1995).

Subfossil records of *P. busbyi* are much more limited due to taphonomic biases. Due to the lower sea level of the LGM, it is likely that forested regions and therefore *P. busbyi* distribution had wider boundaries during this time. Researchers agree, however, that *P. busbyi's* distribution between Auckland and Kaitaia has remained relatively stable and widely spread since this time (Powell, 1946; Spencer et al., 2006).

Translocations of *Paryphanta* populations have occurred since human arrival in New Zealand (Parrish et al., 1995), and these translocations would have also incurred changes in *Paryphanta* distribution. A number of undocumented translocations, which have been communicated orally within the malacology community, have led to the establishment of current *P. busbyi* populations at Āwhitu Peninsula, Kaimai Ranges, Waitākere Ranges, and Warkworth (Figure 1.1; Parrish et al., 1995). Although the exact origins of these populations are unknown (Parrish et al., 1995), COI data obtained and analysed by Spencer et al. (2006) revealed a Warkworth site to have a close evolutionary relationship with populations from both Tangihua Forest and Paparoa. This close relationship may suggest either of these sites as the Warkworth population's translocative origin (discussed further in Section 2.4.2 *Translocated Sites and Their Origins/Known Translocations*). Since knowledge of translocations may be transmitted verbally amongst select malacologists, it is likely that many less commonly known, undocumented translocations have also occurred throughout human history. Through genetic analysis, it still remains possible for translocated populations and their origins to be identified.

1.3.5 *Paryphanta* Taxonomy

Species may be defined by any of multiple proposed species concepts (Frankham et al., 2012). These species concepts are not mutually exclusive, so multiple concepts can be used simultaneously to define species boundaries. The Biological Species Concept is the most well-known concept, considering a species as a group of organisms able to interbreed and produce fertile offspring (Mayr, 1942). However, many groups of organisms that are universally

considered separate species, occasionally even belonging to distinct genera, are capable of successfully interbreeding and producing fertile offspring. Species of abalone (Family: Haliotidae, Genus: *Haliotis*) have been observed to do this in both natural and experimental contexts (Lafarga de la Cruz and Gallardo‐Escárate, 2011), thus violating the Biological Species Concept.

The Phylogenetic Species Concept is a second concept that is commonly applied and defines a species as an irreducible monophyletic group of organisms that are descended from a common ancestor and share defining apomorphic traits (Eldredge and Cracraft, 1980). However, by defining the species as an irreducible group, it denies the existence of any subspecies and has been criticised as overestimating taxonomic diversity while underestimating population size and range (Agapow et al., 2004).

Referring to the two *Paryphanta* morphotypes as distinct species follows the Morphological Species Concept, which is based on Darwin's ideology and defines species as a group of organisms that share a set of physical traits (Aldhebiani, 2018). One drawback of this concept is that it does not consider morphological variation within species or the lack thereof that can occur between species. Presently, no attempts of inbreeding between *P. busbyi* and *P. watti* have been made, and no molecular markers or distinct features, aside from their morphological differences, have been identified. Therefore, these species boundaries may be different if assessed in light of different species concepts.

All the above-mentioned species concepts are frameworks in which taxonomic research can be carried out, though using different concepts for different taxa is one way in which inconsistencies may arise in taxonomy. Integrative taxonomy considers multiple lines of evidence in determining taxonomic positions. This approach was formally introduced in 2005 (Dayrat, 2005; Will et al., 2005) and is considered by many to be the best-case compromise moving forward (Pante et al., 2015). However, a review by Pante et al. (2015) revealed that most species defined between 2006 and 2013 were defined without an integrative taxonomy approach or molecular evidence. Without consistency in how species boundaries are drawn, the ranking of "species" as a taxon has reduced meaning.

Subspecies are defined with even more ambiguity but are usually held to constitute a geographically defined and/or distinctive population or form (Ebach and Williams, 2009). However, the level of distinctiveness is not quantified in any definition; therefore, a distinct group of organisms may be defined as a population, subspecies, or species according to different taxonomists. The Department of Conservation recognises subspecies as management units. While this approach further confuses the definition of "subspecies" in the context of New Zealand conservation, it serves to protect as many populations as possible, preserving both genetic variation and cultural values held by the wider species.

Since *P. watti* is both morphologically and geographically distinct, this population meets the definition of a subspecies at the very least, regardless of what is indicated genetically. On top of that, *P. watti* and *P. busbyi* also meet the definition of species based on the Morphological Species Concept, although given their allopatric distributions, the Biological Species Concept cannot be tested in a natural setting. A degree of evolutionary distinctiveness would further support these clades as separate species and indicate adaptive differences rather than populations with ecotypic differences.

Surprisingly, previous genetic analysis using the mitochondrial cytochrome c oxidase subunit 1 (COI) gene suggested a different phylogeographic split in *Paryphanta* than geographical trends and morphology would suggest (Spencer et al., 2006). The black and green morphotypes seen in *P. watti* and *P. busbyi*, respectively, were found to be paraphyletic, with *P. watti* individuals no more genetically similar to one another than to *P. busbyi* individuals with which they were grouped. Furthermore, significant differences in the COI sequences suggested an east-west split between populations in Northland. Two clades were identified, the first of which included *P. watti* and several *P. busbyi* populations along the east of Northland, ranging from Kaitaia to Taranga/Hen Island and Waipu (Figure 1.1). The second clade was found in the western and southern areas of Northland between Herekino and north Kaipara (Figure 1.1) and included only *P. busbyi* morphotypes. Based on the mean genetic distance between individuals, the date of divergence between these newly identified clades was estimated to be between 1.0 and 3.4 MYA (Spencer et al., 2006). Preliminary ITS-2 data for a subset of the taxa from Spencer et al. (2006) appeared to corroborate the east/west split found in their COI data (Kennedy, pers. comm. 2022).

This inferred phylogeographic split would consider *P. watti* as consubspecific, which was never previously considered. Whether the eastern and western clades proposed are conspecific and are separate subspecies or entirely distinct species, is dependent on further analysis and which species concept is followed.

Prior to the publication of COI data by Spencer et al. (2006), *P. watti* was considered a distinct taxon, whereas today, current official taxonomic classifications recognise *P. watti* as a management unit. Most authors still distinguish between *P. busbyi* and *P. watti* to align with its status as a management unit, treating *P. watti* as either separate subspecies (Stringer and Montefiore, 2000; Stringer et al., 2003; Beauchamp, 2011) or separate species (Spencer et al., 2006; Mahlfeld et al., 2012), while still acknowledging that there is little confidence in these groupings (Spencer et al., 2006; Mahlfeld et al., 2012). *P. watti* is not mentioned in Appendix 1 of Mahlfeld et al. (2012), which discloses the conservation status of New Zealand terrestrial Gastropoda. Instead, the conservation statuses of *P. busbyi* and *Paryphanta* sp. 1 (NMNZ M.305039) are listed, although the latter is known to be from the same collection lot as the western *P. busbyi* specimen utilised in the analysis by Spencer et al. (2006). Therefore, Mahlfeld is presumably referring to the proposed clade consisting of *P. busbyi* east with *P. watti,* and secondly to *P. busbyi* west, as mentioned in Spencer et al. (2006).

The World Register of Marine Species (WoRMS) currently recognises just one taxon, *P. busbyi,* within the genus *Paryphanta,* although this database is compiled based on available literature, which in the case of *Paryphanta* does not include any recent taxonomic analysis. Both *P. watti* and *P. busbyi watti* are included, however, as synonymised names with *P. busbyi.*

It is clear that some authors believe multiple species may exist within the genus, though there has not been concordant agreement on where those species boundaries lie. It is important to acknowledge that with this uncertainty, there are many details that are non-disclosed in the genus descriptions by WoRMS and that this situation is convoluted.

With Northland geological history and many terrestrial animals in the area reflecting a physical north-south split (as described in Section 1.2.3 *Phylogeography/Northland Phylogeographic*

Trends), genetic differences between the northern and southern *Paryphanta* morphotypes at the molecular level would seem entirely plausible. This hypothesis is further supported by the morphological ranges of genus *Paryphanta* that are consistent with the geological history of the region. Alternatively, while the east-west phylogeographic split revealed by Spencer et al. (2006) was unexpected, this pattern has been observed in other taxa (e.g. *Oligosoma smithi*; Hare et al., 2008; *Entelea arborescens*; Shepherd et al., 2019) and is therefore worth investigating further.

1.3.6 Accuracy of the Currently Proposed Phylogeographic Split

The apparent genetic divergence between eastern and western populations is difficult to explain as these results do not correspond with what is understood about ancient Northland geology. Additionally, this distribution pattern does not match that of other Northland land snails. In two revisions of New Zealand land snails, 14 other species were listed that occur within the distribution of *P. watti*. Of these 14 species, only three also occurred within the distribution of *P. busbyi*, with all three of those species occurring broadly across the distributions of both the eastern and western *P. busbyi* clades. The same revisions listed another 14 species with similar distributions to *P. busbyi,* and 10 of these species occurred across the distributions of both the eastern and western clades (Marshall and Barker, 2007; Marshall and Barker, 2008). The distribution patterns of other rhytidids also suggest the inaccuracy of the east-west *Paryphanta* split, as *Amborhytida dunniae* occupies the almost exact distribution as *P. busbyi*, while *A. duplicata* occupies an almost exact distribution as *P. watti* (Spencer et al., 2006).

Analysis of the COI gene in isolation may not have indicated the same phylogeographic structuring as if multiple markers were used. When the COI gene was used for DNA barcoding in endemic New Zealand grasshoppers (Orthoptera: Acrididae), genetic differences were identified between geographically partitioned individuals of a single morphotype, as was observed for *P. busbyi* (Trewick, 2008). However, identical haplotypes were shared between morphologically and ecologically distinct species; therefore, it was concluded that the use of DNA barcoding in isolation can provide misleading information (Trewick, 2008). While the COI gene may be of assistance in taxonomic practice, a larger, more comprehensive analysis is preferable to increase confidence in results (Lambert et al., 2005; Trewick, 2008).

Incomplete lineage sorting is the phenomenon in which ancestral variants are shared by descendant populations or species (Cerca et al., 2021). It is expected that over time, complete lineage sorting would occur by means such as drift or selection, resulting in different clades having different genetic variants (Mende and Hundsdoerfer, 2013; Peyrégne et al., 2017). With incomplete lineage sorting, phylogenetic trees produced using molecular data do not match the true lineage history (Wall et al., 2013). This phenomenon occurs due to the retention and stochastic sorting of ancestral polymorphisms (Maddison and Knowles, 2006), particularly when speciation is very recent or rapid (Suh et al., 2015). Increasing the number of loci or specimens can account for these inconsistencies in phylogeographic studies (Maddison and Knowles, 2006), therefore, to determine if incomplete lineage sorting was a factor in Spencer et al. (2006), additional loci and specimens will be used where possible in this project. In the case of the genus *Paryphanta*, it would mean that two separate lineages (either species or subspecies), in fact, share genetic variants, making the molecular-based phylogenetic tree appear paraphyletic. Incomplete lineage sorting would explain the results of Spencer et al. (2006) while still acknowledging that the true phylogeographic split is entirely different.

Lastly, the long branch attraction phenomenon may have been at work in the phylogenetic analysis of kauri snails. Rapidly evolving lineages have high nucleotide substitution rates, but when both lineages independently evolve the same nucleotide substitution, it may appear to researchers to be a single event occurring in a common ancestor. Long branch attraction occurs when two or more long branches are misidentified as sister groups due to these methodological artefacts (Bergsten, 2005). According to phylogenetic researchers, the effects of long branch attraction cannot be truly resolved, making this phenomenon the most detrimental problem faced in phylogenetic reconstruction (Gribaldo and Phillippe, 2002; Kapli et al., 2020). Therefore, this phenomenon must be considered in the context of the research by Spencer et al. (2006), as it may have impacted the seemingly close relationship between *P. watti* and other easterly *P. busbyi* populations. Several strategies have now been suggested to detect and avoid long branch attraction, including the exclusion of certain species with very high evolutionary rates, the removal of loci with very high rates, and the addition of species that may break up long branches on the tree (Brinkmann et al., 2005; Rodríguez-Ezpeleta et al., 2007; Kapli et al., 2020). Since

there are no other species within the genus *Paryphanta* that can be added or removed for this project, long branch attraction will try to be limited by using alternative loci than the COI gene.

Considering these limitations that may have been in effect previously, the *Paryphanta* phylogeographic split identified by Spencer et al. (2006) must be regarded with caution. The taxonomic status of species in the genus *Paryphanta* remains unclear, though most authors agree that two taxa and no more are present in this genus (e.g., Spencer et al., 2006; Malhfeld et al., 2012). The greater uncertainty at present is determining the true number of species within the genus *Paryphanta*, and researchers in this field have yet to conduct a full taxonomic analysis to resolve this. Species definitions have always been contentious, and this uncertainty has been exacerbated by authors not disclosing what they define as a species. Until species definitions have been reviewed, authors should clarify in text which species definitions they follow to avoid further confusion. For the purpose of this thesis, the *Paryphanta* clades will hereon be referred to as either "*P. busbyi*" or "*P. watti*" as reference to their respective green or black morphotype, and further clarification, where necessary, of those populations within "*P. busbyi*" will be referred to by their relative location to one another (e.g. east/west, or north/south) or geographic locations (e.g. Waitākere Ranges).

1.3.7 *Paryphanta* Conservation

Paryphanta populations are considered culturally significant within New Zealand, largely for their endemic status but also for their role in Māori history. Māori have undertaken the role of guardians/kaitiaki of the forests to protect not only this taonga/treasured genus but other native wildlife as well. Multiple populations of unrecorded translocations are known (Parrish et al., 1995), and it is plausible that these translocations were carried out by pre-European Māori to embrace *Paryphanta* individuals themselves as kaitiaki, as is known for other land snails in the region (Stringer and Grant, 2007). Oral history passed down through Ngāti Wai suggests pre-European Māori even consumed pūpūrangi. Te Arawa descendent, Ihenga, traveled to Whangārei (Figure 1.1), where he and his companions consumed pūpūrangi that had been roasted in cooking fires (ahi). Following this, Whangārei Town Basin was given its original name, Te Ahipūpūrangi-a-Ihenga (Cooper, 2016). While pūpūrangi typically refers to

Paryphanta species, its vernacular meaning can also be used in reference to any large snail, including marine snails or flax snails (*Placostylus* spp.) which are known to have been consumed (Hayward and Brook, 1981).

Population Estimates

Population estimates can be made using a number of methods, with each method having its own assumptions and resulting in slightly varying values. *Paryphanta* individuals living in leaf litter can be hard to locate, so the number of specimens found in any given area may be an underestimate of the true population size. Methods of estimating *Paryphanta* population sizes may include a line transect survey (Buckland et al., 2004) or a mark-recapture technique (Jensen, 1994), though it is important to consider that the area size surveyed, habitat surveyed, time spent in the field, and number of researchers surveying can impact the final population estimate.

P. busbyi is listed as "Near Threatened" on the International Union for Conservation of Nature red list (Sherley, 1996), although this categorisation was made in 1996 and requires updating. The giant land snail recovery plan by the Department of Conservation estimates the *P. busbyi* population to include thousands of individuals (Parrish et al., 1995). *P. watti* populations are much more at risk and population estimates vary hugely. Less than 20 live *P. watti* individuals were reported in the ten years prior to 1995 (Parrish et al., 1995), although population estimates of *P. watti* at Te Paki Farm Park suggested a population size of around 10,500 individuals (comprising 5,500 individuals at Te Paki hill and 5,000 individuals at Kohuronaki) (Stringer and Montefiore, 2000). Population estimates at Unuwhao are reported to be less than ten individuals (Parrish et al., 1995). The true number however, remains uncertain since 126 live individuals were reported across three sites within the known *P. watti* range (Stringer et al., 2003), although this report was published 20 years ago with no additional estimates having been published since.

With the population estimates ranging hugely and confidence intervals not provided, the true population sizes are still unknown, though they are expected to be less than 10,000 individuals. Recent observations by malacologists suggest that the Kohuronaki population has recently become extinct (Walton pers. comm. 2022), and if these reports are accurate, it is possible that

the total *P. watti* population could be as high as 5,000 individuals, though considering the proximity of Te Paki and Kohuronaki, factors leading to the decline of population size at Kohuronaki would have also likely affected those at Te Paki. In addition, any genetic variation unique to the Kohuronaki population will have been lost.

Threats

Birds and introduced mammals such as pigs and rats frequently predate on *Paryphanta* individuals (Montefiore, 1994; Montefiore, 1995; Stringer and Montefiore, 2000; Stringer et al., 2003), while other mammalian predators including mice, brushtail possums, European hedgehogs, weasels and stoats also pose a potential threat. Habitat degradation by introduced mammalian grazers, including horses, pigs, deer, and goats, disturbs the undergrowth in which *Paryphanta* occur, leaving them vulnerable to desiccation, especially in warmer months (Stringer et al., 2003). Predatory ant species have been listed as a future threat to *Paryphanta* species due to their expanding range (Stringer et al., 2003), and have since invaded the Aupōuri Peninsula (Ward and Toft, 2011). Furthermore, contending with other invertebrate taxa such as large centipedes, mygalomorph spiders, carabid beetles, and weta are likely to provide some competition for *Paryphanta* populations (Stringer et al., 2003).

Due to their brilliant colouring and large size, *Paryphanta* shells, often still containing the live animal, are taken on occasion for private shell collections. Larger shells are favoured in collections, and overexploitation of adult *Paryphanta* shells by shell collectors may have negatively affected *Paryphanta* population size (Parrish et al., 1995). Juveniles have been seen to supplement their diet with calcium from the shells of deceased snails (Ward, pers. obs. 2022), therefore collection of dead shells can still have negative impacts on living populations. With *Paryphanta* populations now illegal in shell trade due to their protected status, their most prominent threat by humans since the 1980s has been the destruction of native forests for farmland and pine tree plantations (Goulstone et al., 1993; Stringer et al., 2003). The effect of this threat on *Paryphanta* populations is not fully understood. Although *Paryphanta* individuals have been recorded within pine forests in Northland (Ballance, 1985), it remains clear that, with the reduction of their native habitat, *Paryphanta* range has become more fragmented.

Conservation Management

Previous recovery plans for *Paryphanta* populations were made by the Department of Conservation, but with *Paryphanta* being regarded as culturally significant within the Māori community, Northland iwi have also contributed hugely to the protection of the genus. *P. watti* exist within the rohe/boundaries of multiple iwi and in 2014, Ngāti Kuri settled under Te Tiriti documenting their guardianship/kaitiakitanga of *P. watti* (Te Hiku O Te Ika Iwi, 2014). Although, additional iwi, namely Te Aupōuri, who's rohe also encompasses *P. watti* distribution, share guardianship/kaitiakitanga of *P. watti*. With *P. busbyi's* larger distribution, populations are located in many iwi's rohe. Today, population management aims to involve both the Department of Conservation and the local iwi for each population.

The Department of Conservation Giant Land Snail Recovery Plan (Parrish et al., 1995) acknowledged the restricted distribution of *Paryphanta* populations and listed four options for recovery, as follows:

1) Do nothing
2) Maintain and increase island populations and some selected mainland populations across their geographical and genetic range.
3) Endeavour to maintain all existing taxa and every population through management *in situ*.
4) Translocations of snails to less vulnerable locations.

The second option, whereby plans were to maintain and increase island and selected mainland populations, was chosen and it was decided that further surveying of *P. watti* would be necessary to accomplish this recovery goal (Parrish et al., 1995). *P. watti* occurs mostly at the three peaks of Te Paki, Unuwhao, and Kohuronaki, although Parrish et al. (1995) acknowledge that populations exist in the areas between these locations as well. Whilst acknowledging these additional populations, the Department of Conservation did not consider them for immediate intervention in their Giant Land Snail Recovery Plan.

Before 1995, little management of *Paryphanta* populations had been undertaken, although forest restoration of Te Paki Farm Park may have allowed for range expansion of *P. watti* in the wider area (Parrish et al., 1995). Translocations of *P. busbyi* have occurred historically, and although many were undocumented, their success is inferred from the establishment of the populations at Āwhitu Peninsula, Kaimai Ranges, Waitākere Ranges, and near Warkworth (Parrish et al., 1995). Captive breeding however has proven ineffective for Northland's other large endangered land snails, *Placostylus* spp., and was not recommended for management of *Paryphanta* populations (Parrish et al., 1995). No follow-up report has been made since the publication of The Department of Conservation Giant Land Snail Recovery Plan although the hunting of pigs in a region west of Te Paki hill was thought to be reducing pressure on *P. watti* populations in the area (Stringer and Montefiore, 2000; Stringer et al., 2003). It is unclear whether this reduced pressure has had a positive impact on relevant population sizes over the last 20 years, although it can be assumed that this form of management on its own is ineffective or not widespread enough due to concerns surrounding the possible extinction of the Kohuronaki population.

Since thorough surveys to determine distribution range, population estimates, and current threats have not been done in over 25 years (Parrish et al., 1995), it is essential that a review be undertaken and an updated management plan be formed in order to best conserve the genus.

1.4 Science Communication

1.4.1 Importance of Science Communication

Scientific research is important for conservation and ensuring the survival of a species, although to completely reach the potential outcome of research, it is essential that the knowledge gained is clearly communicated to both a scientific and general audience. The planning, conducting, and follow-through of research often require contributions from external sources, aside from the scientists directly involved in the research. Therefore, it is important to communicate science throughout all stages of research to engage interest in science from the general public. Increased

interest in turn may result in enhanced quality of research, increased knowledge shared, increased funding, and a better outcome for the conservation management of endangered species.

A significant issue faced in conservation is the misallocation of conservation efforts and funding towards a select "type" of taxa. Due to taxonomic bias, "charismatic" animals, particularly vertebrates such as mammals and birds, tend to receive more investment compared to invertebrates, including the genus *Paryphanta* (Lydeard et al., 2004; Donaldson et al., 2016; Mammola et al., 2020). Science communication has the power to raise awareness of this issue and draw focus toward the less represented taxa. One example that has brought attention to lesser-known species within New Zealand is Radio New Zealand's weekly afternoon show, Critter of the Week, which has highlighted over 300 species of plants, animals, and fungi since the show's debut in 2015 (Toki and Mulligan, 2023). This show has also engaged the public, relying on volunteers to create or improve the Wikipedia articles that correspond with the current week's critter. This example demonstrates the potential for science communication that may be carried out for the genus *Paryphanta*.

Science communication often requires extra time and financial resources, which may be less prioritised in research that focuses on underrepresented invertebrates, especially those without an excess funding allocation. Furthermore, different methods should be used to reach various audiences, for example, other scientists, museums, iwi, Northland locals, and tourists visiting Northland. However, as seen by the positive impact of science communication for "charismatic" taxa, which often receive a greater amount of resources provided to conservation efforts, the benefits of prioritised science communication are sure to outweigh the costs.

Relaying information may be achieved through various types of outreach. "Outreach" is considered to be any scientific communication that engages a wider non-specialist audience outside of academia (Poliakoff and Webb, 2007). Outreach may include any event or activity that aims to either inform the public or provide opportunities for multidirectional interactions between scientists and members of the general public. Interactions between scientists and other scientists may also be referred to as outreach, or as "inreach." The form of outreach should be catered to the specific target audience, therefore, communicators may use various types of

language or methods of reaching target audiences and unique strategies may be incorporated in an effort to relate to these groups. Forms of outreach include, but are not limited to, short videos, documentaries, stories, radio show appearances, scientific publications, or conference presentations - all of which may be utilised for genus *Paryphanta*. Outreach can also be achieved to varying degrees of active engagement with the audience, typically following one of three models: the deficit model, contextual model, or participation model. Each of these models result in different levels of understanding and engagement of science from the general public. The models will be explained further in the context of both public understanding of science (PUS) and public engagement in science and technology (PEST).

1.4.2 PUS versus PEST

Both public understanding of science (PUS) and public engagement in science and technology (PEST) are considered the two levels of science communication. While some science communicators use the terms interchangeably, there has been a shift from "PUS" to "PEST" in recent years, both in terms of the actual terminology used in literature, as well as the approach used to communicate science. While many science communicators consider this transition to PEST a simple change of language, the original application of PUS neglected the engagement and participatory aspects that PEST approaches prioritise (Bastos et al., 2019). Therefore, in the case of this project, the two are considered different.

PUS describes the unidirectional sharing of information from the scientific community to the general public (Bastos et al., 2019). Two well-known models of science communication, these being the deficit model and the contextual model, can be understood as having PUS approaches as both models involve unidirectional communication from the scientist to the general public. The deficit model assumes that public skepticism about science is caused by the public's lack of relevant knowledge and therefore aims to alter individuals' attitudes, beliefs, or behaviours by simply imparting scientific information to the general public (Lewenstein, 2010; Suldovsky, 2017). The contextual model takes PUS a step further by understanding and considering the audience's prior understandings, beliefs, and attitudes to encourage greater receptiveness from

the audience (Bucchi and Trench, 2014). Nevertheless, this model is still focused on simply providing information and fails to make use of public engagement (Lewenstein, 2010).

While a PUS approach may be efficient for quick, rapid responses from the general public, it may also result in secondary negative impacts by disregarding and underestimating the expertise of the general public, reinforcing the view that "scientists know best". The unidirectional nature of these models is less likely to decrease the gap between scientists and the general public or promote active engagement in science by the general public (Lewenstein, 2010).

A step further than PUS in terms of communication effectiveness is PEST, which involves facilitating the relationship between science and the general public and encouraging active engagement and participation from the public (Bastos et al., 2019). Building a relationship between the science community and the general public involves multidirectional communication, which can decrease the power imbalance and increase understanding, receptiveness, and productivity across all parties (Jucan and Jucan, 2014).

The results of scientific research affect all of society, not only scientists. Furthermore, it is often the general public that initially brings scientific questions to light, and as mentioned earlier, are often responsible for funding scientific research. With science both impacting and being driven by society, the dynamic between science and the public is clearly complex, and thus a unidirectional form of communication does not suffice.

The participation model of science communication, also referred to as citizen science, involves a PEST approach. Ideally, participatory science should be inclusive, flexible, and adaptive throughout all stages of research, including planning, conducting, and sharing of results (Senabre Hidalgo et al., 2021).

The understanding that both members of the general public and scientists have valuable information to be shared increases the strength of research, while simultaneously decreasing the gap between scientists and the general public. The maturation of science communication has led researchers to understand that many members of the general public are experts in their own right,

and regardless of their scientific background, may benefit scientific research. Bringing in their own expertise, the public can contribute to research in a variety of ways, from aiding with methodology and providing more context to assist with interpretations of results, to acting on results to enhance the outcome of the research. By learning to communicate science effectively beyond scientific peers, we are sure to benefit society in ways that researchers are unable to do alone (Kuehne et al., 2014).

Participation from the public can occur across a variety of levels. Members of the general public can easily participate in conservation science with little effort, although their contribution still first requires public understanding and appreciation for the science at hand. Several science projects already exist that encourage general public engagement, with these projects often referred to as citizen science. One example used towards the Predator Free 2050 goal is that locals can keep pest traps within their backyards, meaning pest control for conservation can be reached more effectively across a much wider area (Predator Free 2050, 2024). Science or government institutions can monitor traps and continue conservation research without relying further on the general public beyond this small level of participation, although it is clear that public engagement in this case allows for more effective results. A second example is the general public's ability to contribute to datasets using online resources such as iNaturalist, an online database that uses scientists and the general public to map and share observations of biodiversity that then become publicly available (iNaturalist, 2022).

Although participatory science will increase financial costs and time associated with consultations, it also increases rewards. The level of participation, however, is a continuous scale; therefore, considering the numerous levels of participation optionable for the general public, scientists may decide the optimal level at their discretion.

1.4.3 Effective Ways of Communicating Research

While science is communicated to 'the public', this terminology does not describe a uniform group of people, but rather, a more diverse target audience than is usually expected. Therefore, several factors must be considered when communicating science, including the form of outreach

most likely to reach the audience, the audience's prior understanding and knowledge of scientific jargon, and the individual experiences and values held by the audience. Oftentimes, different groups within the public will respond to communication differently, thus multiple approaches should be used to target as many groups as possible.

Form of Outreach

As mentioned above, several forms of outreach may be chosen depending on the information intended to be shared and the target audience. The types of media the general public is inclined to notice, as well as the events they are likely to attend, should be considered. When approaching a larger target audience, multiple forms of outreach may be most beneficial to reach a more diverse public and to reinforce the information shared.

While publishing results in peer-reviewed scientific journals is important in gaining credibility within the scientific community, it rarely allows research to reach the wider target audience, especially members of the public with a non-academic background. *Paryphanta* phylogeography, taxonomy, and conservation are topics relevant to Northland locals with varying degrees of scientific background, so outreach should include a combination of active and passive forms within the area. Since local iwi are kaitiaki of *Paryphanta* and therefore play an important role in this conservation, a more active form of outreach, such as hui, is typically used to provide opportunities for information to be shared between scientists and the iwi, while simultaneously building and maintaining trust between the two parties.

Māori culture places great value on the inclusion of a face-to-face meeting, or hui. Given today's available technology, many iwi also appreciate the opportunity for long-distance hui/kōrero via video calls. While hui are often equated to Western meetings conducted before business, this description minimises the complex cultural dimensions and importance of such an event (O'Sullivan and Mills, 2009). These engagements should not solely focus on the research, nor should it be the main focus of the hui. Rather, conversations in which all parties can understand one another's experiences and values should be held as these interactions can inform a great deal more than conservations focused on "business".

Hui should occur within the first stages of research and should use a PEST approach. Since PEST-based outreach involves multidirectional communication and the understanding of values held by the target audiences, hui should aim to build a relationship between each party. Scientists must be able to convey their reasoning for *Paryphanta* as a genus of interest and make obvious their respect for Māori culture. Scientists should also aim to receive knowledge, by learning more about how *Paryphanta* populations are valued within the community, understanding the role of *Paryphanta* in Māori history, and why the genus *Paryphanta* is considered taonga. Given the importance of face-to-face interactions within Māori culture, hui and other relationship-building engagements should occur in person, where possible, or via video call. In-depth discussions can later include the current and predicted health of populations, project proposals, and concerns from both parties, although trust and a developed relationship are essential before focusing on these topics.

Following the formation of relationships between scientists and iwi, additional types of outreach may be carried out. Presentations given to local communities or newsletters may be used to update the iwi on the progress of the research. Additionally, scientists may work together with iwi in the planning and conducting of research, or to develop material for the education of children/tamariki that will encourage learning about taonga taxa, including the genus *Paryphanta,* by the next generation.

Several other groups involved in conservation may also be receptive to the information on *Paryphanta* conservation, for example, Forest and Bird, the Department of Conservation, and local and regional councils. Due to the scientific background of many members within these groups, outreach such as presentations and conferences are frequently held and allow information to be conveyed while also providing opportunities for networking and answering any questions.

While effective for a proportion of the Northland public, these forms of outreach would fail to reach tourists, hikers, and locals without a connection to local iwi or science groups. Furthermore, meetings and consultations often discuss specific information of little to no interest to other members of the general public. Northland's general public who are not likely to attend

meetings and consultations would be more receptive to passive outreach such as information signage. Signs and informative posters are placed at many trails and reserves in which *Paryphanta* populations exist, including interesting facts and simple ways in which the public can preserve the current populations, for example discouraging the removal of *Paryphanta* individuals.

This passive act of outreach, despite following a PUS approach, allows the general public to gain scientific knowledge while inspiring interest in conserving *Paryphanta* populations. As discussed above, PUS is still necessary as an initial step in PEST, therefore a combination of both passive outreach and a PEST approach of active outreach provides greater opportunities for an overall increase of shared knowledge.

Choice of Language

To achieve PEST, it is important to communicate research, including results, interpretations, and implications, in a manner that is understood by those interested in these topics despite their degree of scientific background.

Scientific jargon is typically used in written and verbal communication amongst scientists and those with an academic background. Given the experiences that are shared amongst scientists and those with an academic background, scientific jargon can efficiently and thoroughly explain components of interest such as hypotheses, research methods, results, and research outcomes.

While the inclusion of jargon is efficient in discussions between scientists and those with an academic background, the use of jargon can be detrimental to communicating science when aiming toward a non-academic-based audience. Jargon can make it unnecessarily challenging for the full content to be understood by those with specialties in alternate fields, which can also create a sense of inferiority within the audience. Effective communication with those outside of the specific field of research requires the language to predominantly include layman's terms. Not only does this allow for the information to be fully understood by a wider audience, but it also

builds trust between scientists and the community as it allows for a sense of inclusivity across all parties.

It is important to note that using layman's terms does not mean the information should be "dumbed down." Not knowing about a certain topic does not equate to not understanding, although unnecessarily using jargon in attempts at conveying information does not provide the audience with the full opportunity to understand. The use of particular words that inform without patronising is essential in ensuring the audience remains receptive and feels valuable to the cause. Therefore, choice of language is essential for effective science communication.

The signage and posters around *Paryphanta* habitats that are frequently visited by tourists are an example that highlights the effective use of inclusive language that can convey information to a general audience without jargon. These signs explain the issues that *Paryphanta* populations face and provide recommendations for tourists that will contribute to *Paryphanta* conservation. Recommendations may be, for example, remaining on paths and refraining from collecting shells. The explanation alongside recommendations can give the audience a fuller sense of how their actions can impact *Paryphanta* populations, therefore increasing interest and encouraging the audience to do their part.

Relating to Target Audience

It can be unnatural for individuals without a strong scientific background to make decisions based on scientific facts alone. Instead, they may prioritise the immediate benefits of their community. Scientists must appreciate that those with different experiences have different values. Thus, in working towards a shared end goal, scientists need to be able to see other perspectives as valid and appreciate alternative values.

It is up to the scientists to help others understand how science-based decisions can benefit the community directly. Considering the priorities and values that might motivate a community can encourage others to actively engage with science, though achieving engagement requires first building a relationship based on trust and understanding.

Working Alongside Iwi

Conservation of New Zealand's endemic biodiversity is highly valued by Māori people, Crown research institutes, and the New Zealand government alike. Although, despite having the same overarching goal of replenishing and conserving the unique ecosystem, the approach toward achieving said goal often varies, resulting in parties fighting against one another.

New Zealand has been home to Māori for over 700 years (McWethy et al., 2014) and until European settlers arrived around 150 years ago (McWethy et al., 2010), Māori were the sole kaitiaki of New Zealand. Māori people are still kaitiaki today, though in this post-colonial time are not given 100% of the control and trust that comes with protecting New Zealand's ecosystems and taonga species. While academic research and conservation of endemic taxa are prioritised by the New Zealand Government, the expansive pre-colonial history holds much knowledge and the first step in academic research should always be engagement with Māori people.

Engagement should never be a "tick the box" step. Researchers should not expect any public citizen, indigenous or otherwise, to feel as though they must blindly trust based on research alone. Working with taonga species especially requires a high level of trust - a factor that is earned through smaller shared opportunities to work together, learn from one another, and understand one another's goals and values. Engagement should instead be an opportunity to initiate this relationship, which should continue throughout and upon completion of the research. Engagement is not simply for permission to study taonga species, but to build a mutual learning and working relationship - a key intention missed by many researchers.

While all New Zealand conservation research should include Māori involvement, this inclusion is especially important for taonga taxa, including the genus *Paryphanta.* With local iwi being kaitiaki of *Paryphanta* populations, close cooperation and coordination are crucial, not only to research but to the continued conservation of the populations.

Prioritising Information

When given large amounts of information at once, people have a tendency to lose interest quickly. Instead, three points that the audience will easily understand and have a positive reaction to should be identified and prioritised. Individual experiences and values held by the audience should be taken into consideration when choosing which pieces of information to prioritise, as any audience will only be able to retain limited information, and will naturally be more or less receptive to different pieces of information dependent on their prior views.

Numerous studies in psychology have demonstrated that people are better able to retain three or four pieces of information in their working memory (Luck and Vogel, 1997; Cowan, 2010). The rule of three can often be seen in marketing, influential articles, and even in fairytales due to its proven success in attracting and maintaining interest.

This method of prioritising three important pieces of information can be applied to various forms of outreach, including initial consultations, presentations, and posters. The information prioritised may vary depending on the form of outreach, the stage of research, and which points are more likely to be understood and considered interesting to the target audience. Once an opinion is made regarding a certain topic, it can be significantly harder to alter that opinion. This bias may be beneficial or consequential for scientific research and depends on the order the information is presented. By first presenting the audience with information that makes sense to them, they are not expected to blindly trust scientists. From there, their interest will grow and additional information can be presented later.

When communicating with scientists and academics about research, such as this research on the genus Paryphanta, the aims, research methods, and results are typically prioritised and highlighted as key points in publications and presentations. Alternatively, when communicating with iwi, especially before a relationship is built or during the planning stages of research, information on the project values and how the research might involve and benefit iwi is highlighted. Finally, when communicating with a non-academic-based general public, such as tourists within the area of a protected taxa, might be more receptive to how their actions impact

the environment and in return how changes in the environment may impact them. In this way, each target audience can receive less, albeit more relevant information, thereby preventing information overload, while also receiving specific information they are most receptive to.

1.5 Justifications for Research

1.5.1 Benefits for *Paryphanta* Conservation

Aotearoa New Zealand has a diverse and unique biota (Myers et al., 2000; Taylor-Smith et al., 2020); thus it is imperative that this biodiversity is conserved. Much cultural importance is placed on protecting native wildlife, which includes the genus *Paryphanta*. This endemic genus was present in New Zealand prior to human arrival, based on fossil records of syntopic species (Brook, 1999), and iwi have made clear the value of *Paryphanta* populations by offering themselves as guardians/kaitiaki for this threatened genus. The maintenance of *Paryphanta* populations will not only support New Zealand's biodiversity but also its cultural richness.

To take appropriate steps toward the conservation of the genus *Paryphanta*, we must first develop a reliable understanding of its phylogeography and taxonomy. Very limited literature is available on *Paryphanta* population structure and distribution. Only one research paper has been published on *Paryphanta* phylogeography that includes the use of genetic data (Spencer et al., 2006), and while this study was well designed and executed, the research produced unexpected results that do not align with the geologic history of Northland or previous morphology-based taxonomic conclusions. No further research has since been published on the phylogeography or taxonomy of the *Paryphanta* genus, meaning the phylogeographic patterns revealed by Spencer et al. (2006) remain unexplained and uncorroborated. Furthermore, as the COI gene remains the only locus analysed, we cannot be sure that the east-west Northland split is the most likely phylogeographic structuring for this genus. With Next Generation sequencing (NGS) techniques now available, we can use genotyping by sequencing (GBS) to achieve improved sequencing output while being more time- and cost-efficient than what was previously possible (Kumar et al., 2019).

With the total *Paryphanta* population being small (Parrish et al., 1995; Stringer and Montefiore, 2000), any further population decline will likely have a greater impact on genetic variation than a proportionately similar decline in a larger population. It is essential for the survival of *Paryphanta* that as much genetic and morphological variation be conserved across the genus and across Northland. Greater genetic variation provides more resilience and capability for adaptation in the face of changing climate or landscape (Hughes et al., 2008; DeWoody et al., 2021). With less room for error, well-informed research is necessary for effective conservation management and monitoring, as it will tell us which populations must be prioritised to conserve genetic variation.

Furthermore, understanding the genetic makeup of each population will be informative for any translocations that may take place in the future. Translocation should be a controlled and meticulously planned process, making sure to keep genetically rarer populations distinct to prevent the loss of rare alleles via outbreeding depression, while also aiming for genetic rescue (Whiteley et al., 2015). Translocations have been used as one of the chosen methods for the management of *Pl. ambagiousus* populations in the region, although with little success (Parrish et al., 1995). For this reason, it is important to have abundant information on distribution and population structure so that any *Paryphanta* translocations and other future conservation efforts may be done efficiently, effectively, and well documented.

Research on *Paryphanta* population structure and genetic makeup has been negligible in the past two decades. With available funding, relevant researchers to advise, and advanced genetic techniques, now is an appropriate time to continue previous research and make active efforts toward *Paryphanta* conservation. Not only will research improve the current understanding of *Paryphanta* population structure, but it will also generate information that can be used as a foundation for future research and management plans for the genus.

1.5.2 Benefits for Science Communication as a Research Tool

Science communication has been shown to positively impact receptiveness, engagement, and understanding of science by the general public (Jucan and Jucan, 2014; Bastos et al., 2019). However, science communication is rarely used to its fullest extent. By making a conscious note of the science communication used in this research, a greater understanding of its potential will be gained, which may then be utilised in planning science communication for future projects. Aspects of science communication that are deemed beneficial can be highlighted, as well as aspects that may be improved or adjusted for future research. Recommendations for researchers can then be made from these observations.

1.6 Thesis Aims and Summary

1.6.1 Thesis Aims

(1) This thesis aims to test, through genotyping by sequencing, the phylogeographic pattern of *Paryphanta busbyi*, and determine if the east-west split of genus *Paryphanta,* revealed by Spencer et al. (2006), is correct. The thesis will exclude *P. watti* from conclusions due to inability to collect *P. watti* specimens.

(2) In doing so, it is aimed that an in-depth understanding of the population structure within *P. busbyi* will be achieved, translocated populations will be identified and inferences about the origins of translocations will be made.

(3) Science will be communicated with science groups, museums, and iwi during the planning stages and duration of research. Additional recommendations for science communication will also be made to make accessible the knowledge gained, allowing it to be used for eco-restoration and conservation of the genus in the future.

1.6.2 Thesis Summary

Chapter Two: Genotyping by Sequencing (GBS) Reveals Phylogeographic Structure in *Paryphanta busbyi*

Mitochondrial and nuclear DNA have been known to infer different evolutionary patterns due to their different forms of inheritance (Birky Jr. et al., 1995; Irwin, 2002; Smith, 2016), evolutionary rates (Avise et al., 1987), and size (Roderick, 1996; Gregory, 2005; Dufresne and Jeffery, 2011). Therefore, it is beneficial in phylogeographic studies to compare both mitochondrial and nuclear DNA. Genotyping by sequencing (GBS) is a cost-effective approach to identify single nucleotide polymorphisms (SNPs) across the nuclear genome (Davey et al., 2011; Narum et al., 2013), and is proven to be successful in phylogeographic studies (Bagley et al., 2020; Stubbs, 2022).

In Chapter Two, it is detailed how DNA was extracted and SNPs were identified for 34 *P. busbyi east* and *P. busbyi west* specimens using GBS in combination with bioinformatic analysis. SNPs were genotyped for each specimen and were compared against each other to reveal the phylogeographic structure within *P. busbyi.* Results were then related back to the mitochondrial COI data obtained by Spencer et al. (2006).

Chapter Three: Science Communication in Phylogenetic Research

Science communication is an important aspect of planning and conducting research, as well as sharing research results and working towards active management plans for conserved taxa.

Chapter Three discusses how science communication was considered throughout all stages of this project. The chapter reviews different methods used to approach common challenges in phylogenetic research, including planning research, building relationships, discussing opposing views, collecting samples, and continually relaying information.

This chapter also discusses alternative approaches and recommendations for other researchers that may be used in future phylogeographic research on taonga species, as well as suggestions on how results and general information may be presented to the public in a manner that will aid in the continued conservation of genus *Paryphanta*.

Chapter Four: General Discussion

Chapter Four follows through on the research presented in the previous chapters by discussing the findings of this research, including possible explanations and implications for results. The General Discussion also considers the limitations of the methods used and their effects on the research. Conclusions and explanations of these conclusions are made regarding the thesis research question. Finally, questions and considerations for future research surrounding the topic of *Paryphanta* phylogeography, taxonomy, and conservation are presented.

Chapter Two

Genotyping by Sequencing (GBS) Reveals Phylogeographic Structure in *Paryphanta busbyi*

Abstract

Paryphanta are a genus of large, carnivorous snails that exist in Northland, New Zealand, with two described species - *P. busbyi* and *P. watti*. Morphological patterns within the *Paryphanta* genus exhibit a north-south split, whereas the current phylogeny based on COI data exhibits an east-west split. Discrepancies between the morphological patterns and the genetic phylogeny have resulted in uncertainty surrounding the phylogeographic structure within genus *Paryphanta* and the taxonomic status of both species. To investigate these discrepancies further, specimens were collected from 12 sites across the Northland and Auckland regions, including Warkworth and the Waitākere Ranges, both of which have known translocative origins. *P. watti* representatives were unable to be obtained so population structure analysis was performed solely on *P. busbyi* specimens. Genotyping by sequencing (GBS) was performed on nuclear data to assess the phylogeographic structure, determine site of origins for known translocations, and identify additional translocated sites and their origins. GBS data was assessed using a phylogenetic maximum likelihood approach, principal component analysis, and structure analysis; these analyses revealed three distinct clades (northern, southern, and Waitākere) as well as an additional intermediate form. A clear north-south phylogeographic pattern was observed between the northern and southern clades, with the intermediate form sharing genetic similarities with both clades. The Waitākere clade was exempt from this north-south trend, presumably due to its translocative origins. Both known translocated sites were inferred to have originated from Tangihua Forest, and a further two additional translocated populations and their potential translocative origins were identified.

2.1 Introduction

Each individual has their own unique pattern of nucleotides that make up their complete genome, which is inherited biparentally or uniparentally (for nuclear genomes and mitogenomes respectively; Hartl and Clark, 1997; Smith, 2016). Genomes can be compared between individuals or against a reference genome to answer a range of questions regarding, but not limited to, population structure, relatedness, and evolutionary history (Weir et al., 2005; Speed

and Balding, 2015; Kim et al., 2017). Single nucleotide polymorphisms (SNPs) are base-pair substitutions that occur at specific points within the genome and are the most common genomic variation observed (Smigielski et al., 2000). Other genomic variations include insertions, deletions, duplications, inversions, and translocations of large chromosomal regions (Feuk et al., 2006; Alkan et al., 2011). The process of identifying and comparing SNPs among individuals is called genotyping, though it is important to note that the term "genotype" can also refer to an individual's entire genetic constitution with specific reference to allelic variations of a gene (Przełęcki, 1964; Watson, 1970).

Historically, conservation genetic research was mainly limited to specific, known regions of the mitochondrial or nuclear genomes due to the time and cost limitations of First Generation sequencing (Narum et al., 2013). Genotyping by sequencing (GBS) is an application of Next Generation sequencing (NGS) that has encouraged the use of genetic research for improved conservation management of species due to its increased accessibility and cost-effectiveness over other genetic methods.

GBS has the ability to newly discover, identify, sequence, and genotype thousands of genome-wide SNPs at the population level (Narum et al., 2013) by sequencing a targeted fraction of the genome (a reduced representation library [RRL]) without requiring sequencing or bioinformatic analysis of the entire genome (Davey et al., 2011; Narum et al., 2013; Friel et al., 2021)). GBS thereby decreases the cost and time associated with genomic research compared to whole-genome sequencing (WGS), while still providing high levels of coverage of the genome in which loci can be sequenced (Davey et al., 2011).

WGS was traditionally used for discovering SNPs through comparison between a sequenced genome and a reference scaffold genome (Li et al., 2019), though oftentimes, obtaining a base-by-base description of the entire genome is unnecessary. GBS reduces the complexity of the genome using restriction enzyme digestion (Rowan et al., 2017; Wickland et al., 2017; Hess et al., 2020), which targets specific base pair sequences to cut the genome into smaller pieces. Fragments within a given size range then undergo PCR enrichment before being sequenced using NGS and genotyped bioinformatically (Hess et al., 2020; Stubbs, 2022).

With tens to hundreds of gigabases of sequence data being generated per run (Narum et al., 2013), GBS analysis pipelines, which are the sequential bioinformatic tools used to automate the genetic analysis of sequence data, are necessary to reach a point in which the data can be interpreted by the researcher. These tools assist by demultiplexing samples, filtering out "noise" and inaccurate sequence calls, identifying undescribed SNPs, and genotyping the individuals sampled (Narum et al., 2013; Rochette and Catchen, 2017). A popular pipeline for GBS analysis, used in this study and other areas of molecular ecology and population genetics, is the Stacks pipeline (Catchen et al., 2011; Rochette and Catchen, 2017). This approach typically requires a minimum of one week, however, a month is commonly regarded as the standard timeframe (Rochette and Catchen, 2017).

Successful applications of GBS have made genetic data more accessible within the zoological context. GBS has greatly advanced conservation research on population-genetic structure (Larsen and Matocq, 2019), maintenance of genetic variation in translocated populations (Jahner et al., 2019), phylogeographic analysis (Bagley et al., 2020; Stubbs, 2022), and a wide range of other topics (Narum et al., 2013). The design of GBS protocols allows for a greater number of samples to be processed at a time (using individually barcoded GBS libraries) and can also be applied more easily across different species, making comparisons between species using a single procedure possible (Rowan et al., 2017).

The methodology has been applied to a wide range of plant and animal species (Larsen and Matocq, 2019), including critically endangered taxa such as kākāpō (Foster et al., 2021; Stubbs, 2022), molluscan species (Jiao et al., 2014; Wang et al., 2016), and non-model species without reference genomes (Andrews et al., 2016; Wright et al., 2019), all of which are criteria that apply to genus *Paryphanta*.

Although GBS is associated with immense benefits, this sequencing method results in extensive amounts of genetic data being lost (Wickland et al., 2017). Consequently, GBS cannot be applied to studies dependent on obtaining full genome sequences (Kumar et al., 2012; Narum et al., 2013; Ulaszewski et al., 2021). Additionally, GBS has added time constraints associated with

bioinformatic analysis, necessitating the inclusion of available time when planning genetic research (Wickland et al., 2017).

2.1.1 Chapter Aims

This phylogeographic research aims to evaluate the reliability of the mitochondrial COI data published by Spencer et al. (2006) by incorporating nuclear data in the analysis of *Paryphanta* populations. GBS was selected as the method of choice for this due to its cost-effectiveness, prior success in phylogeographic studies, and its ability to be performed without a reference genome, given that genomes of *Paryphanta* specimens are yet to be sequenced entirely.

This chapter aims to identify multiple nuclear markers through GBS that may be used for future phylogenetic and phylogeographic research on *Paryphanta* spp., which would overall contribute known markers for future phylogenetic research.

Next, it is aimed that phylogeographic inferences could be made using GBS data that would provide greater overall context on the true phylogeographic patterns in genus *Paryphanta* and lead to greater confidence in the true taxonomic status of populations within this genus.

Finally, it is aimed that population structure would be revealed and inferences could be made regarding the origin of the translocated Warkworth and Waitākere Ranges populations (Parrish et al., 1995). It is expected that additional populations with an undocumented translocative history may also be identified and that inferences could be made regarding these populations' origins.

2.2 Methods

2.2.1 Sample Collection/Curation

In order to obtain high quality DNA for GBS, fresh or well preserved tissue was required and was sourced from a combination of fieldwork and the Museum of New Zealand Te Papa Tongarewa collection.

A Wildlife Act Permit, issued by the Department of Conservation, was required to collect and euthanise specimens. Specimens were held and genetic research was conducted under the Department of Zoology's Department of Conservation permit. As a prerequisite for these permits, engagement was undertaken with Northland iwi. While the Department of Conservation was able to carry out engagement with relevant iwi and hapu, additional engagement was carried out in order to build relationships with multiple Northland iwi and hapu, including Te Parawhau and Te Roroa, and to strengthen existing relationships with Ngāti Wai and Ngāti Kuri (discussed further in Section 3.2.2 *Building Relationships*).

A two-week fieldwork expedition was conducted in the Auckland and Northland regions to collect fresh specimens. Sites were selected based on known locations of populations (e.g. Spencer et al., 2006; museum records), documented observations (iNaturalist, 2022), known translocated sites (i.e. Warkworth and Waitākere Ranges; Parrish et al., 1995), and recommendations from locals based on their knowledge of *Paryphanta* distribution within their rohe/region. Kauri and rimu forests were typically searched, especially those with ferns (e.g. *Dicksonia* spp.), nikau *(Rhopalostylis sapida*), and kanuka *(Kunzea ericoides*).

Upon arrival at native forests with suspected *Paryphanta* populations, an inspection of the road-side ditches was carried out, since *Paryphanta* individuals are frequently washed down the banks from the forest above during heavy rainfall. Shell fragments and perished or dying snails are therefore indicative of a live population nearby. Searches were conducted by looking under

leaf litter in close proximity to ferns rather than kauri trees (as in Montefiore, 1994; Montefiore, 1995; Stringer and Montefiore, 2000).

Multiple methods of obtaining DNA from identified *Paryphanta* specimens were considered, including periostracum fraction (Armbruster et al., 2005; Morinha et al., 2014), DNA swabs (Armbruster et al., 2005; Morinha et al., 2014), tissue clips (Mahlfeld, pers. comm. 2022), sampling of deceased animal tissue (Walton, pers. comm. 2022), and ancient DNA extraction techniques on shell (Psonis et al., 2022; Walton et al., 2023). However, each of these techniques had a large number of limitations, specifically added time constraints and added difficulty of sequencing degraded DNA. Euthanisation was chosen as it provided the added benefits of being able to obtain high-quality DNA and preserve specimens for future subsampling, which would absolve the need for additional sampling for future projects.

All sites were searched during the day, with Whangārei and the Waitākere ranges also being searched at night. As *Paryphanta* are more active nocturnally (Montefiore, 1995), these night searches were very successful, although night searches were not conducted in rural areas due to unfamiliarity with those locations. *Paryphanta* specimens were euthanized on-site using 90% ethanol, a procedure chosen for its speed and efficacy in preserving high-quality DNA in land snails (Gilbertson and Wyatt, 2016).

After two days, the animal was removed from the shell using physical manipulation and was subsampled. Ethanol was replaced to maintain the quality of the specimens and subsamples. All subsamples used for GBS were catalogued in an Excel spreadsheet for efficient management throughout the project. Identification numbers used for this project, collection sites, storage location, subsample storage location, identification numbers used in previous studies, preservation quality, research steps taken, and notable results were recorded for each specimen.

A total of 35 specimens were collected from 12 sites across the Northland and Auckland regions (Figure 2.1), representing different areas within the *P. busbyi* east and *P. busbyi* west distributions. To limit pseudoreplication, as many sites were visited as possible, though this number was limited due to the threatened conservation status of *Paryphanta* populations.

Figure 2.1 Site locations across Northland, New Zealand, from which fresh tissue specimens of genus *Paryphanta* were obtained for phylogeographic analysis. Black pinpoints indicate site locations from which specimens were collected for genotyping by sequencing (GBS) analysis (n = 35), with sample size for each locality indicated within pinpoints. Red and yellow pinpoints indicate site locations used in Spencer et al. (2006), with their respective clades for reference: *P. busbyi* east (red) and *P. busbyi* west (yellow).

Four of the sites sampled for GBS were geographically close to sites used in Spencer et al. (2006), these being Tangihua Forest, Diggers Valley, Waipu Caves, and Warkworth (Figure 2.1). These sites were chosen so that comparisons could be made between GBS data and the COI data produced by Spencer et al. (2006). Warkworth was prioritised for comparison due to its known translocative history (Parrish et al., 1995), whereas the other three sites were included due to their accessibility.

a)
b)
c)

buffer that catalyses the chemical breakdown of cell membranes to allow for the release of target molecules from the cell.

20 µl of Proteinase K was added for protein digestion and removal of contamination, and the samples were mixed thoroughly by vortexing and incubated at 56°C on a rotator overnight, which increases the surface area to volume ratio, therefore speeding up lysis. Following this process, the lysate, being the product containing the lysed cells, did not appear gelatinous which was a good indication that the process worked well.

Samples were vortexed for 15 seconds then a master mix containing 200 µL of >96% ethanol, which extracts proteins from the solution, and 200 µL of Buffer AL, which redissolves any precipitates, was added to each sample. The solution was then vortexed to remove gelatinous lysate and ensure a homogenous solution.

The mixture, including precipitate, was then pipetted into DNeasy Mini spin columns placed within 2 mL collection tubes and centrifuged at 6000 x g for 1 minute. The flow-through and collection tubes were then discarded and the DNeasy Mini spin columns were placed in new 2 mL collection tubes.

500 µl Buffer AW1 was added to each sample and the samples were centrifuged for 1 min at 6000 x g. The flow-through and collection tubes were then discarded and the DNeasy Mini spin columns were placed in new 2 mL collection tubes. 500 µl Buffer AW2 was added to each sample and the samples were centrifuged for 3 min at 20,000 x g in order to dry the DNeasy spin column membrane. Both buffer AW1 and AW2 are wash buffers which are used to remove contaminants, including proteins and salts, from the sample. The centrifuge step removes all residual ethanol as this can interfere with subsequent steps, including high temperature steps such as PCR in which remaining ethanol will cause the denaturation of DNA molecules. The DNeasy Mini spin column was then removed carefully to ensure the column did not come into contact with the flow-through as this could result in accidental contact with ethanol. Any contact with ethanol would require an additional centrifuge step at 20,000 x g for 1 minute to once again

dry the spin column membrane. The flow-through and collection tubes were then discarded and the DNeasy Mini spin columns were placed in 2 mL Eppendorf DNA LoBind tubes.

Flow-through was discarded appropriately each time in a waste container separate from bleach waste. This was done to avoid contact between Buffer solutions and bleach as the acidic Buffer solution combined with bleach produces a harmful chlorine gas, posing severe health and safety risks.

200 µl of Buffer AE, i.e. the elution buffer, was pipetted directly onto the DNeasy membrane to ensure maximum contact of the elution buffer with the DNA on the membrane. The samples were then incubated at room temperature for 1 minute before being centrifuged for 1 minute at 6000 x g to elute, which due to the added Buffer AE, removes the final DNA molecules from the extraction mix and encourages its transition into the Eppendorf DNA LoBind tubes. Since the preservation quality of the samples was good, the elution step was not repeated.

The spin column was discarded following the centrifuge and the remaining product was the usable DNA extract.

Quantification

Fluorometers use fluorescent dyes that emit signals when bound to DNA or RNA, avoiding free nucleotides, degraded nucleic acids, and protein contaminants. Fluorometers are beneficial over spectrophotometers due to their greater precision and accuracy, particularly at low concentrations, and due to their nature in which readings are less likely to be affected by contaminants. Limitations, however, mean fluorometers are more expensive (due to requiring specific kits and thin-wall 0.5 mL tubes), more time-consuming, require more DNA, and do not provide information on DNA quality.

Spectrophotometers differ from fluorometers as they are also able to assess DNA quality using ratios of ultraviolet light absorbance values, as indicated by 260/230 and 260/280 ratios. These

values would be considered within the “normal” range between 2.0-2.2 and ~1.8 respectively, while values significantly lower or greater than this are indicative of contamination.

Fluorometer values were required for later calculations to determine the amount (µL) of DNA extract required for library preparation, but with the Spectrophotometer being less expensive, less time-consuming, requiring less DNA extract, and being able to assess DNA quality, this method of quantification was used initially despite its lower accuracy.

The amount of DNA in raw extracts was quantified by placing 1 µL of DNA on the spectrophotometer after first using 1 µL of Buffer AE as a “blank” (Supplementary Table 2.1).

The DNA concentrations of just nine samples, representing a range of DNA concentrations from the spectrophotometer, were then measured again using the Qubit® 3.0 Fluorometer, following the Qubit® dsDNA Broad Range Assay protocol as follows. The high and low standards used for the Qubit® contained 190 µL of qubit solution and 10 µL of either standard solution 1 or standard solution 2 which were added to two 0.5 mL qubit tubes.

The samples were prepared by combining 198 µL of qubit solution and 2 µL of DNA extract in individual 0.5 mL qubit tubes. All 0.5 mL tubes were mixed by vortexing then briefly spun down on a benchtop centrifuge before components of the tubes were measured by the Qubit®. This was done only on a subset of samples in order to obtain a reference concentration that could then be used to extrapolate Qubit® values for all samples (Supplementary Figure 2.1). The extrapolated values were then used to calculate the volume (µL) of DNA extract required to obtain 500 ng of DNA which was the required amount for library preparation (Supplementary Table 2.1).

2.2.3 GBS Library Preparation

Digestion and Ligation

The digestion and ligation steps bind adapters to DNA, these being the barcode and common adapters (Table 2.1). Each well within the 96-well plates used has a unique combination of adapters, i.e. each DNA strand has one adapter flanking each of its 5' and 3' ends. A unique barcode adapter is used for each of the wells, while common adapters may be one of eight sequences (with each lettered row containing a unique sequence, though only five rows were used in this case). Adapters are able to ligate to the DNA strands as each adapter contains complementary sequences to the DNA strands following DNA cuts catalysed using the specific restriction enzyme used (PstI-HF). However, there is a chance of two of the same adapters binding to one DNA strand, i.e. two common adapters or two barcode adapters flanking a single DNA strand. If this happens, it is considered noise and is ignored.

Digestion

The purified DNA (500 ng) was added to 96-well plates containing the two previously added adapters in each well. The Eppendorf Concentrator Plus was used to dry plates at 45°C on the V-AQ setting for 1.5 hours.

The DNA was digested using PstI-HF restriction enzyme (40 units) from New England Biolabs (which forms a 4 bp long sticky end at the 5′-CTGCA/G-3′ cut site) and Cutsmart Buffer from New England Biolabs (2.0 µL). The master mix was brought up to a total volume of 20 µL per sample by adding dH_2O (17.8 µL). The mix was vortexed and spun down in the mini centrifuge before being poured into a trough from which 20 µL was added to each well using a multichannel pipette.

The plate was then put in a MJ PTC-200 Thermal Cycler at 37°C for 2 hours, as this is the optimal temperature for PstI-HF activity, and was followed by a cooling phase at 4°C for 1

minute to pause the reactions while prolonging PstI-HF life to ensure it remains active for the subsequent ligation step.

Table 2.1 Unique PstI-HF barcode adapters used for each fresh tissue *Paryphanta* sample in genotyping by sequencing (GBS) library preparation.

Well	Sample	Site Locality	GBS Barcode Adapters	GBS Common Adapters
A1	CW.021.01	Tangihua Forest	CTCG	ATGAAAG
A2	CW.021.02	Tangihua Forest	TGCA	ATGAAAG
A3	CW.021.03	Tangihua Forest	ACTA	ATGAAAG
A4	CW.022.01	Waipu Caves	CAGA	ATGAAAG
A5	CW.023.01	Lonsdale Park	AACT	ATGAAAG
A6	CW.023.02	Lonsdale Park	GCGT	ATGAAAG
A7	CW.024.01	Warkworth	CGAT	ATGAAAG
B1	CW.024.02	Warkworth	CGCTT	CTCG
B2	CW.025.01	Whangārei	TCACG	CTCG
B3	CW.026.01	Waima Forest	CTAGG	CTCG
B4	CW.026.02	Waima Forest	ACAAA	CTCG
B5	CW.026.03	Waima Forest	TTCTG	CTCG

Table 2.1 Continued.

B6	CW.026.04	Waima Forest	AGCCG	CTCG
B7	CW.027.01	Puketi Forest	GTATT	CTCG
C1	CW.028.01	Mangamuka	ATTGA	ACGACTAG
C2	CW.028.02	Mangamuka	CATCT	ACGACTAG
C3	CW.028.03	Mangamuka	CCTAG	ACGACTAG
C4	CW.028.04	Mangamuka	GAGGA	ACGACTAG
C5	CW.029.01	Diggers Valley	GGAAG	ACGACTAG
C6	CW.030.01	Totara North	GTCAA	ACGACTAG
C7	CW.031.01	Whangārei	TAATA	ACGACTAG
D1	CW.031.02	Whangārei	TAGGAA	ACTA
D2	CW.031.03	Whangārei	GCTCTA	ACTA
D3	CW.031.04	Whangārei	CCACAA	ACTA
D4	CW.032.01	Warkworth	CTTCCA	ACTA
D5	CW.033.01	Waipoua Forest	GAGATA	ACTA
D6	CW.033.02	Waipoua Forest	ATGCCT	ACTA

Table 2.1 Continued.

D7	CW.033.03	Waipoua Forest	AGTGGA	ACTA
E1	CW.033.04	Waipoua Forest	CTCG	CGCTT
E2	CW.033.05	Waipoua Forest	TGCA	CGCTT
E3	CW.034.01	Waitākere Ranges	ACTA	CGCTT
E4	CW.034.02	Waitākere Ranges	CAGA	CGCTT
E5	CW.034.03	Waitākere Ranges	AACT	CGCTT
E6	CW.035.01	Waitākere Ranges	GCGT	CGCTT
E7	CW.035.02	Waitākere Ranges	CGAT	CGCTT

Ligation

While the DNA was digesting, a master mix of T4 DNA ligase buffer (5 µL) and T4 DNA ligase from New England Biolabs (400 units) was prepared for each sample's ligation phase. The final volume of the master mix was brought up to 30 µL by adding dH_2O (24 µL). The master mix was poured into a trough and 30 µL was added to each well containing digested DNA using a multichannel pipette.

The plate was placed in the PTC-200 at 16°C for 30 minutes, 37°C for 2 minutes, 16°C for 30 minutes, 37°C for 2 minutes, 16°C for 30 minutes, followed by a denaturation stage at 80°C for 30 minutes and a final cooling phase at 10°C for 1 minute to conclude the reactions. Each 16°C ligation phase attaches the adapters to DNA strands, however, the nature of the ligation steps means DNA strands may recombine. The digestion phases of brief, intermittent 37°C increases in temperature allow PstI-HF to re-separate the genomic DNA strands that are yet to have

adapters ligated. PstI-HF does not digest DNA from adapters during these phases as the adapter sequence does not include the full restriction site, rather, it only contains the matching sticky end. The final product is the DNA strands with attached adapters.

Purification

The plate was centrifuged to remove liquid from the tops of the wells then 50 µL of the digested-ligated (DIG-LIG) DNA was pipetted into a Qiagen MinElute 96-well PCR purification plate using a multi-channel pipette, which enables pipetting of multiple wells simultaneously. The MinElute plate was then placed in a Presto™ Vac 96 Well Vacuum Manifold at 60 cm/Hg for 5 minutes to dry wells. Excess liquid was dabbed with a paper towel from underneath the plate before a second vacuum was done for 2 minutes until there was no longer any excess liquid underneath.

The plate was then washed with dH_2O (30 µL) in each well and was again placed in a vacuum at 60 cm/Hg for 5 minutes, and again for 2 minutes since excess liquid was pooled underneath after the first vacuum step.

23 µL of elution buffer (EB; 10 mM Tris-Cl, pH 8.5) from the Qiagen DNeasy blood and tissue kit was added to each well in the minelute plate and mixed 30 times using an Integra Viaflo electronic multichannel pipette. The EB and DNA mix was then transferred to a fresh 96-well plate using the Viaflo.

Polymerase Chain Reaction (PCR)

For each sample, 10 µL of DIG-LIG-PUR DNA was put into a fresh 96-well plate. A master mix was made by combining 2x MyTaq HS Master Mix from Bioline (25 µL) and each of the following PCR Primers (0.2 µM): (1) 5’-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT, (2) 5’-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT. Primer sequences were taken from Elshire

et al. (2011). The final volume of the master mix was brought up to 50 µL by adding dH_2O (13 µL).

Primers contained complementary sequences for amplifying restriction fragments with ligated adapters, with the forward primer attaching to the start codon of the template DNA (the anti-sense strand) and the reverse primer attaching to the stop codon of the complementary strand of DNA (the sense strand). The 5' ends of both primers bind to the 3' end of each DNA strand. Primers also contained complementary sequences for binding PCR products to oligonucleotides that coat the Illumina sequencing flow cell and priming subsequent DNA sequencing reactions (Elshire et al., 2011).

All reactions were prepared on ice. Again, the amounts were multiplied by 40 to allow for pipetting error. 40 µL of the master mix was then added to each well to combine with the 10 µL of DIG-LIG-PUR DNA.

A PCR negative was also made using 40 µL of the master mix, though an extra 10 µL of dH_2O was used in place of the DIG-LIG-PUR DNA. The Eppendorf Mastercycler Pro was set to 75°C for 5 minutes; then performed initial denaturation at 95°C for 60 seconds; 24 cycles of denaturation, annealing, and extending at 96°C for 30 seconds, 65°C for 30 seconds, and 72°C for 30 seconds respectively; followed by a final Taq extension step at 72°C for 5 minutes and a final cool-down period of 4°C for 1 minute.

Visualisation of DNA

To assess the concentration and length of PCR products, samples were centrifuged then run on 1% agarose gel containing SYBR Safe from ThermoFisher Scientific in 1X TAE Buffer at 250 volts for 15 minutes and photographed before being run for a further 15 minutes (Figure 2.3). Each well contained 10 µL of PCR product. For easy size comparison, 2 µL of the GeneRuler DNA Ladder Mix (100 bp) from ThermoFisher Scientific was added to two of the wells. To ensure the DNA extraction step did not pick up contamination from alternate sources of DNA, the extraction negative was also included in one of the wells.

The fragment size range of the PCR product to be sequenced was between 200 bp and 500 bp, as this was long enough to hopefully contain a SNP whilst still being short enough that it could be sequenced with the chosen sequencing platform as described in Section 2.2.4 *Sequencing*. The image taken of the gel electrophoresis (Figure 2.3) revealed that all samples did contain fragments of the desired length, and CW.024.02, CW.026.04, CW.031.02, CW.033.03, CW.035.01, CW.034.01, CW.035.02, and CW.034.02 were brighter between the 200 bp - 500 bp range, meaning they had a higher concentration of fragments within this size range.

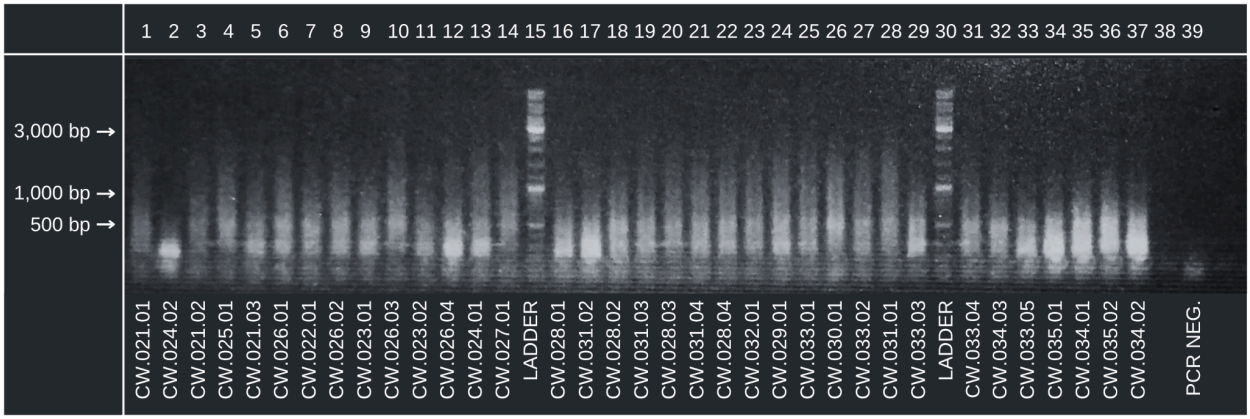


Figure 2.3 Gel electrophoresis showing the PCR product of genotyping by sequencing (GBS). The sizes of brighter bands in the ladder are indicated on the left.

Pooling of PCR Products

PCR products were pooled into a 1.5 mL tube for sequencing. To normalise concentrations between the 200 bp to 500 bp range, only 4 µL of PCR product was pooled for samples with a brighter band within this size range (CW.024.02, CW.026.04, CW.031.02, CW.033.03, CW.035.01, CW.034.01, CW.035.02, and CW.034.02), whereas 8 µL of PCR product was pooled for all other samples. The pooled DNA was then vortexed vigorously for 10 seconds and centrifuged.

Purification of Pooled Library

The pooled PCR products were purified using the iNtRON MEGAquick-spin™ Plus Total Fragment DNA Purification Kit to remove leftover primers and unincorporated dNTPs as these interfere with DNA sequencing reactions. To allow for human error and to ensure the most optimally purified DNA was sequenced, the pooled PCR product was purified twice separately, with 100 µl undergoing the same purification process as follows.

100 µl of PCR product was transferred to a 1.5 mL Eppendorf microcentrifuge tube and 500 µl of BNL Buffer / Plus, which is an agarose gel lysis buffer, was added and mixed well by vortexing. The BNL Buffer was handled with care due to it containing chaotropic salts, which are irritants. The vortexed sample was then transferred to a column within a collection tube and centrifuged at 11,000 x g for 30 seconds. The flow-through was then discarded.

750 µl of Washing Buffer / Plus (with ethanol previously added) was added to the column within the collection tube and centrifuged at 11,000 x g for 30 seconds. Then flow-through was again discarded. The sample was then centrifuged again at 18,000 x g for 3 minutes in order to dry the column membrane before the column was inserted into a new microcentrifuge tube.

40 µl of Elution Buffer / Plus was pipetted directly onto the column's membrane centre. The column was left for 1 minute before being centrifuged at 18,000 x g for 1 minute to elute the DNA. The spin column was then discarded. The final purified DNA was vortexed to mix for 5 seconds before being centrifuged for a final time at 18,000 x g for 1 minute to ensure all sample was properly mixed and spun down.

Quantification

Both 1.5 mL tubes containing purified DNA were quantified using spectrophotometry and fluorimetry, as detailed in Section 2.2.2 *DNA Extraction/Quantification*. This was done to assess quality and concentration of DNA before deciding which of the two purified pooled DNA samples should be sent for sequencing (Supplementary Table 2.2).

"Tube 2" was determined to have a higher DNA concentration, as indicated by the ng/µl value. Furthermore, "Tube 2" was determined to consist of better quality DNA, as indicated by the 260/230 ratio. Despite being outside the ideal 260/230 ratio of 2.0-2.2, "Tube 2" had a much lower ratio than "Tube 1" (2.40 versus 3.27 respectively). This suggests that "Tube 1" had greater contamination by unwanted compounds. Therefore, "Tube 2" was selected for sequencing.

2.2.4 Sequencing

The pooled library was sent to the John Curtin School of Medical Research at the Australian National University (ANU) and run on a NextSeq 2000 using 150 bp paired-end sequencing.

2.2.5 Bioinformatics

SNP Calling

Raw fastq files were quality-checked using FastQC v0.11.9.. Adapters and reads shorter than 95 bp were removed using *cutadapt* v4.4. Reads were demultiplexed using the *process_radtags* module in *Stacks* v2.61. Reads were then concatenated using *Python* v3 custom scripts.

Genotyping

Stacks v2.61 was used for SNP calling. In the absence of a closely related reference genome, SNPs were called *denovo* using the *denovo_map.pl* was used with default parameters (-M = 2, -n = 2). Following genotyping, *VCFtools* v0.1.15 was used to assess the quality of samples based on missing data. Sample CW.021.02 was removed from subsequent analyses due to its high proportion of missing data (>80%).

SNPs were filtered using *VCFtools* v0.1.15, and SNP loci that appeared in 80% of sampled individuals were retained (vcftools --vcf M2_final/populations.snps.vcf --max-missing 0.8

--recode). Finally, all SNPs were thinned within 100 bp to ensure only one SNP per locus was kept ().

Population Structure

Figures were then generated to visualise *P. busybi* population structure, which was assessed through maximum likelihood phylogenetic analysis, principal component analysis, and *STRUCTURE* analysis.

An unrooted maximum likelihood (ML) tree was constructed using IQ-TREE 2 v.2.2.5 and visualised using FigTree v1.4.4, which suggests the fewest evolutionary steps and branches between individuals as the most likely evolutionary relationship. This was able to easily visualise relationships between individuals without the added complexity of genetic variation between individuals.

The *pcadapt* R package (v4.2.3; R Core Team, 2023) was used to perform a principal component analysis (PCA), which reduces the dimensionality of the dataset to a few core axes that summarises most of the variance. The greatest and second greatest principal components of variation between site localities were displayed for easier interpretation of genetic variation.

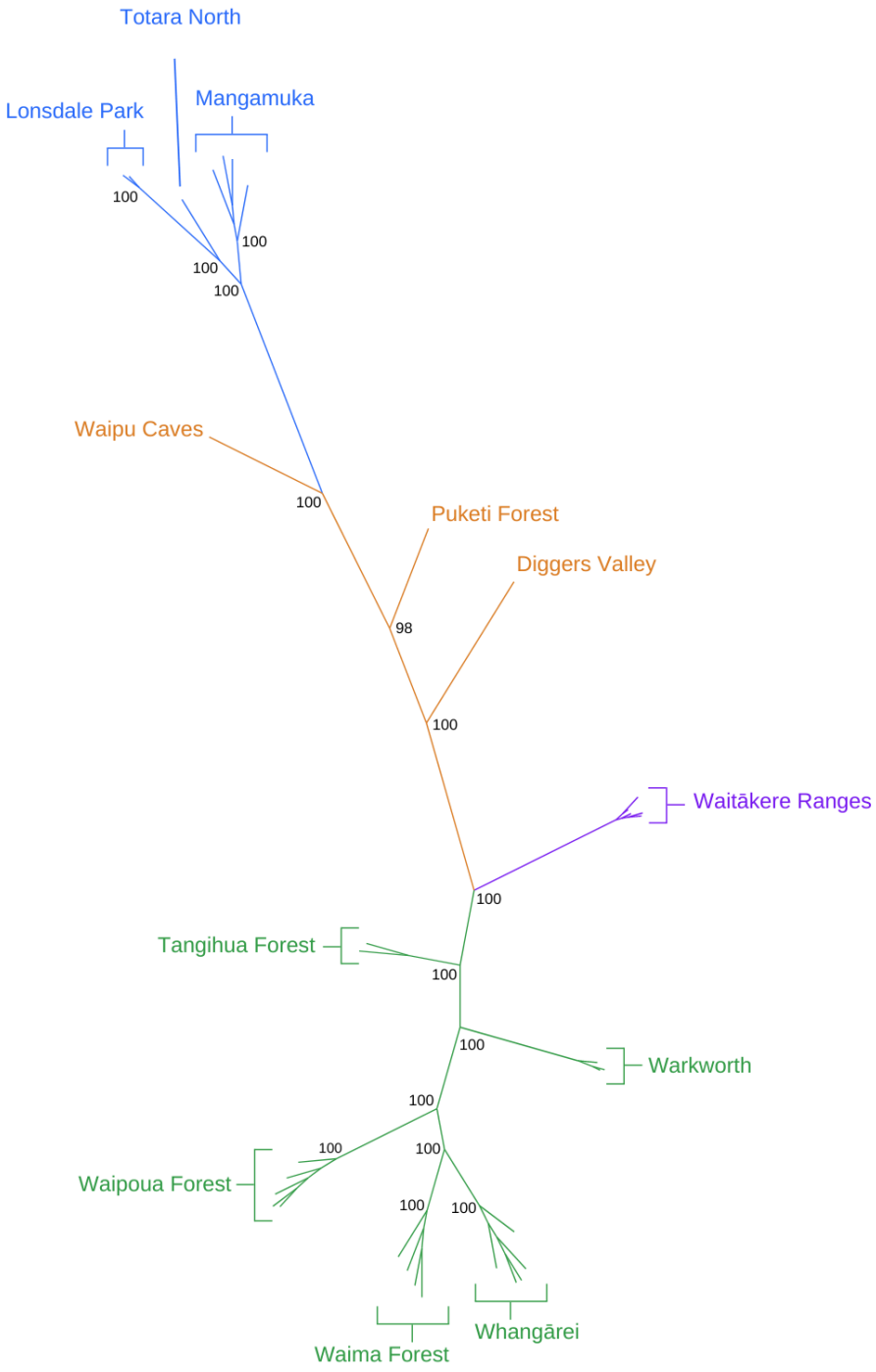
STRUCTURE v2.3.4 was to investigate population structure with three replicates for each K value between 2 and 12, given that there were 12 sample collection sites. Default parameters were mostly applicable but parameters were adjusted from default settings for the number of replications (numreps = 200,000) and the burn-in period (burnin = 50,000), with the latter setting the number of steps discarded wherein the Markov chain moves from its unrepresentative initial value to its equilibrium distribution. An Evanno test to identify the most likely K was run using Structure Harvester (Earl and vonHoldt, 2012). The mean value of likelihood was looked at to determine how well the model fit the data for each of the three *STRUCTURE* runs and the most likely replicate was chosen for visualisation.

2.3 Results

Using GBS, we generated genome-wide SNP data for 35 specimens of *P. busbyi*. GBS produced 32,389,454 raw reads. After discarding one Tangihua Forest sample (CW.021.02) with high SNP call missingness and filtering out SNPs found in less than 80% of the population, 17,412 SNPs across 34 individuals remained.

A maximum likelihood (ML) phylogeny, based on SNPs that were obtained through GBS, showed evolutionary relationships between individuals and supported three clades (northern, southern, and Waitākere) as well as an additional intermediate form, with high bootstrap support ≥92% (Figure 2.4). The northern (Lonsdale Park, Totara North and Mangamuka) and southern (Waipoua Forest, Waima Forest, Whangārei, Warkworth and Tangihua Forest) clades followed a north-south phylogeographic pattern, with the ML tree placing the intermediate form (Waipu Caves, Puketi Forest, and Diggers Valley) between the northern and southern clades. The intermediate form appeared to share genetic elements with both the northern and southern clades, with Waipu Caves genetically more similar to the northern clade, followed by Puketi Forest, and finally Diggers Valley, which appeared to be closest to a true "intermediate". The intermediate nature of these populations was based solely on these genetic elements as no morphological differences were observed.

The Waitākere Ranges appeared to be more related to the southern clade but were labelled as a distinct clade in Figure 2.4 due to genetic differences revealed from principal component analysis and *STRUCTURE* analysis. Despite the Waitākere Ranges existing further south than those populations grouped within the "southern" clade, it was decided that the Waitākere clade should not be named based on its relative geographical placement due to its translocative origins (Parrish et al., 1995; discussed further in *Discussion/Translocated Sites and Their Origins*).


Totara North
Lonsdale Park
Mangamuka
100
100
100
100
Waipu Caves
100
Puketi Forest
Diggers Valley
98
100
Waitākere Ranges
100
Tangihua Forest
100
100
Warkworth
100
100
100
Waipoua Forest
100
100
100
Whangārei
Waima Forest

Principal component analysis (PCA) explained 60% and 20% of variance through PC1 and PC2 respectively (Supplementary Figure 2.2). The greatest levels of variation (PC1) were between the northern and southern/Waitākere clades, with the intermediate form positioned between the two groups in terms of genetic variation (Figure 2.5). Of the "intermediate" sites, PC1 revealed the Waipu Caves population to be the most genetically similar to the northern clade, followed by the Puketi Forest and Diggers Valley populations respectively, while PC2 revealed genetic similarities between the Waipu Caves and Tangihua Forest populations.

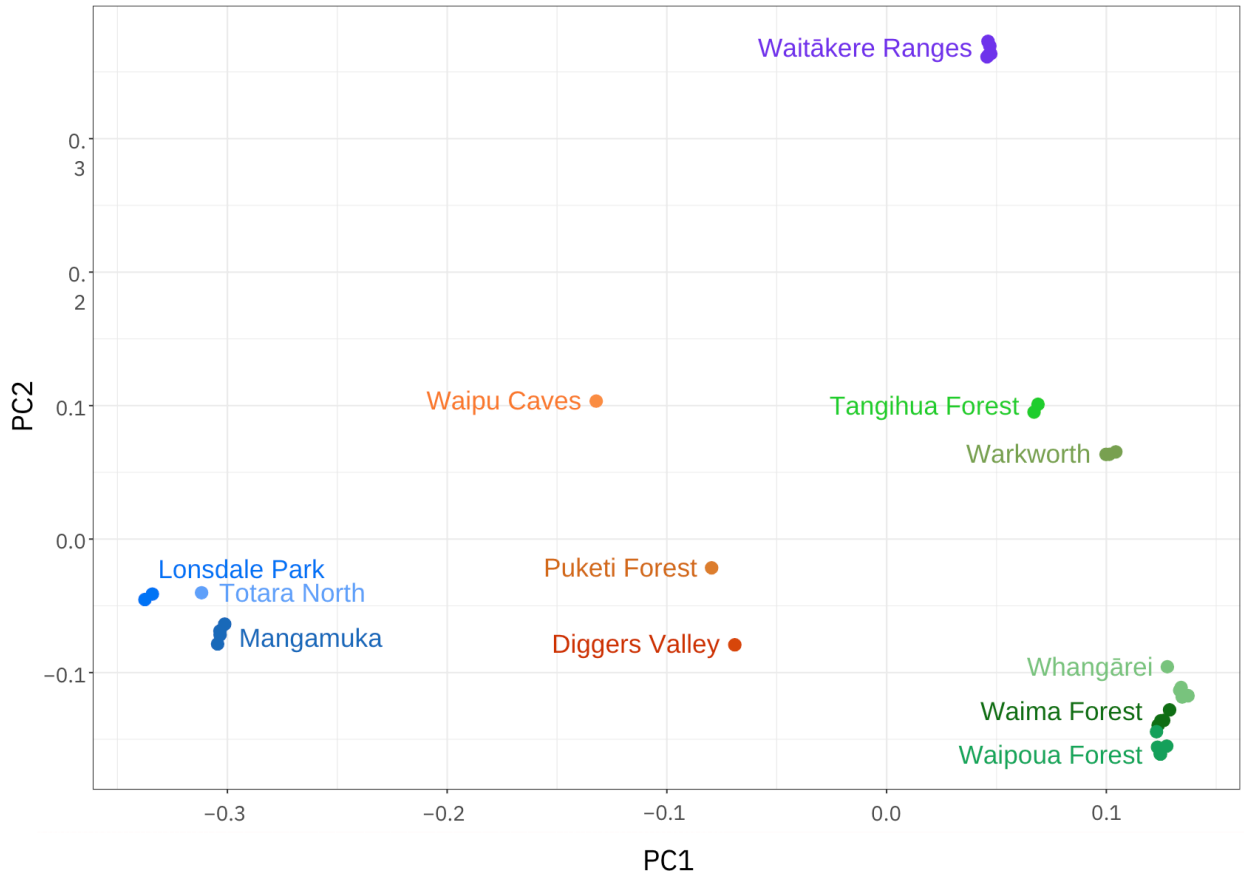


Figure 2.5 Population structure of *Paryphanta busbyi* (n = 34) depicted by principal component analysis (PCA), generated using the *pcadapt* R package (v4.2.3; R Core Team, 2023). The greatest and second greatest levels of variation between site localities are represented by the X and Y axes respectively. Colours indicate the three proposed clades: northern (blue), southern (green), and Waitākere (purple), as well as the intermediate form (orange).

PC2 showed the Waitākere Ranges population as a genetically distinct clade. The Waitākere clade was most similar to the Waipu Caves, Tangihua Forest, and Warkworth sites (Figure 2.5), corroborating its positioning on the ML phylogeny (Figure 2.4).

Most specimens collected from single locations appeared to be more similar to one another than to specimens from other locations, although the PC2 did suggest that one specimen from Waipoua Forest may be closely related to samples from Waima Forest and that one specimen from Whangārei appeared to be more diverged than other Whangārei specimens. Overall, the Whangārei, Waima Forest, and Waipoua Forest populations were positioned closely on both axes, therefore, slight variations between individuals are minuscule in terms of how the individuals fit into the *P. busbyi* population structure as a whole.

STRUCTURE analysis showed the highest support for four genetic clusters (K = 4), these corresponding with the northern clade, southern clade, intermediate form, and the Waitākere clade (Figure 2.6). These same clades were supported when K = 3 and K = 5, although with varying proportions of genetic variation within clades. These patterns align with the clades identified based on ML phylogeny (Figure 2.4) and PCA (Figure 2.5). The northern and southern clades were apparent and genetically distinct from one another when K = 2, with the Waitākere clade grouped as "southern".

All samples from the intermediate form demonstrated genetic similarities with both the northern and southern clades. Of the intermediate forms, the Waipu Caves population appeared closest to the Puketi Forest population, as did the Diggers Valley population. Both the Waipu Caves and Puketi Forest populations also showed similarities with the Warworth, Tangihua Forest, and Waitākere Ranges populations, whereas Diggers Valley did not share these same similarities.

When K = 3, the Waitākere clade appeared as distinct from the southern clade, and genetic similarities held between the Waitākere clade, sites within the intermediate region, and certain sites within the southern clade (specifically Warkworth and Tangihua Forest) became pronounced. The observed patterns remained for K = 4 and K = 5, providing further evidence for the Waitākere clade's placement in the ML phylogeny (Figure 2.4) and the PCA (Figure 2.5).

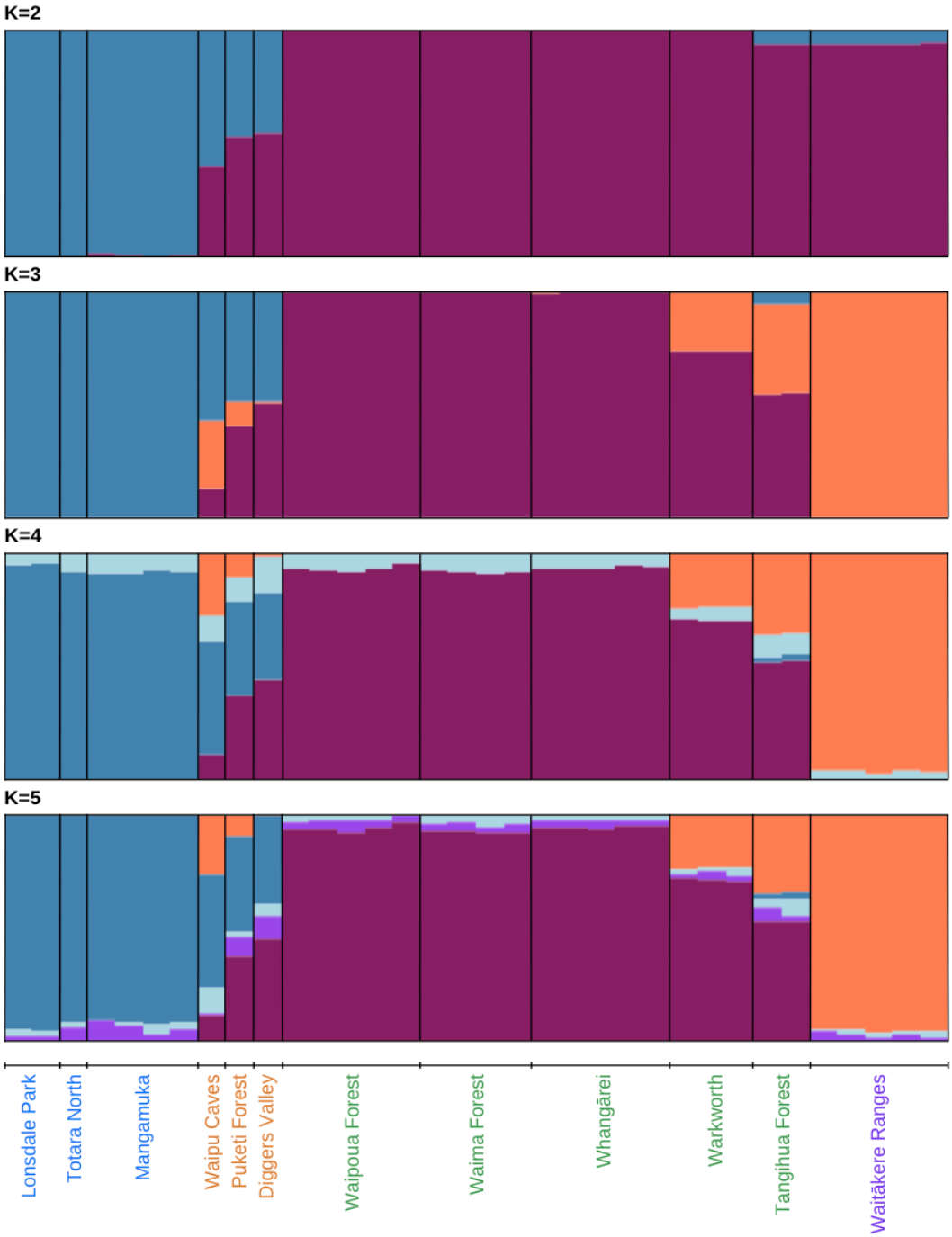


Figure 2.6 Individual clustering assignment of *Paryphanta busbyi* (n = 34) as determined by *STRUCTURE* analysis for K = 2-5 (with greatest support for K = 4). Individuals are represented by vertical bars with their accordance to each genetic cluster indicated by different colours (plum, steel blue, coral and light blue). Coloured labels indicate the three proposed clades: northern (blue), southern (green), and Waitākere (purple), as well as the intermediate form (orange).

The three clades (northern, southern, and Waitākere) and the north-south intermediate form based on ML phylogeny (Figure 2.4), PCA (Figure 2.5), and *STRUCTURE* analysis (Figure 2.6), demonstrated a clear phylogeographical trend when mapped (Figure 2.7).

Figure 2.7 Site locations across Northland, New Zealand, from which specimens of *Paryphanta busbyi* were collected and used for genotyping by sequencing (GBS) and subsequent bioinformatic analysis (n = 34). Colours indicate the three proposed clades: northern (blue), southern (green), and Waitākere (purple), as well as the intermediate form (orange). Sample size for each locality is indicated within pinpoints. Note: one specimen from Tangihua Forest was excluded from the original sample during bioinformatic analysis (see Section 2.2.5 *Bioinformatics* for explanation). Map was created using National Geographic Map Maker.

The genetic distinctness between the northern and southern clades correlated with the clades' geographical placements (Figure 2.7). Additionally, the genetic likeness of the intermediate form to both the northern and southern clades was found to be consistent with its geographical location between the "northern" and "southern" sites (Figure 2.7). However, despite appearing to be the most genetically similar "intermediate" specimen to the northern clade (Figures 2.5 and 2.6), Waipu Caves was geographically closer to the southern clade than other "intermediate" sites (Figure 2.7). Finally, the population structure of the Waitākere clade correlated with its geographical positioning (Figure 2.7) as the Waitākere clade showed greater genetic similarity to the southern clade and intermediate form respectively, with the least genetic similarities seen between the Waitākere and northern clades (Figures 2.5 and 2.6).

2.4 Discussion

GBS was used to discover novel SNPs in the nuclear genome and obtain information on the population structure of *P. busbyi*, which could then be used to infer the phylogeographic pattern as well as identify and determine the origin of translocated populations.

Although this research aimed to assess the phylogeographic patterns across the entire *Paryphanta* range, *P. watti* specimens were unable to be obtained. Consequently, inferences can only be made about populations of *P. busbyi*. For the purpose of this discussion, the population structure and phylogeographic pattern of genus *Paryphanta* discussed will exclude that of *P. watti*.

It must also be considered that considerable geographic sites within central and western Northland were not able to be sampled. These sites could harbour important genetic variation not represented in sampled populations. Additionally, given the small sample sizes for many sites, the results, especially those from PCA and *STRUCTURE* analyses, should be interpreted with caution.

2.4.1 Population Structure and Phylogeography of Genus *Paryphanta*

GBS analysis revealed three distinct clades (northern, southern, and Waitākere) as well as an additional intermediate form (Figures 2.4, 2.5, and 2.6). The northern and southern clades were the most geographically and genetically distinct, sharing little genetic variation and not clustering (Figures 2.4, 2.5, and 2.6).

The intermediate form revealed genetic variation and clustering that was close to both the northern and southern clades (Figures 2.5 and 2.6). The "intermediate" nature of this form was further supported by the intermediate populations' geographical positioning between the ranges of both the northern and southern clades, with one population (Waipu Caves) being clustered within the range of the southern clade (Figure 2.7). Although the Waipu Caves population could be geographically assigned to the range of the southern clade, this population was more similar genetically similar to that of the intermediate forms', which indicates a potential translocation from a site between the northern and southern clades (discussed further in *Discussion/Translocated Sites and Their Origins*). Therefore, if this population's geographical positioning is disregarded, the intermediate form can be considered truly "intermediate" to both the northern and southern clades in terms of both genetic makeup and geographical positioning, thereby suggesting a north-south phylogeographic pattern across Northland.

The Waitākere clade was revealed to be most genetically similar to the southern clade, specifically the Tangihua Forest and Warkworth populations (Figures 2.4, 2.5, and 2.6). Despite Warkwork and Tangihua Forest being the most proximal sites to the Waitākere Ranges, this clade is geographically distinct from all southern clade populations (including Tangihua Forest and Warkworth) to correlate its genetic similarity with geographic proximity. Instead, given the Waitākere Ranges known translocative history (Parrish et al., 1995), this genetic similarity between the Waitākere clade and certain southern clade populations likely indicates its translocative origin rather than natural population expansion (discussed further in *Discussion/Translocated Sites and Their Origins*). Due to being translocated, the Waitākere clade, which exists within the Auckland region, does not align with the exact phylogeographic pattern that is observed across Northland.

The northern and southern clades, in combination with the intermediate form, reveal a clear north-south phylogeographic pattern (Figure 2.7), which contradicts the east-west split reported by COI data and unpublished nuclear data (Spencer et al., 2006; Kennedy, pers. comm. 2022). GBS methods provide higher coverage compared to the COI and ITS-2 genes alone, suggesting that the north-south pattern across Northland, identified through GBS analysis, is the most accurate.

Since *P. watti* was unable to be included in GBS analysis, the distinct morphotypes between *P. watti* and *P. busbyi* cannot be compared and contrasted against GBS data at this time. However, since significant genetic variation corresponding to the geographical positioning of populations was observed across *P. busbyi* populations, it is likely that given the distinct geographic distribution of *P. watti,* they would likely show high levels of variation compared to *P. busbyi.* While such a finding would not correlate with phylogeographic patterns revealed through previous research including *P. watti* specimens (Spencer et al., 2006; Kennedy, pers. comm. 2022), it remains plausible given the northern distribution of *P. watti* and the north-south phylogeographic pattern revealed by GBS analysis.

Without being able to accurately compare Lonsdale Park (intermediate form) specimens to *P. watti,* the apparent morphological similarities of this population suggest they may share more genetic similarities with *P. watti* than other *P. busbyi* populations (Figure 2.2). If a north-south phylogeographic pattern extends to the distribution of *P. watti*, the Lonsdale Park population may represent an intermediate form between *P. watti* and *P. busbyi.* Given that the Lonsdale Park population is one of the closest populations to the *P. watti* distribution, while also being the most genetically distinct from the southern clade, the hypothesis that this north-south split exists across the entirety of Northland is not unreasonable. Additional GBS analysis including *P. watti* specimens is necessary to verify the extension of the north-south phylogeographic pattern to the distribution of *P. watti.*

2.4.2 Translocated Sites and Their Origins

Known translocations

Both the Warkworth and Waitākere Ranges populations are known to be translocated, although their site of origin is currently undocumented (Parrish et al., 1995). With GBS data now available for both populations, population structure can be analysed to infer the most likely place of origin for Warkworth and Waitākere.

Firstly, the populations in Warkworth and the Waitākere Ranges exhibit low genetic variation within each population (Figure 2.6), which is indicative of past translocations. Nevertheless, low genetic variation appears to be a common issue shared across the majority of *P. busbyi* populations (Figure 2.6), and hence these patterns may instead reflect past bottlenecks from habitat loss or predation, or may be a consequence of low sampling. In the case of Warkworth and the Waitākere Ranges, the low genetic variation supports the literature, which states that Warkworth and the Waitākere Ranges each have a translocative origin.

STRUCTURE analysis revealed high levels of genetic similarity between Warkworth and the Waitākere Ranges (Figure 2.6), which suggests that both populations may have been translocated from the same site. Structure analysis also revealed that both sites shared high levels of genetic similarity with Tangihua Forest, followed by Waipu Caves (Figure 2.6). This suggests that one of these two sites, or a nearby location, would be the most likely place of origin. When looking at the majority of variation revealed by PCA, both Warkworth and the Waitākere Ranges had high levels of genetic similarity to Tangihua Forest and little similarity to Waipu Caves (Figure 2.5). ML analysis placed both Warkworth and the Waitākere Ranges by Tangihua Forest with 100% bootstrap support (Figure 2.4), therefore supporting Tangihua Forest as the site of origin.

Since previous phylogeographic research by Spencer et al. (2006) included samples collected from several similar sites, comparisons between GBS data and COI data can be made to measure the level of support for Tangihua being the site of origin for the populations in Warkworth and the Waitākere Ranges. Samples used by Spencer et al. (2006) included a population from a

second Warkworth site, labeled in Spencer et al. (2006) as "Woodcocks, Warkworth." It can be assumed that the Woodcocks population used in Spencer et al. (2006) is of the same origin as the Warkworth population sampled for GBS as only one translocation in that area has been recorded (Parrish et al., 1995). Unfortunately, Spencer et al. (2006) did not sample from the Waitākere Ranges, so inferences about the site of origin for the Waitākere Ranges population can only be made from GBS.

According to phylogenetic analysis using the COI gene, the Woodcocks population was revealed to be most closely related to both a Tangihua Forest population and a Paparoa population (Spencer et al., 2006). The COI data available for Woodcocks and Tangihua Forest populations aligns with the GBS data obtained for Warkworth and Tangihua Forest. This finding provides support for Tangihua Forest as the place of origin for Warkworth without the need to compare Paparoa samples. If it is true that the Tangihua Forest population was used for translocations to Warkworth, it is entirely plausible that the same site was used for translocations to the Waitākere Ranges too.

Other known translocated populations exist, for example, at Awhitu Peninsula and Kaimai Ranges (Parrish et al., 1995). However, since these populations were not sampled for GBS, further sampling and analysis would be required to determine their origins and success.

Suspected translocations

Lonsdale Park

Lonsdale Park specimens were sampled from a 60,000 m^2 patch of forest located at the Lonsdale Park outdoor education centre, which is surrounded by farmland. Considering the conservation status of *Paryphanta* spp. is currently listed as "Near Threatened" and reports suggest that populations within reserves may have already gone extinct, it is unlikely that a natural population would have survived in such a small patch of forest that has been used recreationally since the centre was donated in 1963 (Lonsdale Park, 2023). Additionally, while sampling *Paryphanta*

specimens, a staff member from the centre revealed they were unaware of the population's presence, suggesting that no active conservation has ever taken place in the area.

If a natural population is unlikely to survive in that area, it suggests that this population may have been translocated from elsewhere. This population's darker and smaller morphotype, which more closely resembles *P. watti* than *P. busbyi*, is a distinguishing feature that also supports the occurrence of a previous translocation.

The genetic structure of the Lonsdale Park population was most similar to the Totara North and Mangamuka populations (Figure 2.6). This similarity correlated with its distribution in the northern region and therefore its placement within the northern clade. However, the Lonsdale Park morphotype does not exactly match the typical *P. busbyi* morphotype seen in the other northern clade populations (Totara North and Mangamuka; Figure 2.2). Based on the results of PCA, the Lonsdale Park population appears to be the most genetically diverged population in the northern clade compared to the southern clade. This suggests that the Lonsdale Park population may have originated from a location further north than both Mangamuka and Totara North, closer to the known distribution of *P. watti.*

Regardless of the exact site of origin, it is likely that this would have been a short-range translocation as all three populations exist within close proximity of one another and display minimal variation in contrast to the greater variation observed within the other clades. The Lonsdale Park's site of origin existing within the northern clade would additionally further support the proposed north-south phylogeographic pattern between the northern and southern clades, and the intermediate form.

Waipu Caves

The Waipu Caves population also showed signs of having a translocative origin. This population was assigned to the intermediate form due to its genetic similarities with both the northern and southern clades (Figures 2.5 and 2.6). The positioning of the Waipu Caves population on the ML tree indicated the northern clade as its most closely related clade (Figure 2.4), which was

supported by its degree of genetic variation (Figure 2.5). Contrastingly, this population clustered within the distribution of the southern clade (Figure 2.7). Notably, the Waipu Caves were geographically more southern than any natural populations in that clade, given that the Warkworth population is known to be translocated (Parrish et al., 1995). The discrepancy may indicate that a founding population had previously been translocated from an area closer to the other intermediate populations that exist between the northern and southern clades.

This conclusion would provide stronger support for the proposed north-south phylogeographic pattern and corresponding intermediate form (considering the translocated Waitākere clade as an outlier). However, the positioning of the Waipu Caves population within the southern clade does not disprove this proposed pattern. It would not be unusual for an intermediate population to exist clustered within another clade it shares genetic similarities with. Additionally, only one specimen was sampled from the Waipu Caves population and sampling was not performed throughout central Northland. Populations from central Northland could reasonably be assigned to either the northern or southern clades, or may be an intermediate form. Therefore, further sampling and GBS analysis of the Waipu Caves, as well as additional populations throughout the northern, intermediate, and southern regions, would provide greater context for the distribution and genetic structure of the intermediate form in relation to the northern and southern clades.

Assuming the Waipu Caves specimen is typical of the entire unsampled population and that the population was indeed translocated, it is likely that the site of origin would be geographically close to the other intermediate populations, given their similar population structure (Figure 2.6). The most likely site of origin would, however, be Puketi Forest, as this population was the most genetically similar based on allelic composition (Figures 2.5 and 2.6) and had the closest evolutionary relationship to the Waipu Caves population (Figure 2.4).

The next likely site of origin was Diggers Valley as the sample from this site is the next most genetically similar based on allelic composition (Figures 2.5 and 2.6) and evolutionary relationship (Figure 2.4) than other sites used in GBS. Older populations typically show greater levels of genetic variation (Templeton, 1997; Martin and McKay, 2004), however, considering the lower genetic variation of the Diggers Valley population, revealed by *STRUCTURE* analysis

(Figure 2.6), it is unlikely that Diggers Valley could be the source population for a potential translocation to Waipu Caves. A nearby Waipu Hills population, as well as a Diggers Valley population, were also included in the phylogeographic research by Spencer et al. (2006), and as expected, COI data did not support a close evolutionary relationship between these two populations.

Alternatively, similar genetic clustering with Tangihua Forest, revealed by PC2 and *STRUCTURE* (Figures 2.5 and 2.6), nominates this as another potential source population. Since Tangihua Forest is a likely source population for both the Warkworth and Waitākere Ranges populations, it is plausible that the same source population would have been used for the Waipu Caves population. Despite the Tangihua Forest and Waipu Caves populations being categorised in distinct clades, the Waipu Caves are geographically closest to Tangihua Forest than other sampled sites (Figure 2.7). However, COI data analysed by Spencer et al. (2006) did not reveal any significant relationship between the Waipu Hills and Tangihua populations sampled for that study.

The phylogeographic research by Spencer et al. (2006) provided no further contenders for a source population. Phylogenetic analysis based on the COI data showed the Waipu Caves as most closely related to Taranga Island, but this island is unlikely a source population as its geographic isolation outside the natural range of genus *Paryphanta* suggests that population may also have translocative origins. Therefore, Puketi Forest (or a nearby site) remains the most likely source for the Waipu Caves population.

Conclusion

GBS analysis of *P. busbyi* revealed three distinct clades (northern, southern, and Waitākere) as well as an additional intermediate form that shared genetic similarities with both the northern and southern clades. Maximum likelihood phylogenetic analysis, PCA, and *STRUCTURE* analysis performed on GBS samples corroborated this pattern. When populations were mapped according to clade/form, a clear north-south phylogeographic pattern was observed encompassing the

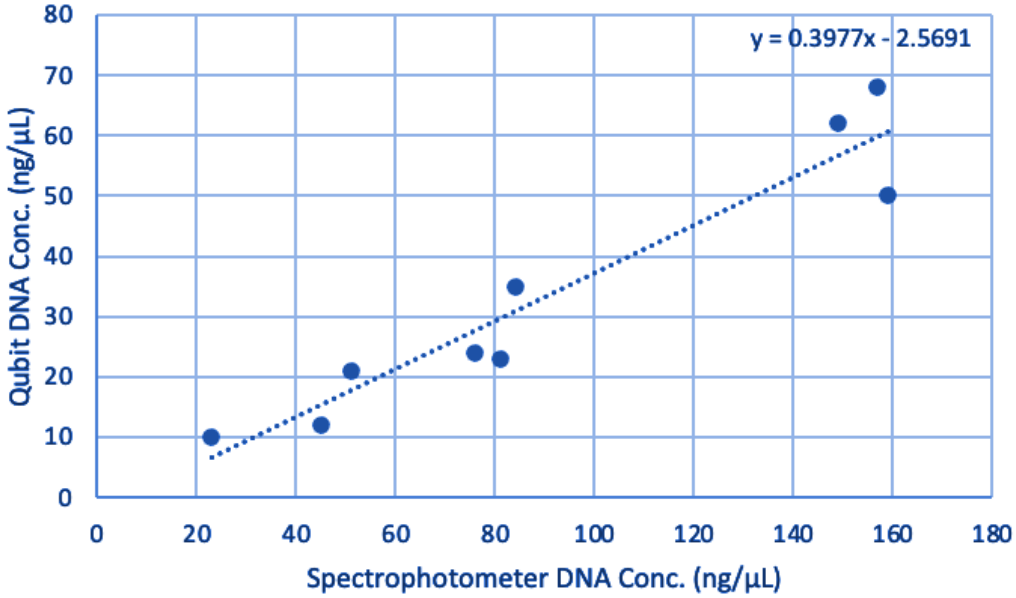
northern and southern clades and the intermediate form. The Waitākere Ranges was considered an outlier in this phylogeographic pattern, based on its translocative history.

The known translocated sites studied (Warkworth and the Waitākere Ranges) most likely originated from Tangihua Forest due to the genetic similarities revealed through PCA and *STRUCTURE* analysis, as well as the phylogeny published by Spencer et al. (2006).

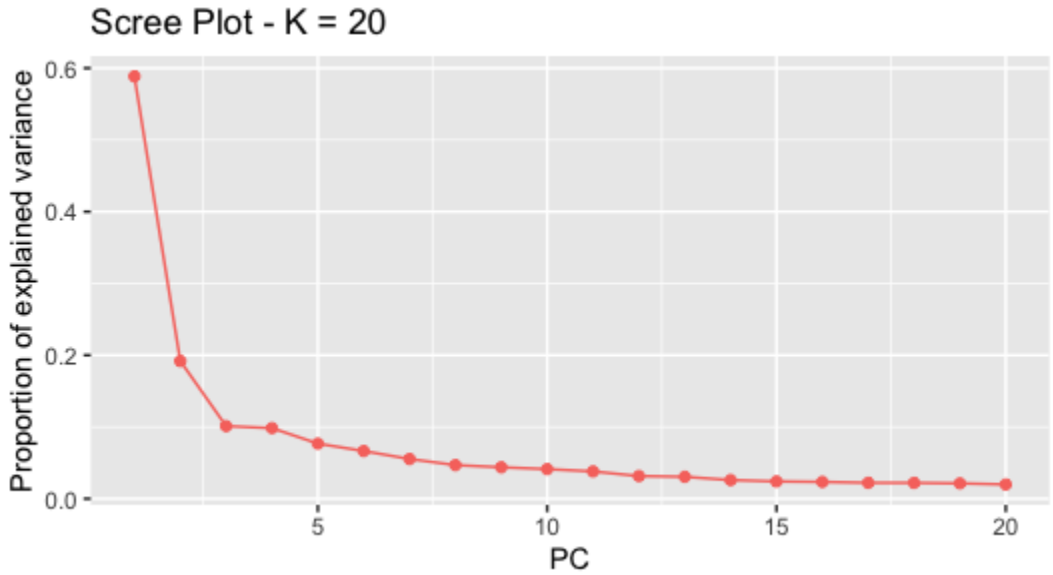
Lonsdale Park and Waipu Caves were identified as sites with a probable translocative origin following comparisons between genetic analysis and geographical placement. Given that Lonsdale Park exists in a small, isolated forest with a unique morphotype, it is plausible that this population may be translocated. The Lonsdale Park population, which resembled the *P. watti* morphotype, appeared to be the most genetically diverged from the southern clade as revealed by PCA, suggesting that if it has translocative origins, this population likely originated from a more northern site than both Mangamuka and Totara North.

The Waipu Caves site was identified as having potential translocative origins due to the discrepancy between its genetic grouping as an intermediate form and its geographical placement deep within the southern clade. *STRUCTURE* analysis revealed Puketi Forest or Tangihua Forest as potential sites of origin due to their genetic similarities, which was corroborated by PC1 and PC2 respectively. The COI-based phylogeny published by Spencer et al. (2006) did not support a Tangihua Forest origin, therefore, Puketi appears the most likely, although Tangihua Forest should not be disregarded as a possible source due to its likely role as a source population for the Warkworth and Waitākere Ranges populations.

Supplementary Figures



Supplementary Figure 2.1 Linear equation based on known Spectrophotometer and Qubit DNA concentrations that were used to extrapolate Qubit concentrations.



Supplementary Figure 2.2 Proportion of variance explained by PCA plot depicted in Figure 2.5.

Supplementary Tables

Supplementary Table 2.1 Concentrations of DNA (ng/µL) as measured on the Spectrophotometer and Qubit; 260/230 and 260/280 ratios as measured by the Spectrophotometer; extrapolated Qubit concentrations (ng/µL); and the amount (µL) of DNA extract required to obtain 500 ng of DNA.

Sample	Spec. Measured DNA Conc. (ng/µL)	260/230	260/280	Qubit Measured Conc. (ng/µL)	Qubit Extrapolated DNA Conc. (ng/µL)	Amount of Extracted needed (µL)
CW.021.01	149	2.19	1.89	62	56.6882	8.8
CW.021.02	157	2.28	1.92	68	59.8698	8.4
CW.021.03	159	2.08	1.93	50	60.6652	8.2
CW.022.01	196	2.29	1.90	N/A	75.3801	6.6
CW.023.01	92	2.20	1.86	N/A	34.0193	14.7
CW.023.02	95	2.36	1.89	N/A	35.2124	14.2
CW.024.01	45	1.96	1.97	12	15.3274	32.6
CW.024.02	51	2.20	1.90	21	17.7136	28.2
CW.025.01	103	2.21	1.92	N/A	38.394	13.0
CW.026.01	109	2.17	1.97	N/A	40.7802	12.3

Supplementary Table 2.1 Continued.

CW.026.02	120	2.14	1.91	N/A	45.1549	11.1
CW.026.03	122	2.19	2.00	N/A	45.9503	10.9
CW.026.04	87	1.74	1.93	N/A	32.0308	15.6
CW.027.01	105	2.19	2.00	N/A	39.1894	12.8
CW.028.01	94	1.94	1.89	N/A	34.8147	14.4
CW.028.02	161	1.94	1.90	N/A	61.4606	8.1
CW.028.03	132	2.12	1.93	N/A	49.9273	10.0
CW.028.04	105	2.20	1.91	N/A	39.1894	12.8
CW.029.01	86	2.15	1.90	N/A	31.6331	15.8
CW.030.01	155	1.97	2.01	N/A	59.0744	8.5
CW.031.01	121	2.13	1.91	N/A	45.5526	11.0
CW.031.02	111	2.18	1.92	N/A	41.5756	12.0
CW.031.03	136	2.33	1.84	N/A	51.5181	9.7

Supplementary Table 2.1 Continued.

CW.031.04	78	2.27	1.90	N/A	28.4515	17.6
CW.032.01	127	2.24	1.99	N/A	47.9388	10.4
CW.033.01	162	2.16	1.89	N/A	61.8583	8.1
CW.033.02	143	2.15	1.89	N/A	54.302	9.2
CW.033.03	23	1.17	1.97	10	6.578	76.0
CW.033.04	81	2.23	1.89	23	29.6446	16.9
CW.033.05	76	2.30	1.90	24	27.6561	18.1
CW.034.01	84	2.03	2.01	35	30.8377	16.2
CW.034.02	79	2.25	1.84	N/A	28.8492	17.3
CW.034.03	57	1.81	1.85	N/A	20.0998	24.9
CW.035.01	100	2.17	1.82	N/A	37.2009	13.4
CW.035.02	51	1.81	1.96	N/A	17.7136	28.2

Supplementary Table 2.2 Concentrations of purified pooled DNA (ng/µL) as measured on the Spectrophotometer and Qubit; as well as 260/230 and 260/280 ratios as measured by the Spectrophotometer.

	Spectrophotometer			**Qubit**
	ng/µl	**260/230**	**260/280**	**ng/µl**
Tube 1	121	3.27	1.77	18.2
Tube 2	293	2.40	1.76	32.8

Chapter Three

Science Communication in Phylogenetic Research

Abstract

Science communication is often considered an afterthought by some researchers, who choose instead to simply impart knowledge gained from research to the general public unidirectionally upon the conclusion of the research. Increased use of public engagement with science and technology (PEST) has demonstrated the benefits of not only aiming to increase public understanding of science but also utilising the public's expertise and potential contribution. This chapter will review the science communication used throughout this research on the genus *Paryphanta* phylogeography, focusing on different methods used throughout the planning and conducting stages. For each instance of science communication used, the review will include the reasoning for what was done, how it was carried out, areas that achieved notable benefits, areas that could be improved, and suggestions that may be applied to future phylogenetic research. Finally, science communication methods that aim to share results and raise interest in *Paryphanta* will be discussed, including recommendations to future researchers that will ensure results reach a wide target audience and are well received to achieve the best possible outcome.

3.1 Introduction

To ensure this research project could be carried out effectively and that findings could be shared optimally, science needed to be communicated throughout the planning and research stages as well as in the sharing of results.

The genus *Paryphanta* is already extremely underrepresented in terms of articles and scientific publications compared to other more "charismatic" taxa that are endemic to New Zealand, such as kiwi (*Apteryx* spp.). While this issue is already apparent by the sheer representation of kiwi that can be seen day to day (e.g. as logos, plush toys, and in story books), it is even more apparent by the number of results collated in a Google Scholar search. Where there were over 12,000 results for "kiwi conservation" (using an advanced search to exclude searches relating to the kiwi fruit), only ~750 results were produced for "kauri snail conservation". Even when including searches for "*Paryphanta* conservation" and "pūpūrangi conservation", the total

number of returned results equated to less than 900. It should be noted as well that each of the searches would likely have returned many of the same papers due to overlap in keywords.

Kiwi, like the genus *Paryphanta,* are endangered and deserve high levels of recognition, with kiwi population estimates being approximately 70,000 individuals (Germano et al., 2018; Westbury et al., 2022). In comparison, the total population size for genus *Paryphanta* is undetermined, although estimates for *P. watti* are assumed to be no more than 10,500 (Stringer and Montefiore, 2000). *P. busbyi* faces the same threats as *P. watti*, and exists within a similar, though more restricted, distribution to the North Island brown kiwi, which has a population estimate of 24,850 (Germano et al., 2018; Westbury et al., 2022). Therefore, it is likely that *Paryphanta* spp. too face similar levels of endangerment as kiwi species and should therefore be more similarly represented.

By highlighting the more "charismatic" features of *Paryphanta* through science communication, awareness of the genus will increase. From increased awareness, the issues faced by *Paryphanta* populations will inevitably become more appreciated by the wider community outside of those already involved in the conservation of this genus. Previous science communication aimed at the general public has been shared via platforms including online newspapers and YouTube videos, and has advertised the interesting traits of the genus *Paryphanta,* including their cannibalism and size (Northern Advocate, 2014; Department of Conservation, 2019). Therefore, science communication for this project intended to highlight these traits amongst others when communicating to a general non-academic audience.

Public engagement in science and technology (PEST) is considered to be the most effective form of science communication (Bastos et al., 2019). Furthermore, given that *Paryphanta* conservation requires cooperation and participation from scientists, local iwi, and tourists within *Paryphanta* distribution, PEST approaches provide the best opportunity for scientists to connect with the general public, learn from their expertise, and encourage cooperation in continued conservation management. For this reason, representatives of the public were included throughout this project where possible.

3.1.1 Chapter Aims

The inclusion of science communication in this project is expected to highlight its benefits throughout all stages of phylogenetic research. The choices for different forms of outreach, choice of language, and methods used to relate to different target audiences will be a common theme throughout this chapter.

This chapter aims to discuss the multidirectional use of science communication throughout this project. Here, common challenges in phylogenetic research are highlighted, including planning research, building relationships, discussing opposing views, collecting samples, and continually relaying information. This chapter aims to discuss how the use of different science communication methods were able to navigate these common challenges and benefit the research.

Finally, this chapter aims to provide recommendations for science communication to be used in future phylogenetic research that will make accessible the knowledge gained, educate the public, and allow results to be used for future eco-restoration and conservation of genus *Paryphanta*.

3.2 Science Communication in Planning Research

Science communication can be used before the commencement of research as it not only aids in the planning stages but also helps to develop relationships that are extremely beneficial later on.

Taking full advantage of science communication in planning means being able to both utilise prior instances of science communication and use specialised approaches to communicate science that is tailored to the individual project. When done well, using science communication in the planning stages of research can ensure the remainder of the research is well-planned, efficient, and successful in terms of achieving optimal outcomes.

3.2.1 Utilising Prior Instances of Science Communication

Scientists regularly use prior instances of science communication during the planning phase of research. Throughout the planning of this project, both peer-reviewed scientific literature and the non-profit social network database, iNaturalist (iNaturalist, 2022), were utilised. These tools aided in the identification of localities in which *Paryphanta* specimens may be available and in developing methods for sample collection and retrieval of DNA from samples. These uses of prior science communication are examples of how scientists may also be on the receiving end of science communication, which in this case enabled time and financial resources to be used more effectively when planning and arranging fieldwork.

Peer-Reviewed Published Literature

The usage of peer-reviewed published literature is commonplace in scientific research, although literature should also be recognised as a form of science communication due to its nature that focuses on sharing information from previous scientific research with other members of the academic community. As explained in Section 1.4.1 *Importance of Science Communication,* the sharing of scientific knowledge may be directed from scientists to the general public, or directly between scientists. Literature reviews examine previous works of science that have been peer-reviewed and published to provide other researchers with access to the science conducted in other projects. These reviews are typically performed before research to obtain background information on a topic that aids in developing research questions, hypotheses, and aims.

Literature was used initially to indicate sites from which *Paryphanta* specimens had been collected in previous research (e.g. Montefiore, 1994; Spencer et al., 2006; Mahlfeld et al., 2012), although the availability of recently published peer-reviewed literature discussing *Paryphanta* distribution was extremely limited. For this reason, already existing databases, collected through citizen science, were also utilised.

Peer-reviewed published literature was also able to provide substantial information on effective search and collection methods of *Paryphanta* specimens (as in Montefiore, 1994; Montefiore,

1995; Stringer and Montefiore, 2000), as well as various methods for sampling and DNA retrieval (as in Armbruster et al., 2005; Morinha et al., 2014; Psonis et al., 2022). This usage of existing literature again enabled time and financial resources to be used more effectively when planning and arranging fieldwork. Not only did this background research ensure samples were located as efficiently as possible, but it also ensured that subsamples from each specimen were able to be retrieved efficiently, thereby inflicting minimal stress upon the specimens before and during euthanasia (see Section 2.2.1 *Sample Collection/Curation*).

Citizen Science

While published works of literature are a classic example of how scientists communicate research with one another directly, scientists may also benefit from the local expertise gained from prior instances of science communication that involved the general non-academic public. Alternative, non-peer-reviewed forms of science communication, such as citizen science, are often also common practice in the lead-up to research. Although, the utilisation of these approaches is often done without much consideration or appreciation of these alternative forms as "good" science. While citizen science was able to benefit scientists in this case, citizen science has the potential to benefit all parties, including those assisting with research. This will be discussed further in Section 3.3.2 *Citizen Science in Sample Collection.*

iNaturalist, which is a publicly available online database, allows the public to map and share observations of biodiversity. This database is an example of PEST, achieved through citizen science, that has been utilised by the public in collecting large amounts of relatively up-to-date data. The availability of iNaturalist data has resulted in increased applications of citizen science in biodiversity research (Di Cecco et al., 2021). Any member of the general public can upload data to iNaturalist, although experts can review and approve identifications. Misidentifications that are not reviewed may occur, for example when images are not provided alongside observation records, therefore identifications from this platform should still be considered with caution. However, when compared with scientific literature, the two platforms could be used to suggest the locality of current *Paryphanta* populations with the added advantage of map points displayed in iNaturalist for easy navigation.

Utilising prior instances of science communication proved to be highly advantageous in not only planning research but ensuring time and financial resources could be used efficiently and responsibly throughout fieldwork. While peer-reviewed scientific literature is widely appreciated within the scientific community, the use of citizen science, specifically the inclusion of iNaturalist data in scientific research, is still cautioned (Di Cecco et al., 2021). This project highlighted how data obtained through citizen science can be used in the planning stages of scientific research, without compromising the final dataset itself. The benefits achieved through the proper execution of citizen science should be considered by future researchers when planning their science communication approaches.

3.2.2 Building Relationships

Science should be communicated in a way that is tailored to the goals of the individual project and the target audience. During the planning stage of this project, science communication approaches were used to build relationships with local Northland iwi, museum curators, and other scientists. Since pūpūrangi (*Paryphanta* spp.) are considered taonga, close relationships with iwi are essential, as all relevant members of the community should be informed and connected to be able to carry out their role as kaitiaki to their best ability.

The planning stages of research often neglect the local expertise in the area. While researchers have advanced scientific knowledge relevant to their proposed studies, the local general public can contribute valuable information regarding the study area. Conservation studies often focus on taxa with a very limited geographic range. While researchers may be very knowledgeable on the taxa, there remains much information that can be provided by locals within that area which would have been gained through years of daily observations. Local experts may be local iwi who are kaitiaki as well as park rangers who operate within forests inhabited by *Paryphanta* populations. Engagement with locals can provide information regarding species' habitats, population trends, or other details that may contribute additional context to the planning of a project and inference of results. While the scientific background of local experts may be limited, their long-term relationship with *Paryphanta* populations and habitats, as well as their knowledge and understanding of these populations should be appreciated nonetheless.

With *Paryphanta* populations being geographically restricted to Northland, engagement with locals was essential to gaining permission to work in the area, as well as to adequately plan the research and maintain relationships throughout the project. Building relationships and maintaining existing relationships with local iwi, museum curators, and other scientists was necessary in order to loan museum samples and build interest with others who may involve themselves in *Paryphanta* phylogeographic or conservation research in the future. The form of outreach, choice of language, and means of relating to the audience were chosen depending on the target audience.

Form of Outreach

Many relationships were in existence prior to this research due to previous research collaborations by other members of the University of Otago's Department of Zoology. These relationships included other scientists, museum curators, and iwi, specifically Ngāti Wai and Ngāti Kuri. The relationships were formed through years of engagement and interaction on several scientific projects and acted as a foundation for the personal relationships developed during this project. When forming the concept for this study, academics within the Department of Zoology were often able to revisit these connections to personally carry out engagement for permit approval. Even still, all iwi listed for consultations before permit approval were personally engaged with by the Department of Conservation as fitting with the Department of Conservation's protocol.

Covid restrictions and long-distance travel with limited funding, unfortunately, added challenges with face-to-face hui being held. So, where face-to-face hui were not an option, video calls were held with the same intentions. Due to many relationships already having been founded and video calls becoming more common practice in iwi engagement, this was an acceptable form of communication and was appreciated by iwi.

In the cases of Te Roroa and Te Parawhau, prior relationships did not exist, therefore engagement for permit approval was carried out by the Department of Conservation alone. Despite permits having been approved, this meant that personal relationships between researchers and iwi/hapu

were not built before the initiation of fieldwork. Although this is often accepted, researchers should allow time for hui or kōrero to build these relationships when requested - even if permitting has been approved prior. Kōrero were undertaken with both Te Roroa and Te Parawhau following permit approval, although it was ensured that kōrero took place before collection in the rohe of these iwi/hapu to ensure a good starting foundation.

Face-to-face meetings with scientists and museum curators were considered less essential, although similar considerations remain. The nature of working within overlapping industries meant many relationships already existed between scientists and museum curators. Therefore, during the planning stages of this project, arrangements to include other scientists and museum curators were made primarily through email, with relationships enhanced through face-to-face visits where possible. This was achieved through a week-long visitation at the Museum of New Zealand Te Papa Tongarewa, in which discussions with scientists and curators were held both within the museum and at lunch meetings.

Choice of Language

The language chosen for each party was tailored to set the tone for future interactions and ensure all parties adequately understood the project. Given their prior involvement in scientific research, scientists and museum curators are naturally more receptive to scientific jargon. When choosing to contribute to a project, scientists and museum curators expected to see a certain level of understanding, and considering their methodological concerns and questions, scientific jargon was often able to convey information more effectively.

Discussions with iwi, park rangers, and other audiences with non-academic backgrounds were approached differently than discussions with scientists and museum curators. Instead, these members of the public provided expertise and contributions to the project that were based on factors other than knowledge of the scientific method. To maintain a positive relationship that reinforced non-academic audiences' value to the project, the language used avoided scientific jargon, while ensuring that all questions were still answered respectfully and in full. The use of inclusive language to describe specialist topics to a non-specialist audience enables open

discussion and enhances relationships. Layman's terms were used to explain scientific concepts including but not limited to phylogeography, population density, and genetic structure. These discussions proved beneficial throughout this project, not only in building relationships but in developing multidirectional sharing of knowledge and collaboration between parties (discussed further in Section 3.3 *Science Communication in Conducting Research*).

Relating to Target Audience

Finally, when discussing the project, individual values and the importance of different research aspects were considered when relating to the target audience.

Engagements with scientists and museum curators were able to include in-depth discussions on research methods, as those with academic backgrounds tend to appreciate the value of new and advanced research. In comparison, when engaging with iwi, research methods would at times conflict with their values, specifically the need to collect and euthanise taonga species. Open discussions with iwi instead focused on sharing world views and values, and it became more apparent as to how cooperation and coordination with local iwi could benefit both parties. Therefore, the reasonings for research and the benefits that would follow were highlighted, rather than focusing on the specifics of sensitive procedures such as euthanasia. It should be noted that the prioritisation of certain topics over others should not involve withholding information. Rather these topics, such as euthanasia, were framed and discussed in a way that highlighted the overarching priorities shared between parties, for example, the conservation of Northland's endemic species. Prioritising information that highlighted shared values with iwi not only helped inform, but also helped both parties relate to one another and avoid disagreement at the onset of engagement. By focusing on building relationships outside of the project, rather than simply building connections for research, this project was much more supported by the wider communities. This benefit was amplified hugely by face-to-face encounters.

As discussed in Section 3.2.2 *Building Relationships/Form of Outreach*, kōrero were held with Te Roroa and Te Parawhau before sample collection in their rohe. Although kōrero were requested and carried out in response to the project proposal, it was still essential to build

relationships outside of the context of this research. Therefore, at the kōrero with Te Roroa and Te Parawhau, conversations largely included non-science-related topics such as personal backgrounds, families, and interests. Again, when the research methods were inevitably discussed, shared values of *Paryphanta* conservation were focused on to highlight the reasoning behind certain methods, including euthanasia. This approach to challenging discussions provided context and reasoning to certain decisions, ultimately allowing parties to relate to one another through shared values (further discussed in Section 3.3.1 *Discussing Opposing Views*).

It is recommended that future researchers should consider the wider benefits of building relationships and personally carrying out engagement, rather than regarding engagement as a "tick the box" step to simply obtain research permits. Researchers should therefore allocate additional time to building relationships and should personally address concerns when they arise.

3.3 Science Communication in Conducting Research

Following the initial science communication carried out in the planning stages of research, additional action should be included throughout the conducting of research to avoid degrading the positive effects already in motion. Science communication conducted during the active research stages should focus on maintaining and enhancing those relationships already built, utilising assistance from others through citizen science, and relaying information to maintain interest from wider communities and prepare for sharing of results upon conclusions being made.

3.3.1 Discussing Opposing Views

Choice of language and ability to relate to the target audience, as has been proven essential in building relationships, is also essential in maintaining those same relationships. Researchers must be able to navigate difficult conversations in a way that conveys reasoning without appearing patronising. While some prior opinions are based on core beliefs, others are based on prejudice and/or lack of information. A key point of education is to challenge and change opinions, although challenging those opinions based on underlying values and core beliefs can be

construed as egotistical. Therefore, instead of taking a forcibly persuasive stance, the most productive discussions were those that centered around providing reasoning for each decision and identifying shared values.

In the case of future research, especially on taonga species, it should be expected that others will hold differing opinions. Ensuring that others always felt comfortable to ask questions and had security in knowing their questions would be answered in full enabled these discussions to take place constructively. Furthermore, by providing opportunities for others to share their opinions, expand on their concerns, and explain their own reasoning for beliefs, these discussions became personal conversations as opposed to neglectful recitations. Therefore, researchers should come prepared to listen to the concerns of others and explain the reasoning behind certain choices. Often when concerns are discussed respectfully, the similar values between parties are revealed naturally and each party can come to an understanding or compromise. By being completely transparent with others about the actions taking place during research and maintaining an open mind to altering plans, relationships already built can strengthen.

Throughout the project, many sensitive topics arose that if not handled with respect and proper etiquette, had the potential to damage those relationships already established. Discussions around euthanising of snails, permanent removal of tissues and shells, and the possible outcomes of sequencing of DNA were all topics that had to be approached in a certain manner. Examples of how these conversations were approached with Te Roroa, the iwi at Waipoua Forest, will be discussed here.

Snail Euthanisasia

The euthanising of snails was naturally a significant concern for iwi due to *Paryphanta* being taonga. This concern was expressed during the kōrero with certain Te Roroa kaumatua at Waipoua Forest and also with a Te Roroa park ranger who aided in sample collection at the same location. During discussions with Te Roroa members, it was highlighted that although other methods of obtaining DNA were considered, euthanising by ethanol was chosen due to its benefits of being able to obtain high-quality DNA as well as its ability to preserve specimens for

future subsampling. Through additional sharing of concerns, the similar values of reducing harm to individuals were realised and all parties agreed that the euthanasia of a limited number of snails would prevent the additional collection of specimens for future research, thereby minimising the harm placed on the entire population.

Although extensive discussions were held before permit approval and sample collection, it remains important to consider that each member of a community will also hold their own opinions, concerns, and values. Those with the authority to permit sample collection and research are not necessarily those who will be present at the time of sample collection. Therefore, each of these discussions are likely to be repeated and researchers should ensure that sensitive topics are thoroughly discussed with each person involved. The importance of including each involved person in discussions was highlighted during sample collection. Although discussions regarding euthanasia had taken place with Te Roroa kaumatua during the kōrero, these discussions did not involve the park ranger assisting with sample collection.

Not all people involved in research will be able to attend meetings in which decisions are made, as was the case for the Waipoua Forest park ranger who assisted with the collection of specimens. Regardless, alternate opportunities should be granted for these contributors to express their concerns and raise questions before contributing. These opportunities were not provided before collection due to time constraints associated with travel. As a result of these time constraints, discussions with the park ranger were not held until after the collection process had commenced. It is recommended therefore that a full disclosure of any sensitive processes, including opportunities for discussion, should be held before any involvement by volunteers. Cultural considerations, including karakia, may then be agreed upon with sufficient time allocated prior.

Removal of Shells

Concerns surrounding the permanent removal of specimens and their shells from the snails' whenua/land also arose during discussions with Te Roroa. While Te Roroa members expressed interest in the snails being returned to their whenua to rest amongst their whakapapa, these

requests misaligned with the shared goal between Te Roroa and researchers of limiting future sampling needs. Upon completion of the research, lodging the shells and tissue in a recognized repository allows for replication of results and further research on the samples. Furthermore, these samples would remain available for future researchers hoping to use either shell or tissue for alternate research, thereby reducing the need for excessive sampling in the future. Once again, the similar values of both parties were highlighted by researchers and all parties agreed on the donation of specimens to minimise any additional harm placed on the entire population.

Sequencing of DNA

The topic of DNA sequencing can induce concerns regarding intellectual property and the possible future outcomes of DNA sequences being publically available. Concerns often centre around monetary IP and bioprospecting. It was explained to Te Roroa that sequencing of DNA was necessary to resolve research questions relating to *Paryphanta* phylogeography due to the discrepancy between morphological and COI data that was available at the time. Had discussions halted at this point, a level of disagreement would have remained between the researchers and iwi. To enable Te Roroa the opportunity to support the research methods, rather than simply understanding the necessity of DNA sequencing, their reasoning behind these concerns was acknowledged and it was explained how their values would be supported. By ensuring that financial gain would not result from this research and that GBS data would not provide an opportunity for bioprospecting, the concerns around intellectual property were relieved. Once these concerns were a topic of open discussion, research was able to move forward with full support and trust, and without hesitation from either party, thereby strengthening the relationships built.

For future research, it may even be possible, in some cases, for iwi members to assist with laboratory work. This would further engage the iwi community in research, allow local scientists to gain experience, and provide opportunities for more knowledge to shared through everyday discussion within local communities.

3.3.2 Citizen Science in Sample Collection

PEST has demonstrated the benefits of working with members of the general public where possible (Bastos et al., 2019). In this project, the general public were able to be included on multiple occasions for specimen identification and collection in fieldwork. This inclusivity not only aided in more efficient searches but also reignited public interest in the project and benefitted relationships developed with included iwi.

By allowing and encouraging local iwi, Department of Conservation representatives, and other members of the general public to assist with fieldwork, casual conversations throughout fieldwork enabled volunteers to better understand our values and motivations and overall lessened the perceived differences between scientists and the public. This approach was used for snail collection at Waipoua Forest, Whangārei, and Lonsdale Park.

By teaching others how to locate specimens, fieldwork also drastically improved in terms of hours spent searching. This improvement was especially notable when engaging in fieldwork alongside locals at Waipoua Forest and Whangārei, where the collection of five specimens (i.e. the maximum number permitted to collect from any site) was achieved. The local expertise enabled us to quickly locate areas in which *Paryphanta* specimens had previously been observed, which with the added person-hours, made a considerable difference. While only two specimens were identified and collected at Lonsdale Park, this sample size should be considered greater than what would be expected without volunteer assistance, given that only one additional empty shell was discovered at the site, indicating that the population density was very small.

Furthermore, the knowledge on *Paryphanta* conservation and search methods that was gained by volunteers could then be relayed back to their respective communities, thereby expanding interest in *Paryphanta* conservation across a variety of communities. Knowledge regarding the *Paryphanta* snails at Lonsdale Park was able to be conveyed to the accompanying Lonsdale Park employee and due to the park's usage as an education centre, this sharing of knowledge was able to set up future education opportunities relating to genus *Paryphanta* and the conservation issues it faces. Additionally, by including a Te Roroa park ranger and a Department of Conservation

representative at Waipoua Forest and Whangārei respectively, knowledge surrounding *Paryphanta* conservation and search methods were able to be shared with members of institutions that may actively contribute to future conservation management of the genus.

While assistance in the field did require time to explain methods, the decision to involve citizen science in fieldwork contributed to an overall increase in productivity. If time is able to be allocated towards seeking out participants and providing instruction on fieldwork, utilising the general public is an option that future researchers should thoroughly consider. Aside from being able to conduct fieldwork more efficiently, any opportunity to increase awareness and appreciation for conservation work should be taken advantage of.

3.3.3 Continuous Relaying of Information

While research projects may include science communication where necessary, it can be harder for researchers to maintain a high level of science communication when the outreach no longer benefits the project to the same degree. Continuous relaying of knowledge can be done to conserve external interest in the project and encourage continued conservation during and after the project.

As with any other form of outreach, the approach depended largely on the target audience. Iwi that had shown particular interest in continued communication throughout the project were updated with a report upon completion of fieldwork, detailing observations including the localities in which *Paryphanta* specimens were identified, the relative health of the forests in which *Paryphanta* specimens exist, and the relative *Paryphanta* population densities. Since the report was to be shared within the local bulletin and was targeted towards a non-academic audience, the language used avoided scientific jargon. Furthermore, the report avoided topics of methodology and statistics, and instead included notes more appropriate for the target audience including the relevance of genus *Paryphanta* in Māori culture, as well as strategies that could be used by the general public for conservation.

Presentations were the primarily chosen outreach when communicating with other scientists. A presentation hosted by the Department of Conservation provided an opportunity to relay updates on the progression of the research, including the outcome of fieldwork, chosen methodology and prospective results. In this case, scientific jargon was chosen as the language used, given that the audience consisted of those with an academic background. Additionally, by delivering a presentation as opposed to a written report, audience members were provided with opportunities to ask further questions that could be answered in real time.

Both the report and presentation were considered successes in terms of outreach as each was able to update on the ongoing advances of the project. However, a limited number of people were able to benefit from the report and presentation as they were restricted to specific target audiences. Expanding the target audience to additional iwi and institutions may have improved the outcome of this outreach in the sense that interest and therefore action towards *Paryphanta* conservation would be enhanced. Therefore, it is recommended that in future research, outreach should be designed to include wider communities. Considering the time constraints already associated with research projects, it may be unattainable to relay large amounts of information regularly, therefore it is suggested instead to ensure the outreach chosen can involve numerous groups. Outreach that targets a wide audience may be done in a timely and financially efficient manner, such as by recording presentations that may be accessible to members of a number of groups or institutions. By allocating time towards planning an event or presentation well, researchers can improve upon the inclusivity of the research, benefit already existing relationships, and network additional professional connections.

3.4 Science Communication in Sharing Results and Gaining Public Interest

Upon completion of research, results should be communicated to a variety of target audiences in order to produce the desired outcome of the research to its full extent. On top of this, by relaying general information about *Paryphanta* spp., interest surrounding the conservation of *Paryphanta* populations and their forest habitats can grow.

The outreach currently used in *Paryphanta* conservation targets a wide audience, including scientists, local iwi, and other members of the general public. To ensure that the best opportunity for successful conservation management is achieved, outreach should continue to be carried out. By publishing current scientific literature, presenting at conferences, working closely with iwi, updating current signage, and including the next generation, results can be communicated in a manner that optimally benefits *Paryphanta* conservation. Providing information in a relatable, simple, and easily accessible form is paramount to this task and is expected to achieve a much greater standard of conservation management than scientists may be able to accomplish alone.

3.4.1 Communicating to Decision Makers

Those making conservation management decisions are often not those conducting the research (Lamb et al., 2018). Therefore, scientific knowledge must also be conveyed to decision-makers in a way that highlights the relevance and credibility of the results (Cook et al., 2013). Funding and active conservation are provided largely by external sources, rather than the researchers themselves. While funding for the actual research is provided before and during research, funding for continued conservation management needs to be obtained upon publication of results. It is clear from the above discussions that including the general public throughout the entire project encourages New Zealand's wider community to be invested in the overall outcome of the research. Therefore, conservation projects are more likely to gain increased funding and commitment towards continuing active management of the taxa of interest, provided the results are communicated effectively.

Advice from scientists is more openly received when researchers acknowledge and share the same values as those making decisions (Cook et al., 2013), for example when information is relayed in a culturally considerate manner (Bickford et al., 2012). Since some *Paryphanta* populations are managed by local iwi, this consideration is especially important in the context of *Paryphanta* conservation management. Working directly with both the Northland iwi and the Department of Conservation to develop an updated conservation plan for the genus *Paryphanta* is of utmost priority. The relationships built throughout this project are immensely appreciated and should be developed further. This project has demonstrated the importance of building and maintaining relationships (see Section 3.2.2 *Building Relationships*), meaning engagement and discussions should be carried out between researchers and iwi rather than relying on external institutions for mediation.

3.4.2 Communicating Advancements in Scientific Method

With genetics being a rapidly advancing field, it is essential to relay information regarding new findings and technologies to encourage continued growth in the quality and quantity of new research. Publications of scientific literature allow other researchers the ability to utilise previously tested methodology. Publications in peer-reviewed journals can detail methods (including their advantages and limitations), results, and conclusions of research and are easily searched for when published online. To communicate the details of this project with other scientists, both this thesis and subsequent papers will be published, ideally as open access to ensure the knowledge gained is as easily accessible as possible.

Presentations, being a more active form of outreach, can be delivered at conferences and are often able to reach additional researchers involved in similar genetic topics. This method will be used to inform other scientists of the research methods used in this project, as well as the results and conclusions obtained. The deliverance of presentations is expected to gain interest from other scientists within the broader field of genetics and conservation as opposed to just those more closely involved in phylogeographic or mollusc-based research.

3.4.3 Communicating to Gain Interest from the General Public

Conservation Signage

Finally, communicating results or even encouraging *Paryphanta* spp. related discussion with the general public can aid in conservation management. In the case of the genus *Paryphanta,* signage targeted towards locals and tourists currently exists around Northland, particularly around nature trails and other regions with high tourism. Signs displayed are informative of *Paryphanta* distribution within the area and the relevance of the genus *Paryphanta* to Māori culture (Cooper, 2016). However, these signs often neglect to explain the issues *Paryphanta* populations face, nor do they adequately advise the public on their ability to contribute to *Paryphanta* conservation. Given that working memory is limited to three or four pieces of information (Luck and Vogel, 1997; Cowan, 2010), it is important to minimise information load in order to produce an optimal response. However, when requesting that the general public take certain actions, such as remaining on the trail and refraining from removing *Paryphanta* shells, mentioning the reasoning behind these requests may encourage the public to better understand their value and role in *Paryphanta* conservation.

It is recommended that signage around trails provide additional information that briefly elaborates on the reasoning behind certain management decisions while maintaining the information load to a minimum. Presented here is an example of signage that could be displayed around *Paryphanta* habitats, aimed at educating those visiting the area on the presence of populations and how they can assist with conservation efforts (Figure 3.1).

Figure 3.1 Example poster that may be displayed around Northland forest tracks in which *Paryphanta* populations exist. Poster designed by Cailie Ward.

Upon observing the poster (Figure 3.1), the audience's eyes should be immediately drawn to the title declaring we must "Save Our Snails," alongside a subtitle stating that "Kauri snails/pūpūrangi live here!" These titles will immediately convey a clear message that these snails are both rare and should be protected. For added context, a large image displaying a *P. busbyi* specimen was included, which may help the audience identify the species of interest in the area while also adding to the visual appeal of the poster.

Since it is understood that an audience is typically able to retain only three to four pieces of information in their working memory (Luck and Vogel, 1997; Cowan, 2010), this poster opted to highlight four facts that may be considered interesting to those on hiking trails. These limited facts spark interest without overloading, encouraging readers to continue reading. From here, arrows can visually draw the eye towards ways in which the public can assist in the conservation of *Paryphanta* spp., with each method relating to one of the four interest points. This positioning of text highlights the essential actions that should be carried out while providing the public with more context as to how their actions may affect populations within the area. In turn, this poster is likely to increase motivation to act without unnecessary information.

All information is presented without the use of scientific jargon, meaning it can be understood by the wider public. Furthermore, all language is inclusive, which draws in the audience and encourages a sense of responsibility. By using phrases such as "*our* snails," "how lucky *we* are," and "*our* backyard," the responsibility is lifted from solely researchers and instead becomes shared by the wider community. While this example was written majoritively in English, it is recommended that this poster, or others similar, also be displayed in Te Reo Māori around Northland. Given that many forests within the *Paryphanta* distribution are operated and managed by local iwi, the use of both Te Reo Māori would increase receptibility to those close to the cause.

While the essential information is presented forwardly, the audience is provided with the option to read additional information on genus *Paryphanta*. Since smartphones are carried by most people, especially as a safety device when hiking, a QR code was chosen to display this additional information. QR codes, or links for online posters, are an effective way of reducing

the time and effort involved for the target audience to access additional information while reducing the information load on the poster itself. Currently, the QR code is set up to provide quick access to the Department of Conservation *Paryphanta busbyi* webpage (Department of Conservation, 2021), although it is recommended that an option be added to donate to a fund, which would be set up before the release of the poster to the public. To maintain convenience, it is recommended that donations should be taken from the same website, whether this be the Department of Conservation *Paryphanta busbyi* webpage or a specially designed page that is able to provide both information and the ability to donate. Finally, the website should be compatible with multiple easy payment methods, such as Apple Pay, Google Pay, and account2account to ensure effort is not drastically increased for the audience.

Finally, with the addition of sponsor logos, the audience may be more inclined to trust the advice presented. Certain communities tend to affiliate more with certain institutions, therefore, by advertising the collaboration between multiple institutions that represent a variety of academic and cultural backgrounds, the information presented is more likely to reach a wider audience and encourage further collaboration by the general public.

Picture Books

Posters and signage may be beneficial in achieving cooperation by visitors within *Paryphanta* habitats, although information regarding the greater issue of *Paryphanta* and forest conservation will unlikely reach a wider audience through this outreach alone. Since *Paryphanta* spp. are found throughout the Northland and Auckland regions and have huge cultural significance within New Zealand, awareness of this genus should be encouraged outside of their forests and to all New Zealanders, regardless of their age and hobbies. By educating the younger generation, a greater sense of understanding of New Zealand's forests and endemic animals will be achieved that they may carry through life. Furthermore, by learning about these issues from a young age, New Zealand's next generation will be able to develop their pride for taonga species. When communicating science to children, it is important to be able to get the message across without any prior scientific knowledge held by the audience. Picture books are an engaging way to communicate issues to children.

Featured here is an example picture book, titled *Dale the Snail*, that may be used to inform children of *Paryphanta* and their threats (Ward and Kaur, 2023; Figure 3.2). The original concept for *Dale the Snail* was originally produced for the University of Otago Science and Creative Non-Fiction Writing course (SCOM403), although the original concept has been extensively revised, re-illustrated, and reformatted for its inclusion and discussion in this thesis.

Dale the Snail was written to communicate the threats faced by *Paryphanta* and to encourage children to consider the impact of human actions on native wildlife (Figure 3.2). The story follows the journey of Dale, a *Paryphanta* individual, who leaves his forest in search of a new place to live, while overcoming challenges that come with specific threats, including deforestation, urbanisation, introduced species, and pollution. The story resolves when Dale discovers a new forest that describes features necessary for optimal *Paryphanta* habitat, these being predator-free, other *Paryphanta* individuals, and plant species typical of a kauri forest.

An earlier version of *Dale the Snail*, was read to 6-7 year old students at the Australian International School (AIS) in Singapore. Although wording, illustrations, and formatting were revised for Figure 3.2, the original storyline was preserved to accurately represent the prior science communication that was carried out. Given that the AIS student body includes a high proportion of New Zealand expatriates, this story was able to provide New Zealand relevant information to those who may otherwise receive little information on New Zealand biology through the international curriculum taught at AIS. The story was well received by the students and the conservation and environmental topics were well understood, as assessed by a subsequent informal class discussion held by Mr. Ian Ward, Assistant Head of Elementary at AIS. Aside from the class discussion, no formal comprehension assessment was undertaken at the time. Comprehension assessments, such as written or oral tests, may be considered in the future to thoroughly analyse the effects of similar science communication.

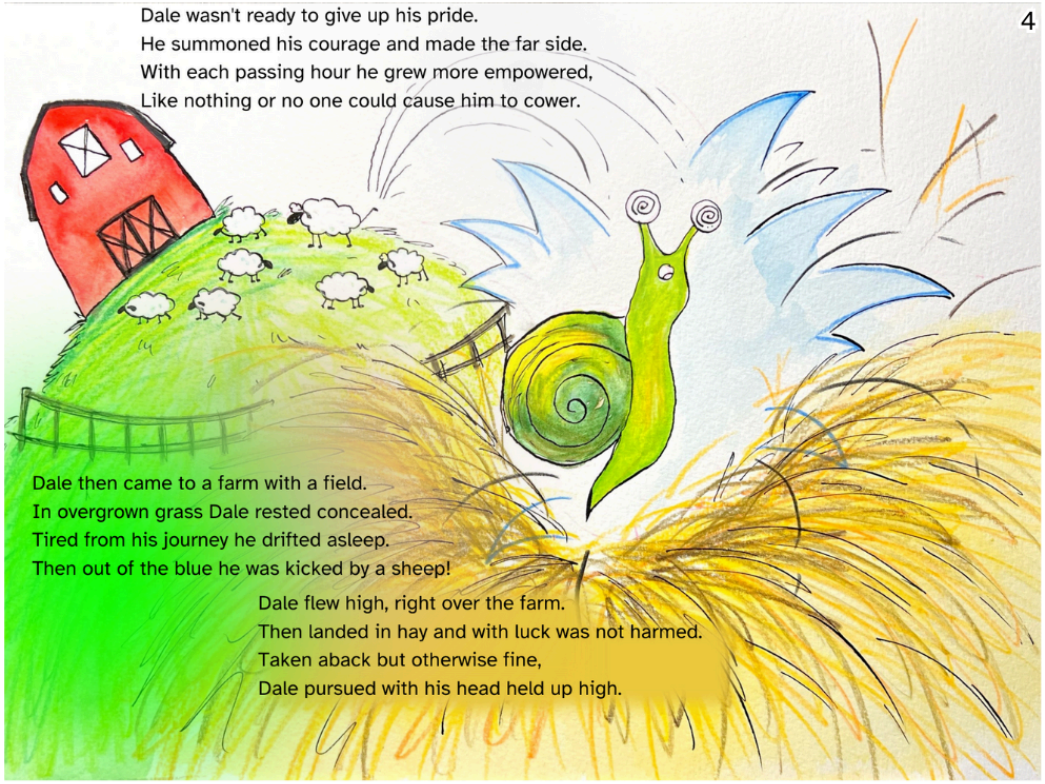
Illustrations included in Figure 3.2 provide an indication of how the story may appear as a published picture book. If published, the information presented through the storyline would become more accessible to children across New Zealand, thereby increasing awareness of genus *Paryphanta* and its threats in the younger generation.

1
Dale the Snail
CAILIE WARD
JASMINE KAUR

2
At the tip of New Zealand where kauri trees grow
Is a forest that's been there since so long ago.
The trees are like giants, growing so tall
But the forest, once huge, is now just so small.
Here lived a snail, all on his own.
He'd already explored every inch of his home.
Where moho, moa, and kiwi once stayed,
The snail had watched all the predators invade.
This snail was treasured, he knew in his heart
But to re-find his worth, he'd have to depart.
He packed up to leave, to write his own tale.
This is the story of Dale the Snail.

3

Dale was excited to start his new feat
But his next great adventure was blocked by a street.
Cars raced by fast and Dale got scared.
How could he cross? He was so unprepared!



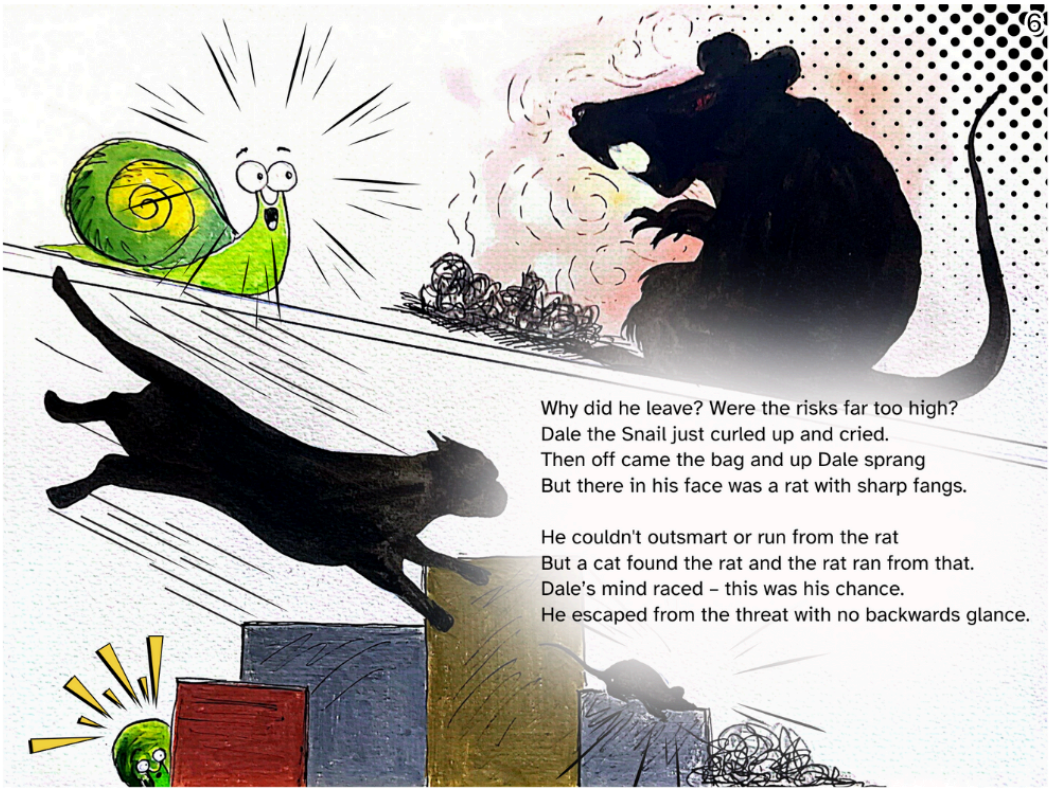
4

Dale wasn't ready to give up his pride.
He summoned his courage and made the far side.
With each passing hour he grew more empowered,
Like nothing or no one could cause him to cower.

Dale then came to a farm with a field.
In overgrown grass Dale rested concealed.
Tired from his journey he drifted asleep.
Then out of the blue he was kicked by a sheep!

Dale flew high, right over the farm.
Then landed in hay and with luck was not harmed.
Taken aback but otherwise fine,
Dale pursued with his head held up high.

Dale set off but it didn't take long
For just one more thing to go dreadfully wrong.
A bag made of plastic flew through the air,
To land right on Dale, causing despair.



Why did he leave? Were the risks far too high?
Dale the Snail just curled up and cried.
Then off came the bag and up Dale sprang
But there in his face was a rat with sharp fangs.

He couldn't outsmart or run from the rat
But a cat found the rat and the rat ran from that.
Dale's mind raced – this was his chance.
He escaped from the threat with no backwards glance.

Dale prevailed and crawled fast and far.
He came to a fence and slipped through the bars.
He noticed a sign saying "Predator Free"
Then saw up above him the tall kauri trees.

After the challenges Dale endured
He finally felt happy and safe and secure.
He'd found his new home which was perfect and grand.
And though it was tricky, he'd done what he'd planned.

It only got better for Dale from there,
When a snail called Gail approached him with care.
Dale and Gail became such good friends.
Finally a snail on whom he'd depend.

Although Dale no longer faced any threat,
The journey to get there he'd never forget.
No one should struggle to feel safe and sound.
And no one should feel like there's no one around.

This is the least all Earth's creatures deserve.
It's important that what we have left we preserve.
All living creatures, whether little or great,
Must be protected before it's too late.

The language used in story books should not include scientific jargon and should refrain from using extensive long words or other words that may be difficult to pronounce, as vocabulary is limited in the target audience. For this reason, *Dale the Snail* used common names of taxa, including kauri snail, moho, moa, and kiwi. Studies have shown that rhyming stories can help children identify sounds in words and improve literacy skills (Wood and Terrell, 1998), especially in children with learning disabilities (Dunst and Gorman, 2011). With pronunciation still being learned by younger children, rhyming stories are often popular as they encourage children to understand, through the context of other words, the pronunciation of new words. Furthermore, for younger children who lack the necessary vocabulary to fully understand the meaning of each word, rhyming stories can still be fun and exciting to listen to, maintaining focus long enough that the overall story is received and understood through context.

The inclusion of pictures alongside a story can provide the necessary context for children to fully understand the story whilst also giving children who learn visually the opportunity to understand without verbal communication. Furthermore, pictures can be educational in that they are able to accurately detail distinguishing features of *P. busbyi* specimens, such as their green colouring. This may enable the audience to recognise this species in the wild and alongside the other information learned through the story, the audience will be better equipped to recognise threats to the populations and the importance of conserving *Paryphanta* and other endemic taxa. It is aimed that with this increased understanding, the next generation will be able to make conscious decisions surrounding conservation, including refraining from collecting *Paryphanta* shells.

The length of a story and the number of details within a story are also important to consider with children as the target audience. The average attention span of a child is typically between two to three times their age in minutes (Brain Balance, 2023), although attention span is significantly increased when reading picture books (Bartan, 2020). Typical storybooks range from 500 to 1000 words to ensure focus can be maintained (Penguin Books Ltd., 2023), which is why *Dale the Snail* was written to fit within this range, totalling 506 words. Finally, as mentioned previously, humans are typically able to only retain three to four pieces of information in their working memory (Luck and Vogel, 1997; Cowan, 2010). For this reason, four challenges were discussed within *Dale the Snail,* with each challenge relating to a prevalent threat faced by

Paryphanta as follows: crossing a road (urbanisation), encountering sheep (deforestation for the development of farmland), becoming stuck in plastic (pollution), and encountering rats and cats (introduced predators).

A typical story structure includes an exposition, rising action, climax, falling action, and resolution. *Dale the Snail* begins by introducing the setting, mood, and characters (exposition), followed by explaining the journey Dale is about to embark on (rising action), narrating the challenges (climax), his escape to the new forest (falling action), and concludes with the resolution (Dale finds safety and a new friend). Each story should also follow a theme, with *Dale the Snail*'s primary theme being the conservation of an endangered, endemic species. This theme is introduced in the exposition, followed through by describing threats that many endangered species face, and is reiterated in the final eight lines of the story. By keeping to a typical story structure, the target audience will be familiar with the flow of the story and can anticipate challenges and ways in which these challenges may be resolved. This familiarity encourages the audience to then notice these issues in the real world where they may be able to relate the information learned about Dale and his journey to consider the wider issues of conservation.

Lesson Plans Following New Zealand Curriculum

Primary through secondary school-aged students will have a degree of scientific knowledge and the New Zealand curriculum includes teachings related to conservation, conducting investigations, and communicating science. By relating these lessons to genus *Paryphanta*, students will be able to gain a thorough understanding of the issues faced by *Paryphanta* populations that can then be applied to a variety of other taxa.

Lesson plans can be designed for teachers in line with the New Zealand curriculum from primary school up to NCEA. Teachers with an understanding of *Paryphanta* may be able to personally design lesson plans, alternatively, scientists with an understanding of the New Zealand education system may be employed to design lesson plan templates to be adjusted in accordance with different school grades. I developed examples of lesson plans for three categories of schooling: primary school Science classes (Figure 3.3), secondary school Biology classes within Northland

(Figure 3.4), and secondary school Biology classes across the entirety of New Zealand (Figure 3.5 and 3.6). Lesson plans were designed to fit concepts relating to *Paryphanta* spp. into both the "Nature of Science" and "Living World" strands that each present scientific concepts in different ways. The "Nature of Science" strand teaches the skills, attitudes, and values to build a foundation for understanding the world, while the "Living World" strand teaches how living things interact with each other and their environments (The New Zealand Curriculum, 2014). Future outreach could involve trialing these lesson plans and feedback gained would allow lesson plans to be refined and incorporated into standard teaching practice.

Lesson Plan 1: Kauri Snail Conservation

Grade: 3 to 5 **Subject:** Science **Strand:** Living World

Strand Focus: Understand the biology of New Zealand, including the sustainability of New Zealand's unique fauna and flora and distinctive ecosystems

Strand Objectives: Understand the processes of life and appreciate the diversity of living things, understand how living things interact with each other and with the non-living environment, and understand the processes that drive change in groups of living things over long periods of time and be able to discuss the implications of these changes

Strand Link: https://nzcurriculum.tki.org.nz/The-New-Zealand-Curriculum/Science/Learning-area-structure

Lesson Objectives:

- Be able to recognise the endangered kauri snail
- Be able to list of threats that have led to the kauri snail becoming endangered
- Be able to come up with solutions to combat threats to kauri snails and other endangered animals that are endemic to New Zealand
- Be able to communicate to the public the issues faced by kauri snails and what we can do to help

Prerequisite knowledge:

- Understand what an ecosystem is
- Understand what a habitat is
- Know different types of threats animals face e.g. predators and pollution
- Understand what "endangered" means

Activity 1:
Class Discussion

- What does endangered mean?
- What are some examples of endangered animals?
- What are some threats they face that makes them endangered?

Activity 2:
Class Activity

Introduce kauri snails by watching a short video (e.g. https://www.youtube.com/watch?v=JUdP81SjMVE) or reading a book (e.g. *Dale the Snail* by Cailie Ward and Jasmine Kaur).

Activity 3:
Class Discussion

Talk about the unique features of kauri snails, e.g. its shell colour, nocturnal nature and habitat.

Activity 4:
Individual Activity

Draw a kauri snail and label its identifiable features.

Activity 5:
Class Discussion

Talk about what threats kauri snails face.

Activity 6:
Group Activity

Brainstorm a conservation plan to reduce or prevent each of the threats mentioned in the discussion. Provide feedback as a group to the class.

Homework:
Individual Activity

Design a pamphlet/poster to save the snails

- Mention three fun facts about the snails
- List three easy things people can do to help the snails and explain how it would help
- Make it visually appealing

Bonus Activity:
Individual Activity

Work on worksheets (e.g. crosswords) that test the knowledge learned from Activities 1, 2, 3, and 5.

Lesson Plan 2: Population Density of Kauri Snails

NCEA Level: 1 **Subject:** Biology **Strand:** Nature of Science **Credits:** 3

Achievement Focus: Investigating in Science

Achievement Standard: 90925 - Carry out a practical investigation in a biological context, with direction

Achievement Objective: Demonstrate investigation skills by collecting, processing, and interpreting primary data in a biological context, with direction.

Achievement Link: https://www.nzqa.govt.nz/nqfdocs/ncea-resource/achievements/2019/as90925.pdf

Lesson Objectives:

- Understand what population density is
- Understand different methods of measuring population density
- Be able to plan a scientific experiment
- Understand how to interpret data and make conclusions
- Produce a report detailing findings from the scientific experiment

Prerequisite knowledge:

- Understand how scientific experiments are planned and carried out
- Understand what makes an experiment scientifically accurate

Lesson Requirements:

- Close proximity of the school to a kauri forest in which kauri snails exist
- Organised meeting with local park rangers, scientist or Department of Conservation representative

Activity 1:
Class Discussion

Discover and evaluate different methods of determining population density, e.g. line transect sampling, quadrat sampling, and mark-recapture sampling.

Activity 2:
Group Activity

Research kauri snails including distribution, habitat, nearby forests with kauri snail populations, and diet then write a proposal for fieldwork to determine population density using the most appropriate method for kauri snails. Choose one additional factor to measure that may influence population density.
Additional factors could be (but are not limited to):

- Predation (e.g. identify predator control traps in the area or identify predation marks on identified kauri shells)
- Demographics of population (e.g. proportion of adults/juveniles identified)
- Habitat (e.g. Identify plant species within the forest and in areas in which kauri snails are identified

Activity 3:
Fieldtrip

Visit a nearby forest where kauri snails exist to receive a talk from a local park ranger, scientist, or Department of Conservaiton representative, explaining more about kauri snails, the threats they face, and how threats affect their population density and abundance. Carry out the planned experiment (after approval of proposal by teacher).

Homework:
Individual Activity

Produce a 2-4 page report including the background information researched, methods used, results, and a discussion on the population density in the area as well as the other factor chosen.

Note: Activity 3 should be carried out separately from Activities 1 and 2, with at least 1 to 2 weeks between segments to allow sufficient time for research proposals to be refined and approved by supervising teachers.

Figure 3.4 Example lesson plan designed for secondary school students studying NCEA Level 1 in Northland. The lesson plan relates the "Investigating in Science" Achievement within the "Nature of Science" Strand to the genus *Paryphanta* to provide students with an understanding of how population density can be measured for invertebrates.

Lesson Plan 3: Conservation Management of Kauri Snails

NCEA Level: 1 **Subject:** Biology **Strand:** Nature of Science **Credits:** 3

Achievement Focus: Participating and Contributing

Achievement Standard: 90926 - Report on a Biological Issue

Achievement Objective: Collect and process data and/or information to report on a biological issue

Achievement Link: https://www.nzqa.govt.nz/nqfdocs/ncea-resource/achievements/2019/as90926.pdf

Lesson Objectives:

- Understand the issues faced by kauri snails including deforestation, predation, shell removal, and human interference (e.g. tourism at kauri forests)
- Be able to identify threats faced by kauri snails and how these threats can be managed
- Make a management plan within a budget for a kauri snail population
- Be able to report on the current threats faced by kauri snails and explain how the management plan designed may be expected to reduce the impact of these threats

Prerequisite knowledge:

- Understand common threats to endangered invertebrate populations
- Understand that conservation management is carried out for the benefit of endangered species and their environments

Lesson Requirements:

- Organised meeting with Department of Conservation representative

Activity 1:
Guest Speaker

Receive a talk from a Department of Conservation representative that explains:

- How organizations receive funding for conservation management
- How species are prioritised for conservation management
- How mammals and birds typically gain more funding and/or interest than invertebrates
- What different approaches are typically used for conservation management

Activity 2:
Group Activity

Process primary and/or secondary data from a range of sources to identify specific threats faced by kauri snails and how these threats are typically managed.

Homework:
Individual Activity

Create a thorough management plan within a given budget for a kauri snail population

- Reporting on the biological issues that kauri snails face
- List different approaches that may be used in a management plan for kauri snails
- Explain how each potential approach may be expected to reduce the impact of these threats
- Identify which management plan would be the most effective and why
- Present management plan to the class

Figure 3.5 Example lesson plan designed for secondary school students studying NCEA Level 1 in any New Zealand school. The lesson plan relates the "Communicating in Science" Achievement within the "Nature of Science" Strand to the genus *Paryphanta* to provide students with an understanding of the issues *Paryphanta* populations face and how populations can be managed.

Lesson Plan 4: Phylogeography of Kauri Snails

NCEA Level: 1 **Subject:** Science **Strand:** Living World **Credits:** 4

Achievement Focus: Evolution

Achievement Standard: 90948 - Demonstrate understanding of genetic variation

Achievement Objective: Demonstrate understanding of biological ideas relating to genetic variation

Achievement Link: https://www.nzqa.govt.nz/nqfdocs/ncea-resource/achievements/2019/as90926.pdf

Lesson Objectives:

- Understand how genetic variation occurs across and within populations
- Be able to describe how traits are inherited
- Understand the concept of phylogeography and be able to determine phylogeographic split in kauri snails across Northland

Prerequisite knowledge:

- Understand the structure and function of DNA
- Understand the heritability of DNA
- Understand phenotypic and genotypic variation and their role in evolution by natural selection

Activity 1:
Class Discussion

Review genetic variation and heritability including the following discussions:

- Revise definitions including gene, allele, genotype, phenotype, dominant, recessive, heterozygous, homozygous, genetic drift, gene pool, and founder effect
- Discover how pedigree charts and Punnet squares can be used to determine traits of interest in certain individuals

Activity 2:
Individual Activity

Complete Punnet squares and pedigree charts for multiple kauri snail populations to identify certain traits in individuals across populations. Be able to explain how genetic variation occurs within and between populations.

Activity 3:
Class Discussion

Review phylogenetic trees and how they may be constructed based on genetic data. Discover how phylogeographic structure reveals the connection between phylogenetics and geographic patterns.

Activity 4:
Group Activity

- Create a phylogenetic tree based on individual genetic sequences provided for kauri snails that represent various sites across Northland
- Use the phylogenetic tree with coordinates of populations provided to determine the phylogeographic structure of kauri snails across Northland

Homework:
Individual Activity

Identify individuals that may be used for a future translocation event making sure:

- To ensure founding and source populations are large enough to limit inbreeding effect
- To ensure genetic variation is maintained in the founding population in a way that represents the variation of the source population
- To ensure the genetic variation of the founding population aligns with the phylogeographic structure of the overall species

Figure 3.6 Example lesson plan designed for secondary school students studying NCEA Level 1 in any New Zealand school. The lesson plan relates the "Evolution" Achievement within the "Living World" Strand to the genus *Paryphanta* to provide students with an understanding of how genetic variation occurs, is inherited, and may be used to determine phylogeography.

Each lesson plan was designed with the target audience in mind to ensure optimal receptiveness regardless of age group, prior understanding, or geographical location relative to *Paryphanta* distribution. The New Zealand Qualifications Authority was referred to in designing these lesson plans to ensure compatibility with the New Zealand curriculum (New Zealand Qualifications Authority, 2023). Additionally, links to achievement standards on the NZQA website included in each lesson plan to provide teachers with direct access to additional resources (Figures 3.3-3.6). Finally, a range of activities were included to allow equal opportunity to learn whether it be through a discussion-based, creative, or hands-on approach.

Given that primary school students will have a limited understanding of science, scientific jargon was not used and opportunities to explain any scientific concepts were included within the lesson plan (Figure 3.3). The lesson was designed to begin with a class discussion revising important background concepts surrounding "endangered animals" before leading into activities specific to genus *Paryphanta.* Given that younger children have a shorter attention span (Brain Balance, 2023), shorter activities were chosen, as well as many discussions that allow teachers to gauge audience understanding of new concepts. A variety of activities were scheduled between discussions to provide children with different learning styles adequate opportunities to understand the information being taught. These activities included visual learning, drawing and colouring, group brainstorming, poster design, and finally worksheets that may be used to engage students with an excess of time.

Students studying NCEA Biology naturally have a greater prior understanding of scientific concepts, as well as increased attention span and ability to critically think. For this reason, all lesson plans targeted towards NCEA Level 1 students involved fewer activities, although activities had increased self-management opportunities (Figures 3.4-6).

Figure 3.4 shows an example of how schools within *Paryphanta* distribution may benefit from being within close proximity to *Paryphanta* populations as the proposed lesson revolves around planning and conducting an investigation to determine *Paryphanta* population density. This investigation allows students to understand scientific concepts and methods relating to population density, while simultaneously gaining hands-on field experience and learning about

Paryphanta conservation. Additionally, the fieldwork was designed to feature a guest speaker to provide students with a real-world perspective on how the concepts discussed are used in conservation research. The knowledge gained about population density is a skill that can be applied to a variety of taxa, although by focusing on genus *Paryphanta,* students will naturally gain an understanding and appreciation for their local endangered invertebrate.

While fieldwork is an effective way of providing unique learning opportunities outside of the classroom, it is not plausible for the majority of New Zealand schools. The opportunity to engage in fieldwork would require approval from guardians as well as the Department of Conservation. Additionally, those schools outside of the Northland and Auckland regions will naturally require huge amounts of resources to visit *Paryphanta* populations, therefore for those unable to travel, two additional lesson plans were designed that can be taught entirely from the classroom (Figures 3.5 and 3.6).

Similar to the NCEA lesson plan designed for Northland and Auckland-based schools (Figure 3.4), the first lesson plan designed for classroom-only-based teaching included a talk from a Department of Conservation representative (Figure 3.5). While the attendance of a guest speaker still involves sufficient planning, it would be beneficial for NCEA students to be provided with opportunities for networking and connecting with working members of the scientific field. Guest speakers can also introduce students to the concept of "Communicating in Science", which was chosen to be the Achievement focus of the lesson plan. Activities relating to this Achievement involved processing data relating to *Paryphanta* threats, as well as designing a management plan for *Paryphanta* populations. These activities will provide students with the opportunity to research and interpret their findings in a practical manner. Finally, fitting with the Achievement focus, students will be able to develop their science communication skills to discuss and justify ways in which they would manage an endangered taxa. Given the benefits of science communication in research, especially in research focusing on endangered endemic taxa such as *Paryphanta,* the ability to communicate science is a skill that should be prioritised in learning environments.

Considering the limited access to guest speakers in some regions, an additional lesson plan was created to ensure that classroom-based teaching may be done without additional resources (Figure 3.6). This final lesson plan relates directly to the aims of this project, by focusing on genetic variation, evolution, and phylogeography with focus on genus *Paryphanta.* This enables the next generation to learn, through guided self-discovery, the outcomes of this project while introducing them to the ways these results can be used for future translocations and conservation management of the genus. Like the other lesson plans, this one includes opportunities for learning through a combination of discussions, group activities, and individual activities. Activities centre around introducing students to basic genetic analyses by teaching them to interpret Punnet squares and phylogenetic trees. Finally, students are provided the opportunity to apply their knowledge by developing a plan for a translocation, using the information discovered through their analyses. With this structure, students are able to both learn about genetics while relating their newfound knowledge directly to *Paryphanta* conservation.

Conclusion

It is apparent that science communication has many benefits when used before, during, and upon completion of research. Science communication can enhance research quality, amplify the sharing of results, and inspire the next generations to continue the research and conservation management of endangered species. Aside from providing scientific benefits, effective science communication provides opportunities for the union of communities through building relationships, as was seen between scientists and iwi, museums, and government institutions such as the Department of Conservation. Given the clear benefits, science communication should be raised to a higher standard in all future phylogenetic research. Therefore, it is recommended that researchers utilise science communication where possible and allocate sufficient time and financial resources to do so.

Chapter Four

General Discussion

4.1 Summary of Research

4.1.1 Aims

The primary aim of this thesis was to reconstruct, through genotyping by sequencing (GBS), the phylogeographic pattern of *Paryphanta busbyi*, and determine if the east-west split within the genus *Paryphanta,* revealed by Spencer et al. (2006), is correct. Through GBS analysis, this research aimed to achieve an in-depth understanding of the population structure within *P. busbyi*, identify translocated populations, and make inferences about the origins of these translocations. Finally, this project aimed to communicate the science prior to, during, and upon completion of the project, involving science groups, museums, and iwi, to ensure all knowledge gained would be accessible and available for use in conservation projects for *Paryphanta* populations in the future.

This final chapter follows through on the research and reviews presented in Chapter Two and Chapter Three by discussing the findings of this research, including possible explanations and implications of results. The General Discussion also considers the limitations of the methods used and their effects on the research, with this leading into a discussion that outlines questions and considerations for future research surrounding the topic of *Paryphanta* phylogeography, taxonomy, and conservation. Finally, conclusions relating to the aims of this project will be made following a thorough discussion on those topics mentioned above.

4.1.2 Summary of Findings

GBS analysis revealed three distinct clades (northern, southern, and Waitākere) as well as an additional intermediate form that shared genetic similarities with both the northern and southern clades. When populations were mapped according to clade/form, a clear north-south phylogeographic pattern was observed between the northern and southern clades and the intermediate form. The Waitākere Ranges clade did not fit this phylogeographic pattern, although this outlier effect can be attributed to its known translocative history (Parrish et al., 1995).

Tangihua Forest (or a nearby location) was identified as the most likely site of origin for both the Warkworth and Waitākere Ranges populations, both of which were known to have translocative origins (Parrish et al., 1995). This inference was supported by the close evolutionary relationship (Figure 2.4) and high levels of genetic similarities (Figures 2.5 and 2.6) that both the Warkworth and Waitākere Ranges sites shared with the Tangihua Forest site. Furthermore, comparisons between GBS and COI data by Spencer et al. (2006) were able to provide additional context that supported Tangihua Forest as the site of origin for Warkworth populations, and given the close evolutionary relationship between the Warkworth and Waitākere populations, it is plausible that the likelihood of Tangihua Forest being the origin of the Warkworth population also extends to the Waitākere Ranges.

The Lonsdale Park population was assumed to have translocative origins due to its genetic structuring with other northern populations and its shared morphological traits with *P. watti.* Given the proposed north-south phylogeographic pattern between the northern clade, southern clade, and intermediate form, the Lonsdale Park's genetic structuring places its likely origin as a site further north than both Mangamuka and Totara North and closer to the known distribution of *P. watti.*

GBS analysis revealed that the Waipu Caves population did not fit with the proposed north-south phylogeographic pattern. Due to the discrepancy between its geographical placement within the southern clade distribution and its genetic placement within the intermediate form, it is assumed that the Waipu Caves population has a translocative origin. GBS analysis revealed Puketi Forest (or a nearby location) as the most likely source of origin due to the Waipu Caves and Puketi Forest populations' shared genetic makeup (Figures 2.5 and 2.6) and close evolutionary relationship (Figure 2.4).

Finally, the capacity and effectiveness of the science communication used throughout this project was also reviewed. It was found that where science communication was used, the project's level of efficiency was improved. Science communication methods included utilisation of prior instances of science communication for planning of research, conducting personalised outreach to build relationships with various target audiences, discussing opposing views with

collaborators (e.g. euthanisation of live animals, removal of shells, and sequencing of DNA), and encouraging citizen science throughout fieldwork. Short-term benefits included the building and maintenance of relationships with other scientists, museum curators, and iwi, as well as improved search methods and increased person-hours during fieldwork. Additional long-term benefits included supporting those relationships already built and informing others of the threats faced by *Paryphanta* populations as well as possible methods to mitigate effects of threats to the populations. Aside from the benefits that science communication was able to provide this project, this project also highlighted aspects of science communication that may be improved for future research, for example, the allocation of added time, where possible, to provide researchers and the public ample opportunity to engage.

4.2 *Paryphanta* Phylogeographic Pattern

The phylogeographic patterns observed in genus *Paryphanta* and other Northland land snails pointed to the inaccuracy of the previously described east-west phylogeographic split. Although the east-west split has been observed in some animals (e.g. *Oligosoma smithi*; Hare et al., 2008; *Entelea arborescens*; Shepherd et al., 2019), this pattern is not apparent in land snails. Therefore, it is likely that in the case of *Paryphanta* phylogeographic research, GBS may be more trusted than COI gene analysis alone due to its ability to sequence thousands of SNPs with high-level coverage (Davey et al., 2011; Narum et al., 2013).

The success of GBS has been proven by its applications within other phylogeography and population-genetic structure projects, in which thousands of SNPs could be identified with high level coverage (e.g. Jahner et al., 2019; Larsen & Matocq, 2019; Bagley et al., 2020). Given that this project was the first to successfully identify thousands of SNPs in *Paryphanta* using GBS, it can be concluded that GBS was an acceptable approach to achieve the aims of this project that centered around *Paryphanta* phylogeography and identification of translocative origins.

Multiple forms of analysis were conducted on the GBS data, these being ML phylogenetic analysis (Figure 2.4), PCA (Figure 2.5), and *STRUCTURE* analysis (Figure 2.6), all of which pointed to the same conclusions which enhances the view that these conclusions were accurate.

Although there are limitations surrounding small sample sizes (further discussed in Section 4.5 *Limitations of Research*), each of the natural populations corroborated the phylogeographic pattern revealed through GBS data, with the exception of the Waipu Caves population. This population may be considered an outlier to this pattern due to the discrepancies between its geographical placement within the southern clade and its phylogenetic placement within the intermediate form. This discrepancy, however, can be explained by a possible historical translocation. Therefore, it is likely that the few samples collected were in fact representative of the wider population and that the north-south phylogeographic pattern revealed can be trusted.

Northland geological history provides an explanation for how species may have evolved in isolation prior to further population expansion. Due to the northernmost region of Northland, Aupōuri Peninsula/Te Hiku o Te Ika, being isolated as an island during the Pliocene, the taxa within this northern region tend to be genetically distinct from those in mainland Northland (Morgan-Richards et al., 2001; Marshall and Barker, 2007, 2008). In addition to this, it is typical for the north-south phylogeographic pattern to extend into mainland Northland for other taxa (Morgan-Richards et al., 2001), which may reveal a north-to-south population expansion and corresponding genetic divergence.

Although *P. watti* samples were not included in this project, the same geological events may explain the north-south phylogeographic pattern revealed by GBS for *P. busbyi.* It is plausible that the divergence of genus *Paryphanta* from its common ancestors may have occurred in the northern regions of Northland due to the isolation of populations within the Pliocene. The availability of Holocene-aged fossils around the far northern region of the Aupōuri Peninsula/Te Hiku o Te Ika, as well as the suggestion that some fossils may be of Pleistocene age (Brook, 1999), supports that divergence had occurred at least by the Pleistocene (>2.58 MYA). The separation of the two *Paryphanta* clades, described by Spencer et al. (2006), was estimated to have occurred between 1.0-3.4 MYA. Despite these clades not aligning with those described following GBS analysis, the divergence date estimates can provide additional context to the proposed timeline and support an older separation date by the mid-early Pliocene.

Range expansion further south likely occurred following the Pliocene upon the formation of land bridges. This broadened distribution would have likely led to further north-south divergence as *Paryphanta* individuals are slow-moving at an observed rate of 10 m in two weeks (Department of Conservation, 2021). This slow rate of migration over time may reveal instances of populations interbreeding between migrations and could explain how the intermediate form arose.

Furthermore, the Lonsdale Park population and how it may fit into the wider context of *Paryphanta* phylogeography should be considered. This northern clade population was shown to have the greatest level of genetic difference from the southern population (Figures 2.5 and 2.6), and even more interestingly, appeared to have *P. watti*-esque morphological characteristics, which have previously only been observed within the *P. watti* distribution at the far northern region of the Aupōuri Peninsula/Te Hiku o Te Ika (Figure 2.2). This population's genotypic and phenotypic makeup would further support the north-south narrative, as well as how *P. watti* might be expected to fit in if *P. watti* samples were included in the study.

It should be considered that the presence or absence of fossils is also dictated by taphonomic biases in the fossil record, for example necrolysis (event of death and decomposition of soft tissues), biostratinomy (events occuring between death and burial), and diagenesis (events occuring after burial), and so the idea that *Paryphanta* specimens existed earlier further south remains a possibility. To account for this, alternate methods should be used to test the narrative of divergence in the northernmost region of Northland with divergence occurring as populations migrated in a southerly direction. Older populations typically have greater levels of genetic variation, so this narrative would typically be tested by comparing the genetic variation of different populations (Templeton, 1997; Martin and McKay, 2004). Considering *Paryphanta* populations have been largely affected by forest fragmentation, predation, habitat destruction, and exploitation by humans (Goulstone et al., 1993; Montefiore, 1994; Montefiore, 1995; Stringer and Montefiore, 2000; Stringer et al., 2003), it is likely that multiple bottlenecks have occurred and much of this genetic variation has been lost.

Analysis of the fossil record could help to reconstruct phylogeny and pre-human genetic diversity and may provide points in time from which *Paryphanta* populations existed in certain regions. The availability of fossil records around the northern region of the Aupōuri Peninsula/Te Hiku o Te Ika reveals that *Paryphanta* populations existed in this region during the Holocene and potentially even the Pleistocene (Brook, 1999). Sand dune deposits, which are effective for the proper preservation of fossils in some instances, are not common in the distribution of *P. busbyi,* therefore, *P. busbyi* fossils are yet to be found in mainland Northland. However, any future identification of *P. busbyi* fossils may be able to provide additional context on the timing of historical populations' existence, from which conclusions regarding this inferred migratory pattern can be made.

Overall, the north-south phylogeographic pattern revealed through GBS analysis appears to be the best supported phylogeographic pattern for *Paryphanta* spp., and is also understandable in the wider context of Northland phylogeography. The sequencing power of GBS, alongside the geological history of Northland, north-south phylogeographic patterns of other Northland taxa, including additional land snail species, and the morphological patterns observed across *P. watti,* Lonsdale Park, and *P. busbyi* specimens, all support a north-south phylogeographic trend. For the first time, population structure, geological history, and morphology corroborate the same pattern; therefore I conclude that this observed north-south phylogeographic pattern is the true structure of genus *Paryphanta*.

4.3 *Paryphanta* Translocations and Their Origins

The phylogeographic pattern and population structure revealed for populations that are assumed to be natural, in combination with the population structure across all individuals and populations, can be used to identify historical translocation events and determine their source. Comparisons can also be made for certain sites that were included in the phylogenetic analysis conducted by Spencer et al. (2006). Finally, the information revealed regarding the site of origin for other translocation events can provide additional context to the information revealed through GBS.

Waitākere and Warkworth

GBS analysis and comparisons with phylogeny revealed by Spencer et al. (2006) identified Tangihua Forest (or a nearby location) as the most likely source location for both the Warkworth and Waitākere Ranges populations. Tangihua Forest is a reserve covering 3,000 hectares of broadleaf and kauri forest that has remained undisrupted by nearby habitat destruction for farming (Department of Conservation, 2023a; Mannion, 2023). These features make Tangihua Forest the perfect environment to maintain a healthy *P. busbyi* population as it is representative of an ideal *Paryphanta* habitat and is unfragmented, meaning genetic bottlenecks are less likely. As a general rule, translocations should be performed with individuals showing high levels of genetic variation, as this is indicative of a healthy population. Although only two specimens were sampled from Tangihua Forest for GBS, a reasonable level of variation was seen between the two specimens (Figure 2.4), and the habitat is considered healthy. Therefore, Tangihua Forest would be considered a good site from which translocations may be made.

If Tangihua Forest is considered the source population for the Warkworth and Waitākere Ranges translocations, genetic variation between the source population and the translocated populations can be assessed to determine any loss of variation caused by these translocations. While the Warkworth and Waitākere populations show low genetic variation between individuals of the same population (Figure 2.6), the genetic variation of the entire Warkworth population appears to be fairly similar to that of the Tangihua Forest population. Given that Warkworth is largely affected by forest fragmentation, it is possible that the Warkworth population's resilience is a result of its retained genetic variation. Populations with lower genetic variation have lower resilience and capability for adaptation in a changing environment (Hughes et al., 2008; DeWoody et al., 2021). Therefore, without high genetic variation, it is more likely that this translocation would not have been effective.

On the other hand, the genetic variation revealed in the Waitākere Ranges is not representative of the Tangihua Forest population. It is possible that the genetic structure of the founding population for the Waitākere Ranges was unrepresentative of its source population. Certain alleles may also have been lost or fixed within the new population due to genetic drift, resulting

in the Waitākere clade's unique genetic makeup. However, with the Waitākere Ranges being a predator-controlled Regional Park consisting of primary forest over 16,000 hectares (Montefiore, 1994; New Zealand Tourism, 2023), the founding population was able to expand their distribution. It must also be considered that unsampled variation exists in the wider population that more closely resembles that which currently appears to be unique to the Waitākere clade.

Lonsdale Park

GBS analysis, *P. watti*-esque morphology, and the proposed north-south phylogeographic clade suggests that the Lonsdale Park population was likely translocated from a site further north. This is supported by Lonsdale Park's use as a recreational centre attracting frequent visitors that may present opportunity for an undocumented translocation occurring through displacement of specimen/s from a location further north. The fragmented state of the Lonsdale Park forest and its frequent recreational use would disrupt the ground cover in which the *Paryphanta* population exists, further decreasing the likelihood of a natural population surviving in this area.

If the Lonsdale Park population is truly translocated from an area closer to the known distribution of *P. watti*, it would disprove the hypothesis that the *P. watti* morphotype is an ecotype. The Lonsdale Park habitat was unlike what would be expected to cause an ecotypic trait shared with *P. watti,* as it differed from the *P. watti* habitat, which is characterised by drier coastal forest (Stringer et al., 2003; Mahlfeld et al., 2012). Rather, the Lonsdale Park habitat was not notably different from the habitat occupied by *P. busbyi* morphotypes, aside from it being incredibly small with frequent recreational use.

The Lonsdale Park population having the most "northern" genetic structure, i.e. being the most diverged population from the southern clade, combined with its morphotype resembling that of *P. watti* (though not completely), indicates that it may be a transitional form between *P. watti* and *P. busbyi.* A transitional morphotype would suggest that there is more continuity between *P. watti* and *P. busbyi* and that they should not be classed as completely distinct species. It may instead be more accurate to assume that *P. watti* and *P. busbyi* are capable of hybridisation and exist on

either end of a spectrum capable of displaying a range of black to green morphs with a corresponding north-south phylogeographic pattern.

If this scenario is accurate, it suggests that a minor genetic variation would correspond to a change in phenotype, as evidenced by the limited genetic variation observed in the three northern populations, despite the significant difference in phenotype observed in the Lonsdale Park specimens. Rather than the alternate morphotypes being a result of different genes or alleles present in *P. watti* and *P. busbyi*, it is possible that these differences may be attributed to a differential expression of a shared gene.

Waipu Caves

Given that many translocations have occurred without documentation, it is plausible that the Waipu Caves population was historically translocated, which would explain the disconnect between the population's geographical and genetic structuring. By fitting the proposed north-south phylogeographic pattern across other sites that are assumed to be natural, it is inferred that the Waipu Caves population originated from an intermediate site between the proposed northern and southern clades, with GBS analysis suggesting this translocation more specifically originated from Puketi Forest (or a nearby location).

Puketi Forest, combined with Omahuta Forest, makes up 15,000 hectares of contiguous tract (Conning and Moors, 1998; Puketi Forest Trust, 2023). Comprised of broadleaf and kauri forest (Conning and Moors, 1998), Puketi Forest is a prime habitat for survival of healthy *Paryphanta* populations and maintenance of genetic variation that could be used as a source population for translocation events. With only one specimen being collected each from the Waipu Caves and Puketi Forest sites, further sampling would be required to provide additional context that could be used to determine the exact source location for the translocation event. Although, given the genetic structure revealed by GBS analysis, alongside the known habitat of Puketi Forest, it can be concluded that the site of origin for the Waipu Caves population was likely either Puketi Forest or a contiguous forest within the intermediate form's distribution.

4.4 Wider Implications of Research

4.4.1 Implications of Phylogeographic Research on *Paryphanta* Populations

Taxonomic Classifications

The phylogeny published by Spencer et al. (2006) raised questions about the true taxonomy within genus *Paryphanta* as it suggested that the widely accepted previous classifications of two species or subspecies (*P. busbyi* and *P. watti*) may not be accurate. While most publications still distinguish between *P. busbyi* and *P. watti* and refer to these groups separately, it is often acknowledged that there is little confidence in these current taxonomic classifications, which have been based on morphology alone (Spencer et al., 2006; Mahlfeld et al., 2012).

GBS analysis revealed three clades (northern, southern, and Waitākere), as well as an intermediate form that exists between the distributions of the northern and southern clades and shares genetic aspects with both clades. Translocated sites should be considered in discussions regarding taxonomic classification as discrepancies between genetic structure and geographical placement can confuse taxonomic boundaries.

Although the Waipu Caves population likely has translocative origins (as discussed in Section 4.3 *Translocations and Their Origins*), its presumed site of origin aligns with its positioning within the intermediate form. Contrastingly, although the Waitākere Ranges population is known to have translocative origins, high levels of divergence have occurred leading to this population now being distinct from the southern clade, from which it is assumed to have been translocated. The Lonsdale Park and Warkworth populations, which were also considered to have translocative origins, appear to have been short-range translocations within the distribution of their proposed clades, which would not affect taxonomic classification.

Given that transitional forms have been identified both genetically (i.e. the intermediate form) and morphologically (i.e. the Lonsdale Park population), it is possible that only one species should be recognised within the genus *Paryphanta.* Although a transitional form is yet to be identified between the Waitākere and southern clades, limited sampling was undertaken in the geographic regions between these two clades (further discussed in Section 4.5.1 *Gaps in Geographic and Morphotypic Representation*).

Paryphanta Conservation

The results obtained from this project can have implications for the management plans of genus *Paryphanta*. *P. busbyi* is currently listed as "Near Threatened" (Sherley, 1996), and with the results suggesting that only one species should be recognised within the genus *Paryphanta,* this listing should refer to both the *P. busbyi* and *P. watti* morphotypes and distributions.

Proposed Management Units

Regardless of the number of species that should be recognised, multiple management units should be identified to ensure that as much genetic and morphological variation is conserved across a wide geographic region. Due to their genetic variation, the three clades identified (northern, southern, and Waitākere) should be prioritised as management units. With each clade representing distinct regions across Northland, the prioritisation of these clades will additionally conserve the overall distribution of genus *Paryphanta.*

Since the intermediate form has genetic aspects of each distinct clade rather than an entirely distinct gene pool, this form is of lower priority. The prioritisation of this clade may drive rarer alleles, which are only represented in a single clade, to extinction through genetic swamping. However, any additional funding could always be allocated to the conservation of the intermediate form in the future.

Morphological variation should also be considered in the development of management units, regardless of their genetic variation. As revealed by the Lonsdale Park population, morphotype

variability does not appear to correlate with clade and the genetic cause is therefore unknown at this stage (see Section 4.3 *Translocations and Their Origins*). Therefore, populations within the *P. watti* and Lonsdale Park distributions should also be prioritised as management units in order to conserve these morphotypes, which differ from that typical of *P. busbyi.*

This project suggests that management units should be defined, with these being: the northern clade (excluding the Lonsdale Park population), the southern clade, the Waitākere clade, the *P. watti* morphotype, and the Lonsdale Park morphotype. Within these management units where multiple populations exist, it is important to focus conservation efforts on those populations that would result in the most effective management. Larger populations typically show greater levels of variation, and as greater genetic variation is able to provide increased resilience and capability for adaptation in a changing environment (Hughes et al., 2008; DeWoody et al., 2021), these populations should be prioritised. In saying that, it should also be considered which populations exist within a relatively stable environment, i.e. forested reserves that protect against habitat fragmentation and predation should also be prioritised. With expansive, stable environments correlating with dense and genetically varied populations, it would be a cost and time-efficient approach to select populations from forested reserves as these populations are likely to have high levels of genetic variation with the added benefit of existing within a protected area. Aside from providing the targetted populations with the best chance for survival, this approach of prioritising highly variable populations would also allow these populations to be used for future translocations, thereby benefiting the genus as a whole.

Habitat Conservation

Predation and habitat degradation by introduced mammals, as well as forest fragmentation, pose an immediate threat to *Paryphanta* populations (Goulstone et al., 1993; Montefiore, 1994; Parrish et al., 1995; Montefiore, 1995; Stringer and Montefiore, 2000; Stringer et al., 2003). With large populations being able to better retain genetic variation, it is important to maintain healthy habitats in order for at-risk taxa populations to thrive.

Observations during fieldwork for this project suggest that large, protected forested areas positively correlate with greater density of *Paryphanta* populations, such as was observed in Waipoua Forest, Waima Forest, Mangamuka, Whangārei, and the Waitākere Ranges. Mataraua Forest is contiguous with both Waipoua Forest and Waima Forest. Combined they make up the largest tract of native forest in Northland and receive significant amounts of funding and active management (Department of Conservation, 2023b). Mangamuka Scenic Reserve, Parihaka Scenic Reserve (Whangārei), and the Waitākere Ranges Regional Park, from which the Mangamuka, Whangārei, and Waitākere Ranges populations were collected respectively, are each protected parks or reserves that also gain funding and active management (Department of Conservation, 2023c).

Active management may vary from site to site, although it typically involves regulated pathways for foot traffic, replanting, predator control, and forms of science communication including signage indicating threats to the forest (Department of Conservation, 2023b). Active management plans can focus efforts towards enhancing the health of populations, and therefore should be carried out for each management unit. This role would be most effective if shared between local council, local iwi, and the Department of Conservation, as each party is adept at providing individual expertise on the matter and funding costs could be divided. Broad areas must be conserved in order to avoid further forest fragmentation and achieve a predator-free environment, which are both factors that threaten *Paryphanta* populations. It can be expected that continued management and cooperation will be required indefinitely to ensure the maintenance of *Paryphanta* populations.

Translocations

The populations that exist in Warkworth and the Waitākere Ranges as a result of translocation events show that translocations have the potential to be useful in *Paryphanta* conservation through the formation of new populations.

Although these translocated populations revealed low genetic variation, this is a feature also observed across natural populations (Figure 2.6). Low genetic variation within populations is

likely due to the occurrence of forest fragmentation and predation that limit population density and expansion, which each have the capability to result in bottlenecks. Regardless, the levels of genetic variation retained in the known translocated populations of Warkworth and the Waitākere Ranges reflect that of natural populations, including Tangihua Forest, which is thought to be the origin of these translocation events. Given that translocated populations typically reflect an immediate loss of genetic variation from what is present in the source population. This retained genetic variation shows that translocations may also be used for genetic rescue, which is the process of moving genes from one population to another to increase genetic diversity and minimise inbreeding.

Since *Paryphanta* individuals are known to disperse at a very slow rate, with this rate being up to 10 m in two weeks (Department of Conservation, 2021), it is not plausible that population expansion leading to broadened distribution would occur naturally. Natural population expansion is even more unlikely given that much of Northland's forests are fragmented due to deforestation for the development of urban areas and farmland.

It is recommended that additional translocation events occur and be documented to amplify the number of *Paryphanta* populations across native forested areas within Northland. Translocation events should coincide with habitat conservation for both the translocated and natural populations, i.e. forest restoration, predator control, and regulations for visitors, which would lead to the existence of much larger and healthier habitats for populations to thrive.

Translocations should be documented and originate from populations within management units in hopes that source populations with greater levels of genetic variation will limit the loss of variation in translocated populations, thereby providing the translocated population with greater resilience to disease and a changing environment. Additionally, given the north-south phylogeographic structure revealed through GBS analysis, short-range translocations should occur where possible. Short-range translocations can ensure the natural genetic structure of all *Paryphanta* populations remains stable across Northland, from which information on the dispersal of species, mating behaviours, delimitation of species, and population boundaries can be obtained (Janes and Batista, 2016).

A population size of 50 individuals is required to cope with demographic stochasticity, as well as the allee effect, which describes how inbreeding depression can reduce average fitness as population size declines (Wittmann et al., 2014). To limit the impact of these processes on both the founding population and the source population in translocation events, genetic structure may be analysed in order to identify multiple closely related populations from which sufficient individuals may be obtained for the translocation. By selecting an adequate number of specimens across closely related populations, the effect that a reduced population size would have on any one source population would be decreased, while still retaining a sufficient population size for the founding population.

4.4.2 Implications of Phylogeographic Research on Additional Taxa

Due to the phylogeographic pattern revealed for the genus *Paryphanta,* this research provides a comparison for other taxa that also reveal north-south phylogeographic trends across Northland (e.g. *Hemideina thoracica*; Morgan-Richards et al., 2001). This research not only supports these north-south trends observed thus far across other species, but may be used as additional context in future phylogeographic research if the same geological processes are identified as relevant in the formation of this phylogeographic pattern. This example of *Paryphanta* may then aid in explaining phylogeographic patterns identified from future research which also investigates Northland geological history and population expansion or divergence of other taxa.

The success of identified *P. busbyi* translocations may be considered future translocations of other plant and animal taxa that exist in the *P. busbyi* habitats. Whether future translocations be done as forest restoration associated with *Paryphanta* conservation or as conservation management of other endangered taxa, the successful past translocations of *P. busbyi* specimens may indicate relevant features of successful past translocations that should be taken into account for any future translocations. The analysis of historical *P. busbyi* translocations has identified specific forests that provide a healthy habitat for founding populations (e.g. Waitākere Ranges), as well as forests in which resilient populations exist that could be used for translocations (e.g. Tangihua Forest). It could be expected, therefore, that if these habitats were successful for use as source and founding *P. busbyi* populations, their success may carry over to other taxa. Of course,

other factors must still be considered in identifying locations for source and founding populations, for example, genetic diversity in each population and the compatibility of ecosystems (e.g. vegetation types and climate).

Finally, this research on *Paryphanta* highlighted the importance of rich, healthy forests for the maintenance of populations as well as for specific populations that require managed conservation. Not only will healthy forests benefit the existing *Paryphanta* populations, but other plant and animal taxa within the same areas. Forest restoration, predator control, and visitor regulation are all possible actions that may be carried out for conservation of *Paryphanta* management units, and by association, other taxa and the forests in which they inhabit too will benefit from these actions.

4.4.3 Implications of Science Communication as a Research Tool

Prior instances of science communication are typically utilised in scientific research as preliminary research tools. Similarly, outreach is also typically used in varying degrees in order to communicate results. However, other methods of science communication are less typical, particularly citizen science to engage the public in active research. This approach was found to be effective during fieldwork as it provided additional aid in locating *Paryphanta* populations and individual specimens that were to be sampled. In addition to enhanced person-hours, citizen science also provided many opportunities for the development of personal relationships, exchange of culture, and sharing of scientific proceedings. Therefore, it was found that citizen science is effective and should continue to be used for *Paryphanta* conservation and in future research.

In turn, by engaging the public in scientific research, it was reaffirmed that interest towards action for conservation is present across a wide number of communities, including local iwi and councils. This interest is an aspect that should be harnessed through science communication to encourage cooperation for improved outcomes of conservation research. Currently, information available on *Paryphanta* population health is limited by the available scientific literature. Iwi and park rangers already have huge influence on the maintenance of land in which *Paryphanta*

populations exist, combined with an immense appreciation for *Paryphanta* populations. Therefore, this project has demonstrated how iwi and park rangers may provide additional continuous contributions to conservation management, for example, by providing updated reports on population density, predator control, and forest health that may then be utilised for continued analysis of managed populations.

Although the full impact of communicating results has not yet been achieved with school-aged children, for example by including *Paryphanta*-focused lesson plans in school curriculums (as in Figures 3.3-3.6), the act of engaging school-aged children through storytelling has already proven to engage interest to some degree (as discussed in Section 3.4.3 *Communicating to Gain Interest from the General Public/Picture Books*). It is therefore worth exploring this avenue of science communication further in future research to provide the next generation with local examples of conservation and genetics. The potential that science communication may provide remains untapped and these examples of reaching a younger generation show that science communication does not necessarily require an expert audience or an audience that is immediately involved in research or conservation efforts. Rather, it is worth introducing these topics to youth early, to engage interest from a young age that may be carried with them throughout their endeavours for years to come.

4.5 Limitations of Research

4.5.1 Gaps in Geographic and Morphotypic Representation

Even when multiple samples can be collected for each site, the sites themselves must be representative of the overall population to avoid pseudoreplication (Lazic et al., 2020). For this project, multiple regions were unable to be sampled for reasons out of our control, including permit regulations, time constraints, and temporary closure of parks.

While a broad range of sites were sampled, these sites did not include any from the distribution of *P. watti,* which manifests a distinct morphotype (Stringer et al., 2003; Department of

Conservation, 2021). *P. watti* are geographically restricted to the northern tip of the Aupōuri Peninsula and are isolated from the distribution of *P. busbyi* by a sand tombolo (Powell, 1947; Montefiore, 1995; Neall and Trewick, 2008). Prior to this research, no *P. watti* morphotypes had been identified within *P. busbyi* distribution. The first example of this morphological similarity was seen in Lonsdale Park populations, which exist in the northern region of mainland Northland, south of the Aupōuri Peninsula.

Given that *P. watti* populations are geographically restricted to the northern tip of the Aupōuri Peninsula and are isolated from the distribution of *P. busbyi* by a sand tombolo, their genetic structure may differ significantly from that of *P. busbyi.* The degree of this difference may even be significant enough that *P. watti* should be classified as a distinct species. Therefore, while inferences can be made based on the combined morphology and genetic structure of the Lonsdale Park population, which might suggest that the north-south phyloegeographic split extends to the *P. watti* distribution, these inferences again cannot be concluded without GBS analysis of *P. watti* specimens.

Samples from certain regions within the *P. busbyi* range were also unattainable. Firstly, sites within the central Northland region around Kaikohe, Kerikeri, Pipiwai, and Helena Bay were not sampled. It is unknown whether specimens from these sites would genotypically cluster within either the southern clade or intermediate form. While it is concluded that a north-south phylogeographic pattern with an intermediate form is present across the distribution of *P. busbyi*, the range of the intermediate form cannot be confirmed due to unrepresented sites.

Secondly, unsampled sites between Waipu Caves, Warkworth and the Waitākere Ranges may have been able to provide greater context surrounding the effect of these translocations and the boundary between the southern and Waitākere clades. GBS analysis revealed the southern and Waitākere clades to be genetically and geographically distinct from one another. Although, given that no sampling of sites occurred between Tangihua Forest and the Waitākere Ranges (aside from the translocated Warkworth site), it remains undetermined whether a transitional form between these two clades exists as is seen between the northern and southern clades. If additional

samples were collected from this region, they may well reveal additional populations with the same genetic aspects shared by the Waitākere, Warkworth, and Tangihua Forest populations.

4.5.2 Small Sample Sizes

On one hand, the use of larger sample sizes in research increases statistical power, improves reproducibility, and allows results to be extrapolated to the overall population more accurately (Faber and Fonesca, 2014; Maleki et al., 2019). On the other hand, proportionately large sample sizes will have added financial, time, and effort constraints and may also pose ethical concerns, especially on threatened or taonga species. Therefore, sample size should be determined in relation to the overall population size.

In the case of this project, the sample size for each site was restricted due to conservation concerns. Threats to *Paryphanta* populations have resulted in low population density in some forests, which often led to challenges in locating and sampling multiple specimens. Furthermore, where it was known that *Paryphanta* populations faced particular threats, such as highly fragmented forests or high risk of predation indicated by damaged empty shells, fewer specimens were sampled due to concerns regarding the effect of sampling on the population as a whole.

A total of 35 samples were collected across 12 Northland and Auckland sites (see Section 2.2.1 *Sample Collection/Curation*), with one sample excluded from GBS analysis (see Section 2.2.5 *Bioinformatics*). To ensure each individual included in the project is representative of their population, researchers should aim to sample multiple specimens from each site. Considering the concerns around *Paryphanta* conservation which limited sampling to a maximum of five specimens per site, which was considered the optimal sample size for each site.

Four sites had a sample size of one, with three of these sites representing the three sites classified within the intermediate form, while the fourth site was Totara North, which was one of only three sites falling within the northern clade.

Furthermore, if one sample requires removal from complete GBS analysis for quality concerns, the effect incurred would be limited by obtaining multiple samples for each site. This was the case for a Tangihua Forest sample as one sample was removed from GBS analysis. The effect of reducing the sample size for this site was negligible compared to if any samples representing the intermediate form were removed.

In the case of threatened or taonga species where sufficient numbers of live specimens are unattainable for genetic analysis, it could be suggested that additional forms of DNA retrieval methods are used alongside euthanasia. However, as discussed in Section 2.2.1 *Sample Collection/Curation*, non-invasive DNA extraction methods such as periostracum, DNA swabs, tissue clips, and DNA extraction from shell provide too many limitations to be feasible methods of obtaining sufficient quality DNA. Since euthanasia can produce higher quality sequences, albeit fewer samples in keeping with ethical standards, researchers may choose to supplement sample size by using less invasive methods alongside euthanasia.

It is still undetermined whether different types of DNA (e.g. mitogenomes vs nuclear genomes) infer the same relationships within genus *Paryphanta*. However, considering the lack of consistency in phylogeographic patterns inferred from COI and GBS data, researchers should account for this uncertainty if deciding to use a combination of DNA extraction methods that require different analysis methods. To test these concerns, it would be beneficial for preliminary research to conduct a study comparing the relationships revealed by different types of DNA (as discussed in Section 4.6.1 *Paryphanta Phylogeography and Population Structure/Mitogenome Research*).

In cases where the seemingly less invasive method is able to provide high-quality samples, for example, tissue clipping, other limitations still apply. While individuals have been observed for up to a few weeks after tissue clipping, any impact that may be revealed exceeding this timeframe is unknown (Walton, pers. comm. 2023). Additionally, the time required waiting for each individual to emerge from its shell requires approximately 30 minutes with only about two-thirds of individuals even emerging (Mahlfeld, pers. comm. 2022; Walton, pers. comm. 2023). With these added time constraints, tissue clipping will often not be a feasible sampling

method alone - much like euthanasia. However, if used alongside euthanasia, tissue clipping may be used to increase the sample number without requiring the euthanasia of excessive specimens, while still allowing for consistency in DNA analysis throughout the project.

4.6 Future Research

4.6.1 *Paryphanta* Population Structure and Phylogeography

GBS on *Paryphanta watti*

With *P. watti* specimens unrepresented in this project, conclusions can only be drawn for *P. busbyi*. Therefore it is of utmost priority that the same methods be repeated, including samples from *P. watti* distribution, so that comparisons can be made across the entire *Paryphanta* distribution. Furthermore, any future genomic research conducted on genus *Paryphanta* should include *P. watti* specimens. Due to their unique morphology, any future research should prioritise management units, which is proposed to include *P. watti*. The inclusion of *P. watti* specimens will ensure that inferences, conclusions, and management plans can include *P. watti* populations.

Mitogenome Research

Given that COI analysis revealed an east-west phylogeographic split (Spencer et al., 2006), while GBS analysis revealed a north-south phylogeographic pattern, whole mitogenome sequencing should be performed in order to determine the cause of this discrepancy. Incomplete lineage sorting and the long branch attraction phenomenon could be considered as possible explanations for the discrepancy between COI and GBS data. Modern genetic analysis methods can account for these phenomena and uncertainty may be resolved following a revised COI analysis that excludes certain species with very high evolutionary rates, removes loci with very high rates, and includes species that may break up long branches on the tree.

It is also currently unknown whether the phylogeographic patterns revealed by COI and GBS analyses were due to the COI gene being nonrepresentative of the mitogenome, or due to the mitogenome and nuclear genome inferring different evolutionary histories. To test for synonymity between phylogeographic patterns revealed by COI, mitogenome, and nuclear data, further analysis should be carried out using all three analyses on a set pool of samples. If it becomes apparent that the COI gene is not representative of the whole mitogenome, and that mitogenome analysis infers the same evolutionary pattern as GBS analysis, this will present future opportunities for non-invasive methods, including the use of ancient DNA methods for mitogenome extraction from shell (Psonis et al., 2022; Walton et al., 2023), to be more frequently utilised in future research for mitogenome analysis on *Paryphanta* and other land snails.

4.6.2 Identification of Genes Associated with Phenotypic Differences

Three morphotypic variations have now been observed across genus *Paryphanta*, these being the darker, typically smaller shell exhibited by *P. watti* (Stringer et al., 2003), the green, larger shell exhibited by *P. busbyi* (Stringer et al., 2003), and the transitional Lonsdale Park form (*P. busbyi*) which exhibited the *P. watti*-esque features of a slightly darker, slightly smaller shell than typical *P. busbyi* shells (Ward, pers. obs. 2022; Figure 2.2).

While a significant phenotypic difference was observed between the Lonsdale Park population and other *P. busbyi* populations, there was limited genetic variation or habitat differences observable between the Lonsdale Park population and other *P. busbyi* populations, especially those within the northern clade with which the Lonsdale Park population is grouped. This observation suggests that the morphological differences may not be distinct features of separate species and instead may be due to a minor genetic variation corresponding to a change in phenotype. Rather than the alternate morphotypes being a result of different genes or alleles present in *P. watti* and *P. busbyi*, these differences may be attributed to a differential expression of a shared gene.

Lake and river populations of *Potamopyrgus antipodarum* exhibit unique morphotypes and phenotypic differences were found to be attributed to genome-wide DNA methylation which altered gene expression between the populations (Thorson et al., 2017). While no significant correlation was seen between the Lonsdale Park, *P. watti,* and typical *P. busbyi* habitats that would be attributed to severe ecotypic differences, slight changes in the environment, rather than the habitat itself, could explain the variation in morphotype. Phenotypic variations in other land snails have been attributed to environmental adaptations. For example, differences in shell size and shape in the pulmonate genus *Albinaria* were found to be adaptations for varying rainfall and temperatures between populations (Giokas et al., 2014). Therefore, subtle environmental differences experienced by *Paryphanta* populations may result in varied gene expression, leading to the morphotypic differences observed.

To confirm or deny this hypothesis, additional specimens should be sampled throughout the distribution of the northern clade and *P. watti*. Further genetic analysis should then be conducted to identify the gene/s attributed to the observed morphotypes. Additionally, an analysis should be carried out to measure possible environmental pressures that could cause this genetic variation. Since identifying a gene responsible for a particular phenotype can involve extensive experiments (Harper et al., 2011), a more effective approach may involve comparisons with known genes associated with morphotypes in other closely related genera or families. This approach would still involve whole genome sequencing, therefore, a faster, though less accurate approach could be to disregard identifying the specific functional gene/s and instead begin focus research on studies that compare shell variation and genetic variation (e.g. Thorson et al., 2017).

Conclusion

This thesis revealed, through genotyping by sequencing, the phylogeographic pattern of *Paryphanta busbyi*, with a high level accuracy previously unobtained. In doing so, the genetic population structure of *P. busbyi* was understood, undocumented translocated populations were identified, and inferences were made regarding the site of origin for documented and undocumented translocations. Finally, science communication approaches were analysed and discussed in the context of conservation research, with emphasis on the importance of public understanding and engagement (PEST). It was encouraged that future researchers take opportunities to involve the public in scientific research, both in terms of collaboration as well as in presenting findings to the general public in an accessible and easily understood manner.

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