Testing the effectiveness of the mt DNA Cytochrome *c* oxidase subunit 1 (COI) gene locus for identifying species of Polychaete worm (Polychaeta: Annelida) in New Zealand

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Abstract

The ability to accurately identify species is fundamental to ecological research and environmental monitoring. Current taxonomic identifications often rely on differentiation of morphologically ambiguous characters, and a process of categorization which is tedious and often leads to misidentifications. This is compounded by the presence of cryptic taxa, which may be prevalent among Polychaete worms (Polychaeta: Annelida). With increased access to genetic techniques, Cytochrome *c* oxidase subunit I has been suggested as a possible aid to assist in the discrimination of species resources.

In this study, I tested the hypothesis that the mtDNA COI gene locus is effective in discriminating morphologically recognised species of Polychaete worms.

A 543 base-pair fragment of the COI locus was successfully extracted for 111 individuals from 16 out of 20 morphologically recognised species. Average intraspecific divergences were 0.8 %, ranging from 0 % to 5 %. Average interspecific variation was 26.4 %, ranging from 13.8 % to 36.8 %. The lowest divergences were found between two *Nereid* species (13.8 %), and two *Glycera americana* species (17.2 %). Relatively high maximum divergences of over 30 % suggest that some species may have reached a divergence saturation level, which may partially explain why familial groupings in constructed trees were not monophyletic. Divergences within the different Nereid species - a group previously known to have morphologically cryptic species – did not reveal the presence of any cryptic taxa. Pairwise comparisons showed a clear divide between percentages of intra- and interspecific divergences, and the suggested threshold of 11 % is effective for the taxa investigated here.

On the basis of these results, I conclude that sequence variation in the mtDNA COI gene locus is effective in discriminating morphologically recognised species of Polychaete worms, but may not be appropriate for deeper (e.g. generic or familial) phylogenetic relationships among taxa.

Acknowledgements

I would like to thank just a few people for making this accomplishment possible:

Carl, my fiancée, who I turned to constantly for help with formatting and multitudes of problems with software and data analysis. He always sets the *best* example and pushes me to try that little bit harder and is always there for me. He supported me when at times it got tough, and showed me that there is light at the end of the tunnel, even if at times I felt like I was going backwards!

My supervisor Ian Hogg for pointing me on the right track, reading, re-reading and editing drafts, never giving up on me, and getting me through to the end. I'm glad one of us has done this before, else I would have been totally lost! Thanks for showing me these fascinating little animals; I never knew they even existed!

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Also my laboratory 'colleagues' Matt Knox and Darrin Sutherland; I couldn't have done this without your help!

And to all the other staff and students at Waikato University that helped me.

Thank you.

Dedication:

I dedicate this thesis to my mum and dad, Kim and Kevin Brett, who have encouraged me to strive to do my best ever since I could walk and talk, and ignited that spark in me for fascination in the natural world by getting your hands dirty and getting up-close and personal with it through scuba diving, fishing. Thanks for encouraging my strange hobbies. This pic is for you, mum!

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1 Introduction

1.1 Introduction

Polychaete worms (phylum Annelida) are present in virtually all marine habitats, including coastal estuarine and rocky shore systems, continental shelf and deep sea benthos, and some pelagic varieties are found in the water column (Glasby et al. 2000). Estuaries are complex environments with varied physical and chemical conditions, causing the existence of many localized micro-environments or niches (Cognetti and Malatagliati 2000). This range of environments promotes rapid speciation (Bilton et al. 2002) as seen in many estuarine species, including polychaetes, where they encompass a range of morphologically diverse types, matching the variety of habitats. Polychaetes can be either mobile or sedentary, and feeding types include predators, scavengers, and filter-feeders (Glasby et al. 2000; Rouse and Pleijel 2001).

Polychaetes are very important members of marine communities in several ways. Firstly, Polychaetes have a high reproductive potential and they can reach very high densities in some areas. For example, Lerberg et al (2000) recorded densities of over 2400 individuals per m^2, for *Streblospio benedicti*. At these densities, polychaetes can contribute to over half of the total biomass in such areas. Consequently, polychaetes play a large role in nutrient cycling (e.g. through digestion), with many species consuming organic particles, through faecal deposition, and when dead, nutrients are released back into the water column. Nutrient cycling is also facilitated by the process of burrowing and tube building in soft sediment by polychaetes, which also increases water movement through the sediment. This effectively aerates the mud to a depth that in most cases would

normally be anaerobic. Waldbusser and Marinelli (2006) found that the polychaete *Abarenicola pacifica* actively increased the advection of porewater through the sediment during feeding by mobilizing the sediment above the feeding area at the tube base and subducting surface material downward. Many soft-bodied polychaete species are also a source of food for larger predators such as fish, that dig in the sediment for polychaetes, crustaceans, and bivalves (Pallaoro et al. 2006). In some areas, polychaetes have been found to be some fish species main food source (Laffaille et al. 2005), and have also been found as part of the diet of cuttlefish (Alves et al. 2006).

Polychaetes affect benthic community structure, as found by Callaway (2006), where in high and low densities (even singular tubes) of the species *Lanice conchilega* caused species richness and abundance to increase compared to areas lacking this polychaete species. Large reefs of these tubes build up in some areas, altering sediment properties, offering refuge, and provide a settlement surface for larvae and small organisms. Such a reef occurs at Monaco in Nelson, New Zealand, and is formed by the sedentary tube-building polychaete *Saballaria kaiparaensis* (see Figure 2.10 in section 2). The soft reef structure provides refuge for many other marine organisms, including other polychaetes such as *Lepidonotus polychroma* and *Perinereis novae hollandiae*. Aeration of the surface sediments through burrowing also allows other sediment dwelling species to subsist in the same area, when they may not otherwise, due to anoxic conditions. Polychaetes affect community recovery time after large-scale disturbances, with species returning in < 1 year, compared to other longer-lived species (Kaiser et al. 2006).

The ability to accurately identify polychaetes is essential for a number of reasons. Firstly, Polychaetes are used as biological monitors, with the presence or absence of species and the increase/decline of a population of species sensitive to polluting factors indicating environmental health. Accurate species identification increases biological security; estuaries appear to be particularly vulnerable to invasions (Wolff 1973, 1999, in Bilton et al. (2002)), where most introductions occur through ballast water transport in ships or through accidental introduction with new aquacultural species. Some species become invasive when introduced to non-native ecosystems, and adverse effects of such invasive species on the local environment includes competition with and/or predation of local indigenous species, and detrimental effects on aquacultural developments. For example, the introduction and population establishment of the polychaete *Marenzelleria* cf. *wireni* in the Dutch Wadden Sea was most likely contributed to shipping activity, and may have caused the decline in local bivalve populations and the resident population of the polychaete *Nereis diversicolor* (Essink and Dekker 2002). Also, species of *Polydora* and *Boccardia* (Read 2001) burrow and cause blistering in the shells of cultivated species such as the Pacific oyster (*Crassostrea gigas*), the green-lipped mussel (*Perna canaliculus*), and the cockle (*Austrovenus stutchburyii*) in New Zealand. The economic consequences of damage such as caused by these burrowing polychaetes in shellfish has previously caused the decline of up to 20% in marketable Pacific Oysters (Handley 1995). Bishop and Peterson (2005) found that the polychaete sp of *Polydora* caused blistering on the shells on 84% to 97% of the oyster *Crassostrea ariakensis* in the United States. The occurrence of such damage caused the entire oyster harvest to be marketed in the low profit 'shucked' category, instead of the high-valued and high profit 'half shell'.

Polychaetes are an economically viable species in some countries. Baitworm fisheries in the U.S.A. generate huge revenue annually, consisting mainly of species of *Nereid* and *Glycerid* (or bloodworm) species of polychaetes. Some are grown aquaculturally (Olive 1999), but most are harvested from the wild. Using species identification to monitor levels of wild populations and keep record of what species are currently present in these environments may help prevent declines in population biomass, like that which occurred in Maine in the 1990's. That specific decline may have been caused by competition from an introduced species (Brown 1993). Invasive polychaete species may also be disease vectors, carrying infections in to new areas and passing disease onto indigenous species. Recent research by Huchette et al (2006) suggests that some shell-boring polychaete species may carry a disease that has been infecting abalone in Australia.

In a recent effort, the Ministry of Fisheries has acknowledged that ballast waters in shipping pose a risk of invasion from new species. As a result, they have composed a list of species thought to be current threats to New Zealand communities, including five species of polychaete worm: *Boccardia proboscidea*, *Neanthes succinea*, *Polydora ciliate*, *Polydora ligni*, and *Sabella spallanzanii* (Mrez 2002). The ability to detect an invading polychaete species early would be advantageous to the preservation of the ecosystem. Some of the species that make up the current New Zealand polychaete biota are ones that have been introduced in the past and have become established, including the *Polydora* species (Cranfield et al. 1998), *Glycera americana*, *Axiothella quadrimaculata*, *Owenia fusiformis*, and several *Nereid* species (Glasby and Alvarez 1999); as little as 20 percent of polychaete species found in New Zealand may be endemic.

1.2 Morphological identification of polychaete species:

Identification of some estuarine polychaete species may have been hampered by their morphological similarity to their fully marine counterparts (Bilton et al. 2002). As such important taxa in the environment, polychaetes are often used to model pollutant effects. Geracitano et al (2004) showed that acute exposures to copper caused morphological and histological anomalies in *Laonereis acuta* (Polychaeta: Nereididae). This could cause problems as morphological changes may affect identification, especially when morphological identifications can be difficult already. Furthermore, the possibility of cryptic taxa, which has been found in some species, may also be problematic. Unfortunately, most methods of taxonomic identification rely heavily on morphological characters. Studies of morphological structures to assign taxonomic identifications can be tedious and can lead to misidentification in cryptic species, and most species morphology is described in detail in the adult stages only. It is well understood that in the area of taxonomy that there is a need for finding new and fast methods for identifying species, and to aid in the discovery of new species. Presently, there are too few taxonomic specialists, with species becoming extinct more rapidly than can be catalogued. The inability to correctly identify species impedes ecological research, including the areas of comparative ecology and biological diversity analysis (Hebert et al. 2003), and an accurate identification system is essential for accurate biological diversity assessments.

The Nereididae are one particular family of polychaetes that are considered one of the most cryptic, with many species complexes all over the world. Morphological

identifications of invertebrates usually focuses on a specific character of the invertebrate body, such as coloration patterns, the structure of wings in wasps (Yu et al. 1992), legs, head and mouth-part arrangement, and genetalia as in spiders (Jocque 2002). Differentiation in these structures can be ambiguous and it can be hard to distinguish between species. Many cryptic species delineation depends on very specific complex structural components whose identification requires close and time-consuming viewing of structure under the microscope. In the Neredidae, structures have been used such as parapodial and chaetal organization (Bakken 2002; Sato and Nakashima 2003), and the number and arrangement of paragnaths on the eversible pharynx (Fiege and Damme 2002; Breton et al. 2004; Bakken and Wilson 2005). Some of the structures used in identification are very small and fragile, such as the parapodia on many species of polychaete. Identification sometimes involves viewing these structures on a slide under a microscope, and any damage to these structures either before collection or during removal will hinder identification.

1.3 Molecular identification and its application to polychaete worms:

With the increased access to genetic techniques the detection of potentially cryptic species complexes within recognized morphological species is now commonplace (Hateley et al. 1992; Abbiati and Maltagliati 1996; Röhner et al. 1997; Sato and Masuda 1997; Manchenko and Radashevsky 1998; Maltagliati et al. 2000; Scaps et al. 2000; Maltagliati et al. 2001; Sato and Nakashima 2003). Genetic differentiation between polychaete species has been shown through systems such as horizontal starch gel electrophoresis to determine allele frequencies (Sato and Masuda 1997); RAPD-PCR fingerprinting analysis (Williams et al. 1990;

Westheide and Schmidt 2003); and electrophoretic analysis of allozymes loci (Maltagliati et al. 2000; Maltagliati et al. 2001). Sato (1999) found evidence of a cryptic species in a complex of Nereididae, with allele substitutions at several isozyme loci detected by electrophoretic analysis. These species were in every morphological respect identical, although further studies revealed significant differences in reproductive behaviour and in egg size. In this case, using genetic differentiation in identifying them as separate species was correct. In such a case of cryptic species, it is doubtful an unspecialized researcher will be able to accurately identify these species morphologically.

When analysed together, the differences between RAPD's and Allozymes are small and most are not statistically significant (Aagaard et al. 1998), and although these methods can show genetic variability between populations and closely species (Stevens et al. 2002; Stevens and Hogg 2003) and have been used frequently to study populations of many polychaete species (Abbiati and Maltagliati 1996; Sato and Masuda 1997; Manchenko and Radashevsky 1998; von Soosten et al. 1998; Maltagliati et al. 2000; Maltagliati et al. 2001; Westheide and Schmidt 2003) neither are suitable for a genetically-based identification system. An accurate genetic ID system would have to be based on a sequence of DNA that is easily attainable and readily analysed. Other molecular techniques have been suggested as a possible alternative to morphological identifications, and genetic barcoding may be one viable solution to this problem.

Hebert et al. (2002) had suggested a section of the mitochondrial DNA gene cytochrome-c oxidase subunit I (COI). Once sequenced, this gene fragment could be used as a 'barcode' to distinguish between species. COI is the best candidate

for this taxonomic tool, as it has a high degree of conservation and insertions and deletions are rare (Moritz and Cicero 2004). It also has many rapidly evolving nucleotide sites, which will allow for differentiation between even recently evolved species (Nylander et al. 1999). Compared to the nuclear genome, the mitochondrial genome lacks introns, has had restricted exposure to recombination, and has a haploid mode of inheritance (Saccone et al. 1999). Hebert et al. (2003) demonstrated that the presence of high level of diversity between species sequences allowed for the successful assignment of 98% of samples of cryptic lepidopteran species. mtDNA sequences divergences have also been successfully used to distinguish between species of North American birds (Hebert et al. 2004b), spiders (Hebert and Barrett 2005), cryptic species of butterflies (Hebert et al. 2004a), mosquitoes (Besansky et al. 2003), leeches (Siddall and Budinoff 2005), springtails (Stevens and Hogg 2003; Hogg and Hebert 2004), beetles (Monaghan et al. 2005), oligochaetes (Nylander et al. 1999), naidid worms (Bely and Wray 2004), extinct moas (Lambert et al. 2005), and various other species of vertebrates and invertebrates (Saccone et al. 1999; Hebert et al. 2002; Hebert et al. 2003).

The ability to use COI to identify species will enable the identification of cryptic and polymorphic (where a single species may exhibit a range of different morphologies) taxa, and also identify and associate individuals of life stages other than adult to their correct species (Schander and Willassen 2005).

1.4 Testing the utility of mtDNA COI sequences for Polychaete worms:

COI fragments have been successfully taken from Polychaete worms previously to study the variation within phylogenies of Nereidiform Polychaetes (Dahlgren et al. 2000), but most studies of Polychaetes using a COI sequence divergence technique are attempting to determine the phylogeny of the studied species, rather than separate between species of morphological similarity. It has not been explored weather DNA Barcoding can be used as an effective identification tool, right down to the species level. High levels of genetic differentiation has been shown between populations of the same Polychaete species (von Soosten et al. 1998; Maltagliati et al. 2001), including the Nereid *Hediste divericolor* (Hateley et al. 1992; Abbiati and Maltagliati 1996; Röhner et al. 1997). It is unknown if such inter-population diversity will affect the use of DNA barcoding to discriminate between species. In this study I will examine several species of Polychaete worms from different areas around New Zealand. This study also has a particular focus on the Nereididae as they are a complex family with many cryptic species.

In this study, I will test the hypothesis that the mtDNA COI gene locus is effective in discriminating morphologically recognised species of Polychaete worms. furthermore, I will test weather: 1) Differences will be found between geographically distant populations of the same species, and if these exceed differences between other species; 2) Interspecific divergences of COI sequences from morphologically identified Polychaete species will exceed levels of intraspecific divergence; 3) Species of morphologically identified species can be resolved into family groups by the COI gene locus; and 4) Differences will be

found among groups predicted to have high levels of cryptic species, such as the Nereididae.

1.5 Thesis outline

The thesis consists of four chapters, starting with the Introduction in Chapter 1, which details the target taxa and the difficulty of morphological identifications, and the need to test the use of a molecular (genetic) identification system. Chapter 2 outlines the methods used in collecting samples of the target taxa, followed by detailing the methods used in the laboratory, in morphological analysis, and DNA extraction and amplification. It then follows on outlining the methods of data analysis used. Chapter 3 deals with the results gained in morphological analysis, with the difficulty in determining some individual type morphologies into species groups. It follows with detailing the results of the molecular analysis, talking about intraspecific and interspecific variation in sequences divergences of COI fragments extracted from the target taxa, then comparing intraspecific and interspecific variation to each other. Chapter 4 discusses the results obtained in chapter 3, starting with the morphological analysis, then the molecular analysis, discussing intraspecific and interspecific variation separately. This is followed by concluding statements, and suggestions for future research.

2 Methods

2.1 Study Areas:

Samples were taken from seven sites mostly around the North Island of New Zealand: Coromandel Harbour, Tauranga Harbour, Manukau Harbour, Raglan Harbour, Napier Harbour, Browns Bay near Wellington, and Nelson Harbour. The locations of these sites in New Zealand are shown in Figure 2.1 below. Study sites were chosen to provide as broad a geographic coverage as possible and considering travel logistics, ease of access to tidal areas, and the presence of mud-flat type sediments within each Harbour. All study areas are relatively sheltered environments, are subject to daily tidal fluctuations, and are brackish water environments.

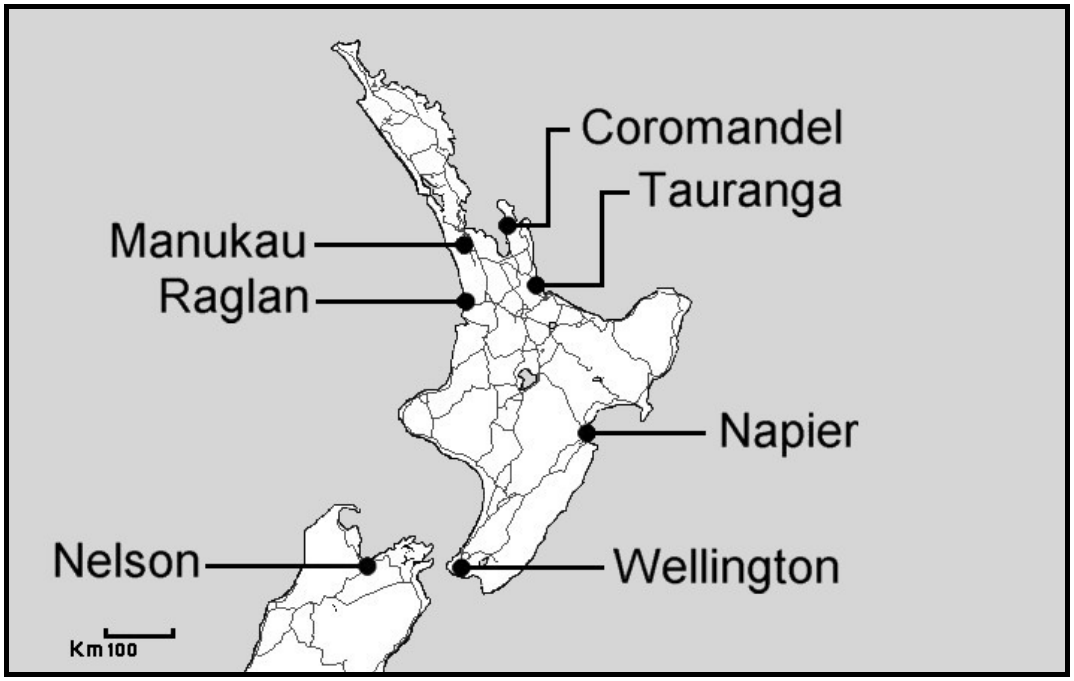

Figure 2.1: Location of study areas on the North Island and northern South Island (Nelson) of New Zealand.

Coromandel Harbour encompasses an area approximately 2.4 km^2, and has a catchment area of steep hills where periodic heavy rainfalls result in heavy silt and clay loads entering the harbour along its many streams including its biggest, the Whangarahi stream. As a result the estuary sediment is soft in many places and the anoxic (black) layer is close to the surface, and is covered in places with large areas of sea-grass. The sampling site is on an extensive mudflat on the southern lobe of the harbour (Figure 2.2).

Tauranga Harbour extends over an area of approximately 189 km^2, and has a catchment which includes the Kaimai mountain range, as well as the large urban area of Tauranga city and outlying suburbs. These areas drain into the harbour via several streams of varying size. This area is subject to flooding only infrequently, and in most areas the sediment is firm with a higher sand content, and the anoxic layer is 10 cm – 25 cm below the surface. Some large areas of sea-grass are present. Tauranga port operates out of Tauranga Harbour, near the city. The area directly around the Port is affected by port operations, with large amounts of organic matter (mainly wood based) and oil products from machinery operation deposited into the water. However, the sampling site was located at the north end of the harbour as shown in Figure 2.3, and so is less affected by these conditions. Some ships visiting Tauranga Port may contain ship ballast water, which poses the risk of introduction of invasive species into Tauranga Harbour.

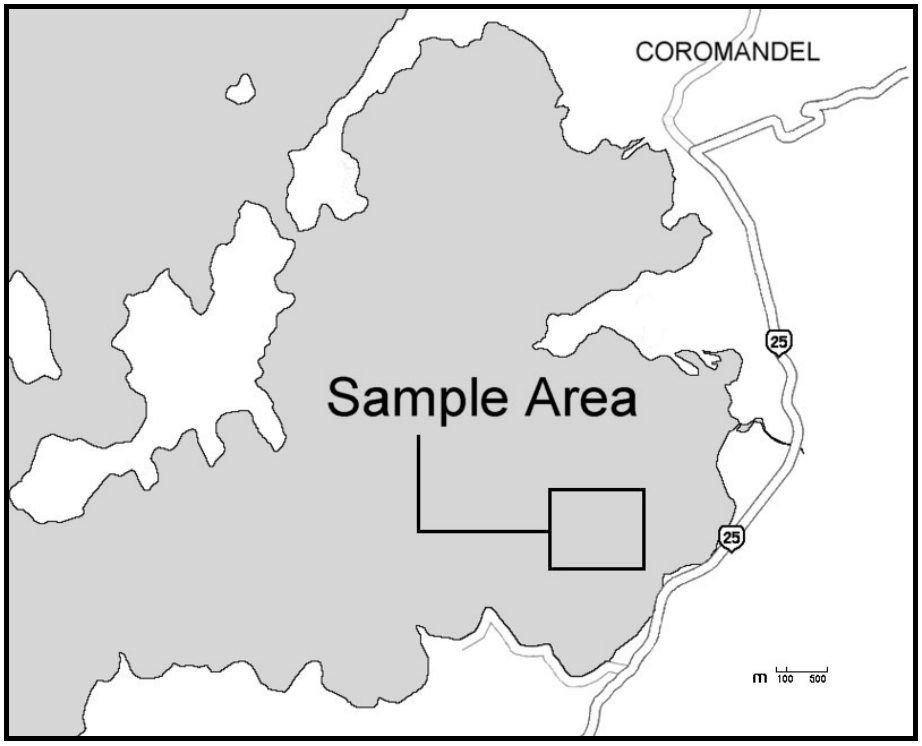

Figure 2.2: Coromandel Harbour showing sampling area.

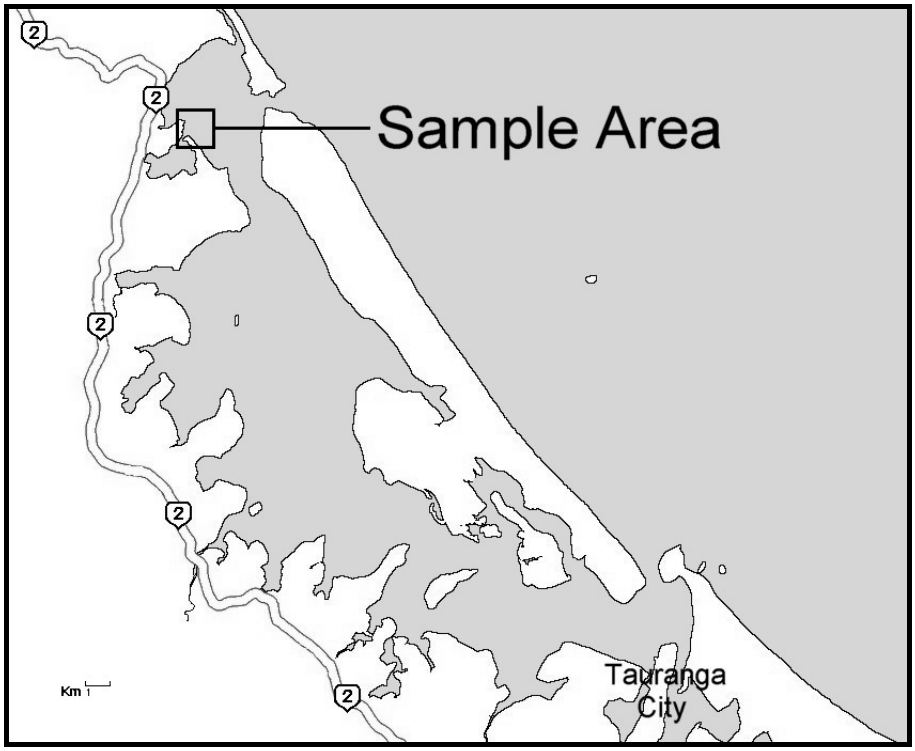

Figure 2.3: Tauranga Harbour, showing sampling area.

Manukau Harbour is located on the west side of Auckland City. It is a very large estuary of approximately 325 km^2, and the sample site is in Weymouth, a region approximately 13 km^2. The harbour is largely influenced by drainage from surrounding farm, rural and urban areas. The sediment at the time of sampling was silty and firm near the shore, but quickly turned to soft mud 20 - 30 m from shore, with no sea-grass in evidence. The sample area in particular is close to State Highway 1 and the city's international airport (Figure 2.4). The area of Manukau Harbour around Weymouth is also subject to the effects of local recreational boating traffic, evident from the numerous small boat-ramps found in the area.

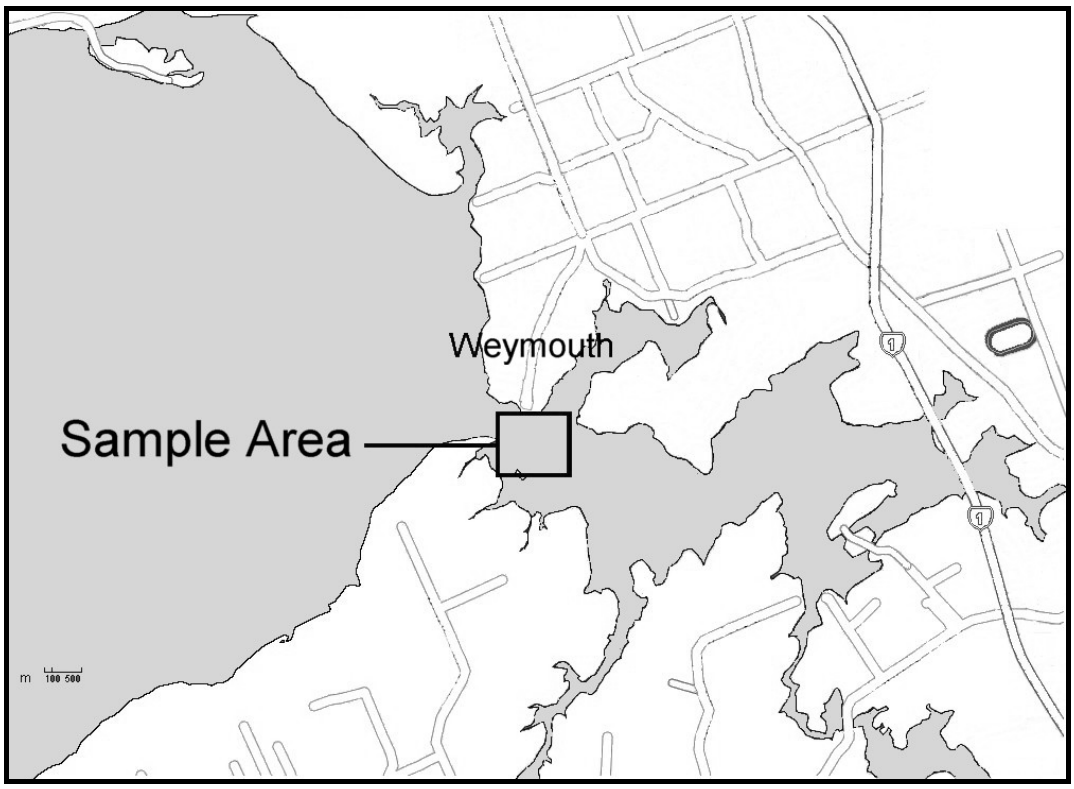

Figure 2.4: Weymouth, Manukau Harbour, showing sampling area.

Raglan Harbour (Figure 2.5) is approximately 30 km^2, and has a catchment consisting of mainly farming land with small patches of forest and urban

township. It contains substrata that is silty and firm in most areas with some small sea-grass beds present. The anoxic layer was approximately 10 – 15 cm below the substrate surface.

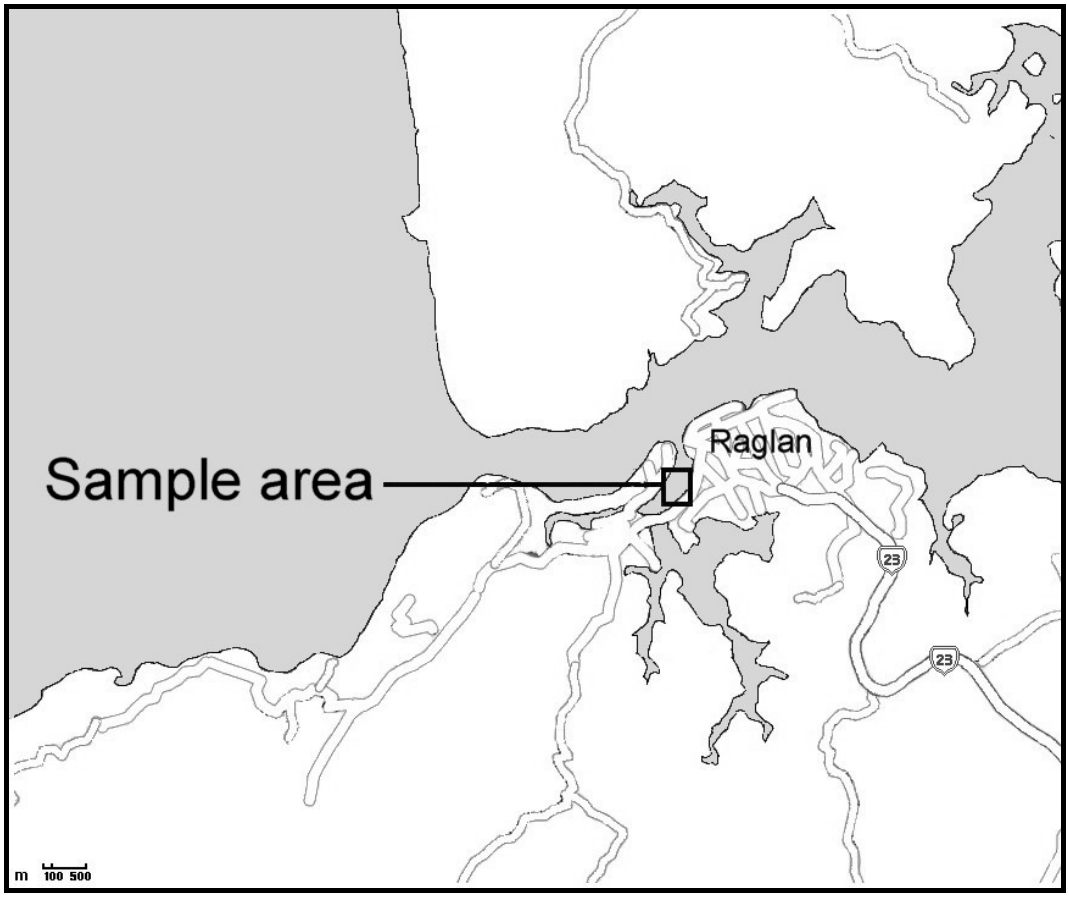

Figure 2.5: Raglan Harbour mouth, showing sampling area.

Napier Harbour consists of a small river outlet approximately 1.25 km^2 (Figure 2.6). The sampling site was located inside the river mouth where the river widens into a small estuary. Accordingly, this sample site may be more affected by fresh water compared to the others. The sediment had a 10 – 15 cm soft layer on top of a firmer layer, with the anoxic (black) layer about 5 cm below that, and no sea-grass was present. Napier Port is located nearby, although effects from this would be reduced as it is located outside the river mouth.

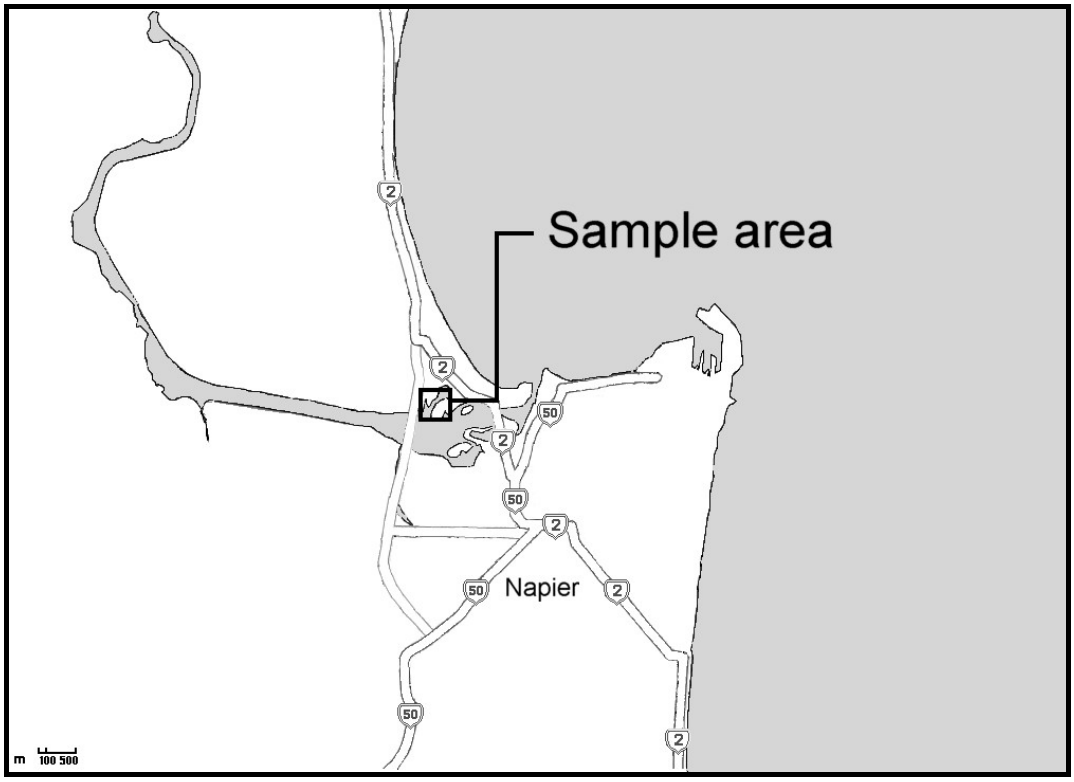

Figure 2.6: Napier Harbour, showing sampling area.

The Wellington sample site in Browns Bay has an approximate area of 7 km^2 (Figure 2.7). The catchment consists mainly of urban township mixed with areas of scrub and farmland. The bay is similar to that of Raglan Harbour, with the sediment at the time of sampling being silty but firm, with the anoxic layer 10 – 15 cm below the substrate surface. Some large beds of sea-grass were present. State Highway 1, an extremely busy motorway, it passes directly over part of the harbour, and it is possible some contamination from motor-travel by-products enters the water.

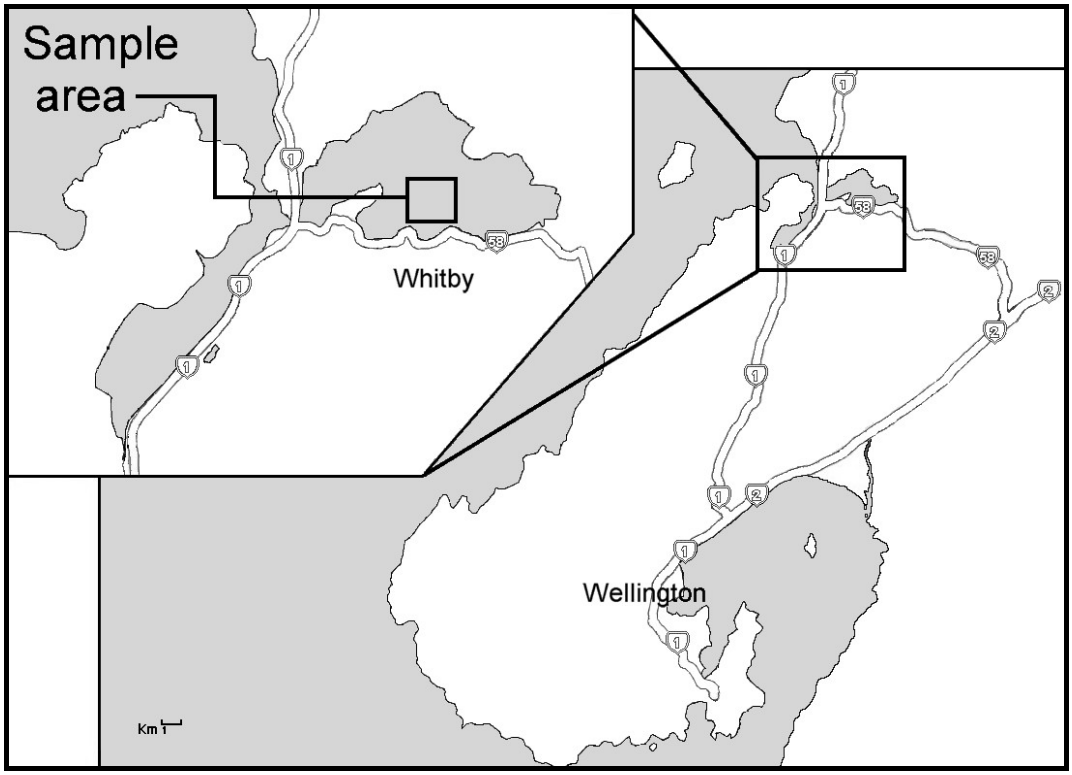

Figure 2.7: Browns Bay, near Wellington, showing sampling area.

Nelson Harbour between Monaco and Rabbit Island has an approximate area of 20 km^2 (Figure 2.8). The catchment consists of the urban area of Nelson city and an area of hills, with numerous streams discharging into the harbour, the largest of which is the Maitai River. The sediment composition and position on the anoxic layer is similar to that found at the Coromandel site, being soft in many places and the anoxic (black) layer is close to the surface, with some sea-grass beds present.

The Port of Nelson is much like at Tauranga, with some ships arriving carrying ballast water. The risk of invasion of estuarine organisms into these areas is therefore high. Marine areas directly around the port are soft, and are affected by port operations including fish offal, wood chips, and oil product deposition into the water.

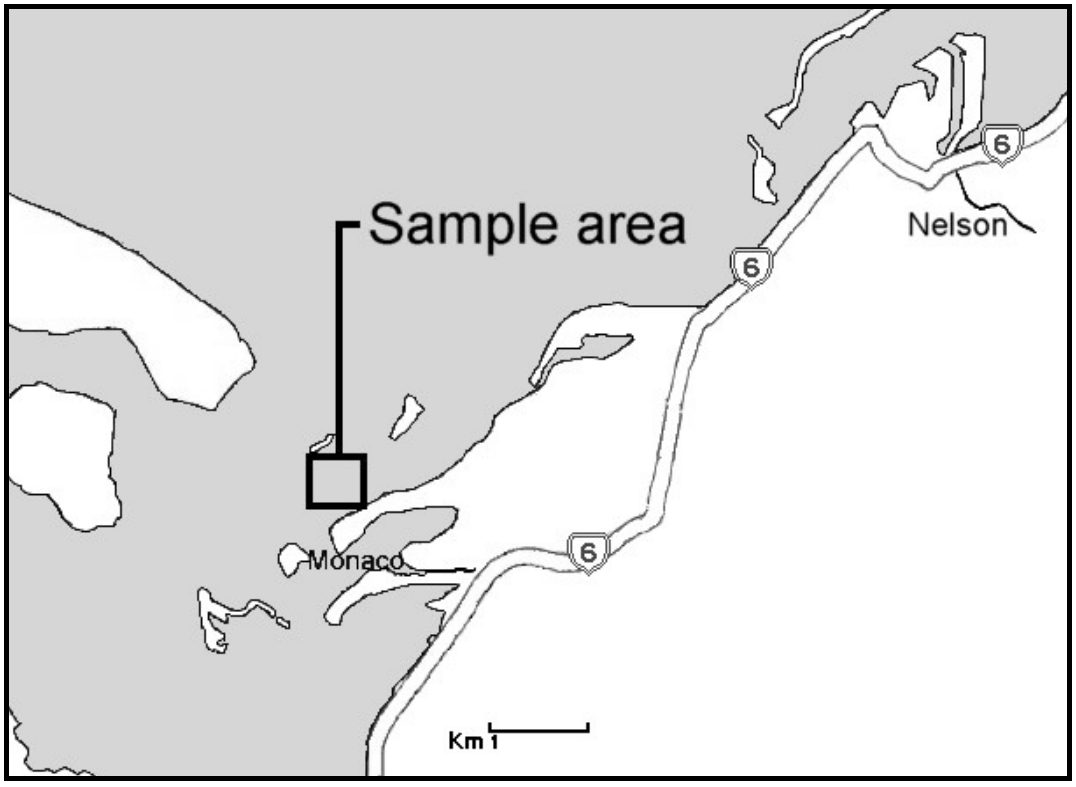

Figure 2.8: Nelson Harbour, showing sampling area.

2.2 Field Methods:

All initial sampling took place in Tauranga Harbour, where soft-sediment sampling methods were tested. Both night trawling of tidal waters and benthic "grabs" using a weighted clamp-jawed sediment sampler proved unsuccessful, while use of a conventional garden spade on sediment exposed during tidal fluctuations worked adequately. Using this method, several sampling events took place over 16 months in Tauranga Harbour. The number of species collected in total increased gradually from the first few initial sampling events, which is shown in Figure 2.9, where the number of new species discovered per additional sampling effort was low.

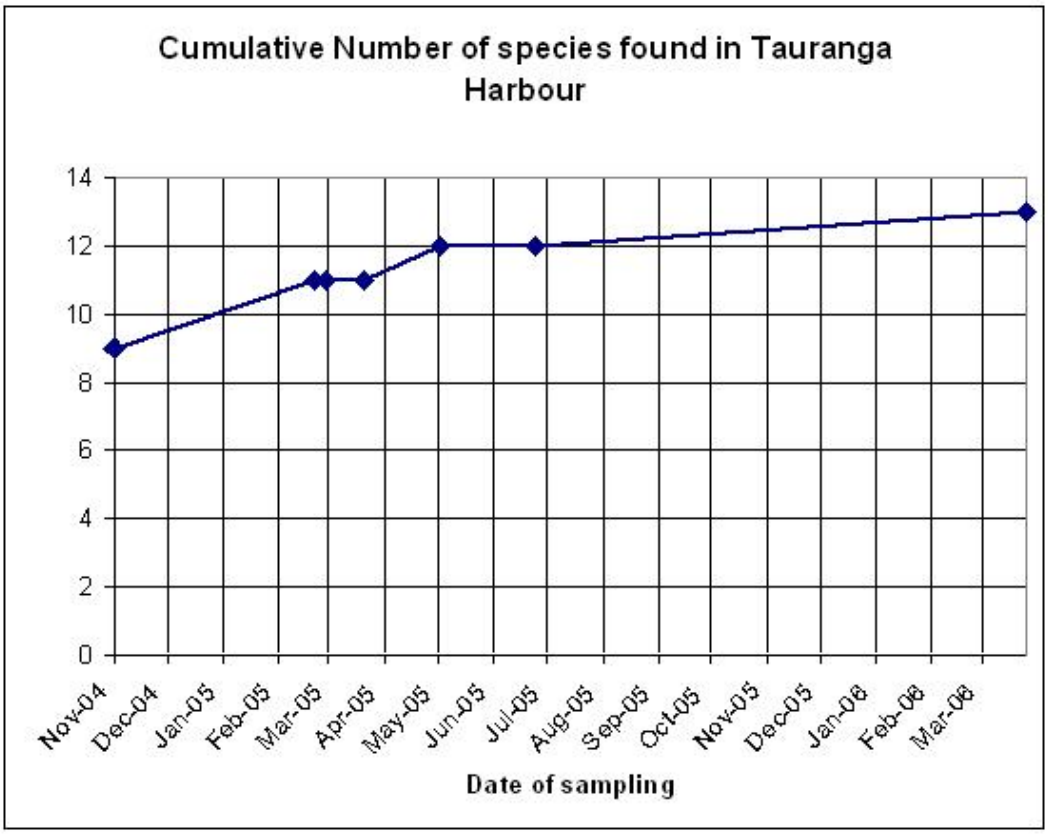

Figure 2.9: Number of species found in Tauranga Harbour over time (sampling effort).

The two new species found in May 05 and April 06 were result of experience gained in locating species at other sites. Therefore, most species sampled could be found with only 1 – 2 sampling events at a site.

Polychaetes were collected from a variety of habitats at each site including soft and hard substrate. Collections from soft substrates such as the one shown in Figure 2.10 were made during low tide followed by cleaning and live sorting in a photographic tray on site. Specimens were either preserved on site using 70 or 95 % ethanol or transported back live to the laboratory in saltwater, as they would routinely survive 24 - 48 hours when contained like this. The latter method proved most useful in species identification (especially for *Nereid* species) as many species require (or are more easily identified with) the proboscis everted. Hard (or rocky) substrate sampling was conducted at anywhere between half and low tide. Some worms were found living in the spaces between live Pacific oysters (*Crassostrea gigas*), or between the tubes of the polychaete *Pomatoceros caeruleus*. These were collected either during the oyster harvesting process or by breaking the tubes off rocks and collecting both the tubeworms and the associated species, followed by sorting back at the laboratory. Figure 2.11 shows some *Pomatoceros caeruleus* tubes (on right) and a Nereid worm (on left), one of types of other polychaete worm collected with them.

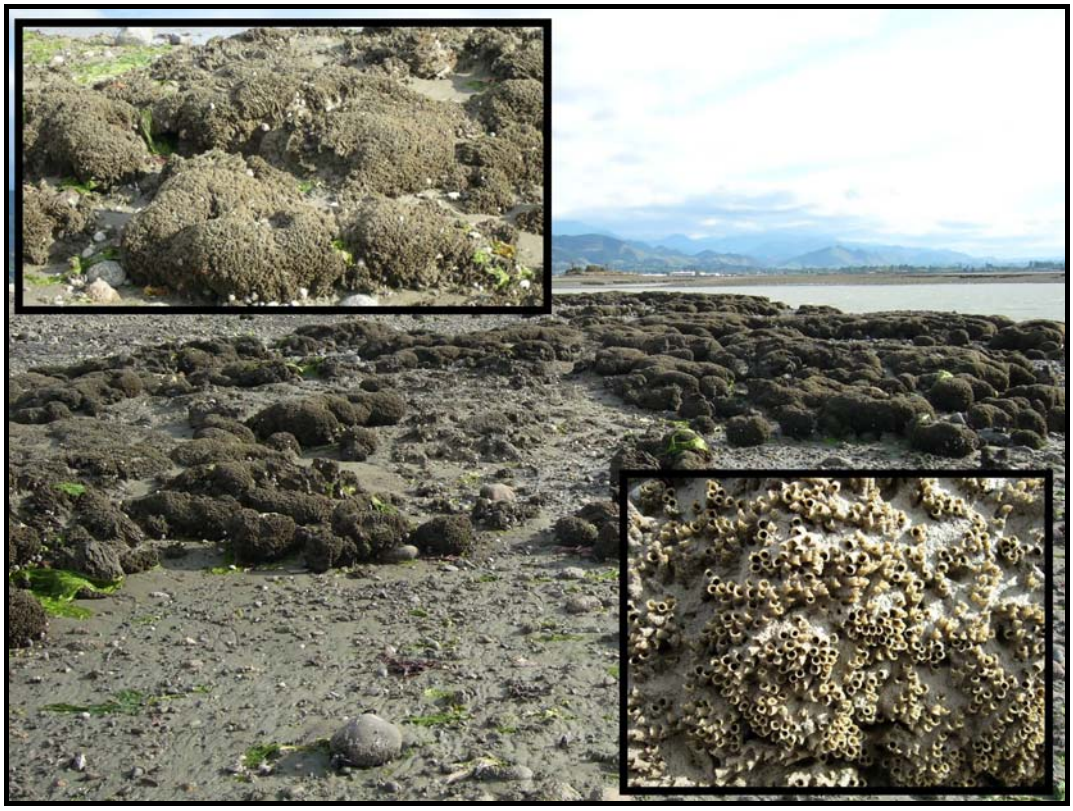

Figure 2.10: Saballaria kaiparaensis tubes forming a large reef at Monaco in Nelson, New Zealand, which is also interspersed with large areas of soft mudflat.

Figure 2.11: Left: an example of a predatory mobile polychaete, a species of Nereid (unidentified) from Napier Harbour, New Zealand; Right: an example of a filter-feeding sedentary polychaete, Pomatoceros caeruleus, its tubes forming on a live farmed Pacific oyster (Crassostrea gigas) in Coromandel Harbour, New Zealand. Mobile species such as the Nereid are often found living between tubes of Pomatoceros caeruleus.

2.3 Morphological Analysis:

All species were identified morphologically prior to DNA analysis. Individuals were separated into glass scintillation vials and preserved in 95 % Ethanol. Live samples were firstly viewed under the microscope to determine morphological features helpful to identification. Species such as *Gylcera americana*, *Aglaophamus macroura*, and all *Nereid* species were made to evert their proboscises by adding a few drops of ethanol to salt water and adding the worms to it and / or, forcing it out by gently squeezing behind the head with a pair of tweezers. This was followed by preservation in 95 % ethanol. Figure 2.12 shows a live individual from the species *Glycera americana*, and Figure 2.13 shows the same individual that has been made to evert its proboscis using a few drops of ethanol. These individuals were then identified using the patterning of chitinous paragnaths on the everted proboscis. Species were identified according to Whitley (1966) and Morton and Miller (1968).

2.4 Molecular Analyses:

For each identified species, up to five individuals were used from each location. Small pieces of tissue were taken from each individual and the DNA extracted using the 'Salting Out Method' (Aljanabi and Martinez 1997). It was found that RnNA-asing did not make any noticeable difference to PCR results. Any RNA-asing was done by adding 0.5 µL of RNA-ase (10 mg / ml) to each sample and incubating in a water bath at 37 °C for one hour.

PCR was carried out using a 10 µL reaction volume consisting of 3.5 µL of Milli-Q distilled water, 1 µL of PCR Buffer (with $MgCl_2$), 2 µL of dNTP's (Boehringer Mannheim), 0.5 µL of 0.005 % BSA (Bovine Serum Albumin), 0.5 µL of each

primers: LCO1490 (5' ggt caa caa atc ata aag ata ttg g 3') and HCO2198 (5' taa act tca ggg tga caa aaa aat ca 3') (Folmer et al. 1994), 0.5 µL of TAQ (1 unit/µL), and 1.5 µL of DNA extract produced from the salting out method. PCR regime was following Folmer et al. (1994): 94 °C for 1 min, 5 cycles of (94 °C for 1 min, 46 °C for 1.5 min, 72 °C for 1 min), 35 cycles of (94 °C for 1 min, 51 °C for 1.5 min, 72 °C for 1 min), followed by 5 min at 72 °C.

PCR product was then run through a 1 % DNA-grade agarose electrophoresis gel made with 1x TBE and 2 µL of Etbr, using 2 µL of PCR product and 2 µL of loading buffer to load each sample into the gel. Successful PCR products showed medium to bright bands, of which the remaining 8 µL of PCR product is then cleaned using the EXOSAP method: 0.1 µL SAP (Shrimp Alkaline Phosphate 1 unit / µL), 0.2 µL EXO I (Exonuclease I 10 units / µL), and 2.7 µL ultra-pure distilled water (Milli-Q water) per sample, and put through a temperature cycle of 30 min @ 37 °C, 15 min @ 80 °C. Samples were then sequenced using the same primers used in PCR amplification (5 µM conc.) on an ICM version 3.1 automated sequencer (MegaBace) at the Waikato DNA sequencing facility, at the University of Waikato. Each sequencing reaction is performed using Applied Biosystems Big Dye v 3.1.

2.5 Data Analysis:

Sequences were aligned using Sequencher (Gene Codes version 4.1.2 for Macintosh) sequence editor. Sequences were then analysed using PAUP* 4.0b10 (Swofford 2002). χ^2 (chi - square) tests were conducted for violation of the assumption of equal base frequencies on all sites, parsimony-informative sites only, and the third codon position only. A neighbour joining (NJ) Phylogenetic

tree was constructed in PAUP*, using the GTR+G+I Akaike model (selected using the program Modeltest 3.5 (Posada and Crandall 1998)), which selected the parameters of –lnL = 7439.2227, K = 9, AIC = 14894.4453; rate matrix: A-C = 1.0000, A–G = 5.6588, A-T = 1.9764, C-G = 1.9764, C-T = 7.4073, G-T = 1.0000; I = 0.3315, G = 0.6972; and base frequencies of A = 0.3000, C = 0.2165, G = 0.1309, T = 0.3526, while all other options in PAUP* remained as default. Nucleotide divergences between locations were calculated in PAUP* using uncorrected distances. Seven polychaete COI sequences were obtained from GenBank (GenBank 1982): two *Pectinaria koreni* (DQ319855 and DQ319840), three *Owenia fusiformis* (DQ319483, DQ319478 and DQ319459), and two *Marenzelleria arctica* (DQ309272 and DQ309269).

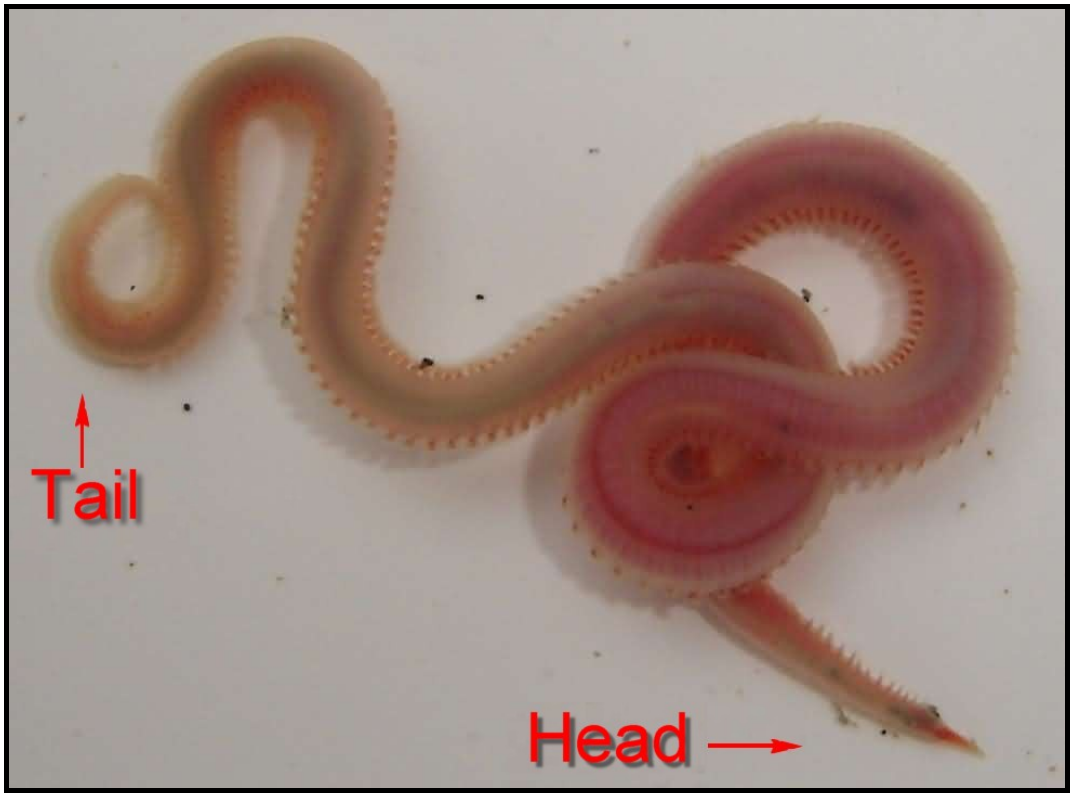

Figure 2.12: A live Glycera americana (the blood-worm) from Manukau Harbour.

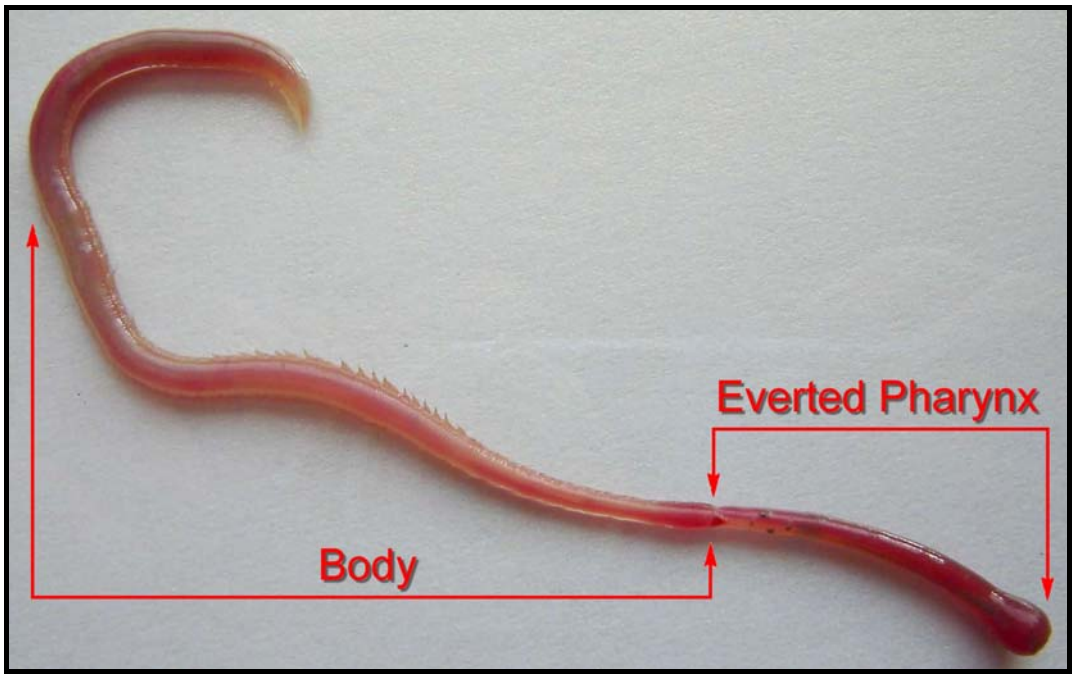

Figure 2.13: The same Glycera americana from above, with pharynx everted.

3 Results

3.1 Morphological Analysis:

Species were morphologically distinguishable, with distinct unambiguous morphological characters (e.g.: *Lepidonotus polychroma*, and *Pomatoceros caeruleus* (see Figure 2.11 in section 2)). In contrast, other species were more difficult as a result of vague descriptions of ambiguous characters in the descriptions. For example, species in the family *Nereididae*, are mainly distinguished by the pattern of chitinous paragnaths on the everted proboscises. However, several individuals collected from both Nelson and Tauranga formed two distinct morphologies, both most closely resembled to *Perinereis nuntia* var. *vallata*, as described in both Morton and Miller (1968) and Whitley (1966). However, they did not match exactly, with individuals from one group missing paragnaths in some areas, and individuals from the other group displaying extra paragnaths in others (Figure 3.1). It was decided therefore to distinguish these groups as *Nereid* sp1, and *Nereid* sp2.

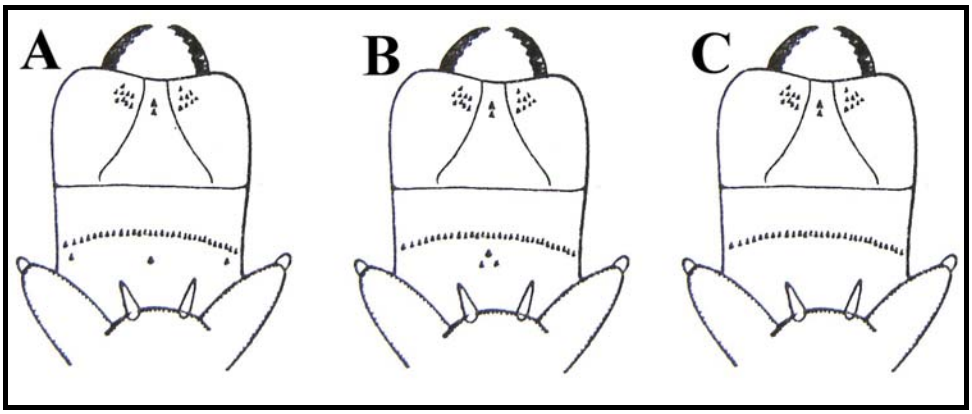

Figure 3.1: Diagram of the head region of three Nereids with proboscises everted. **A**) *Perinereis nuntia var. vallata from Morton and Miller (1968),* **B**) *Nereid* sp1, *from Nelson and Tauranga, and* **C**) *Nereid* sp2 *from Tauranga.*

Another group of individuals from the same family (*Nereididae*) collected from Nelson also showed differences to the closest matching *Nereid* in the two main references used. Accordingly, these individuals were referred to as *Nereid* sp3, and group showed an extra paragnath present at the top-centre of the proboscis compared to *Perinereis camiguinoides*, as well as missing line paragnaths on the lower proboscis. The matching line paragnaths present were also more spaced (Figure 3.2).

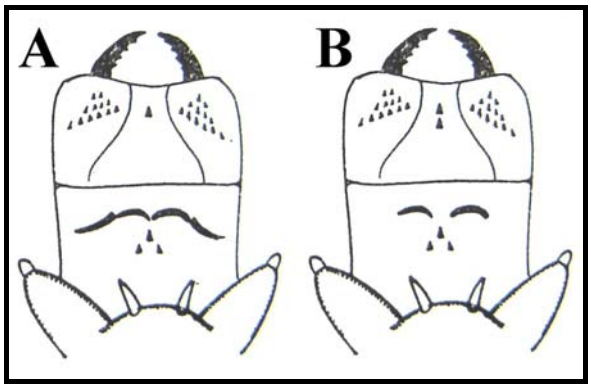

Figure 3.2: Diagram of the head region of two Nereids with proboscises everted. A) Perinereis camiguinoides from Morton and Miller (1968), and B) Nereid sp3 *from Nelson.*

In total, 20 species of polychaete worm were identified using morphological characters, belonging to 16 different families. Of those, 14 species can be found in Tauranga, 6 in Coromandel, 3 in Manukau, 6 in Raglan, 2 in Napier, 2 in Wellington, and 14 in Nelson. Table 3.2 shows a list of the species and their families found at these locations. A total of 14 out of the 20 species were found in more than one sampling location (Table 3.1).

Table 3.1: Polychaete species found in more than one of the sampling locations, where C=Coromandel, T=Tauranga, M=Manukau, R=Raglan, Np=Napier, W=Wellington, and Nn=Nelson,

Aglaophamus macroura (T, R, Nn)	*Pomatoceros caeruleus* (C, T, Np, Nn)
Glycera americana (T, M, R, Nn)	*Axiothella quadrimaculata* (T, W)
Perinereis novae hollandiae (C, N)	*Orbina papillosa* (T, R, M, W, Nn)
Scolecolepis sp (T, M, R, Np, Nn)	*Nereid* sp1 (T, Nn)
Thelepus spectabilis (T, R)	*Eulalia microphylla* (C, Nn)
Nicon aestuariensis (T, Nn)	*Onoscolex pacificus* (C, T)
Pectinaria australis (T, Nn)	*Lepidonotus polychroma* (C, Nn)

Two families had multiple species represented from New Zealand: the *Malanidae* with two species: *Axiothella quadrimaculata* and *Asychis theodori*; and the *Nereididae* with five species: *Nicon aestuariensis*, *Nereid* sp1, *Nereid* sp2, *Nereid* sp3, and *Perinereis novae hollandiae*.

Table 3.2: A list of species found at each sample site (Area) with family, the total number of individuals collected from each species, the number of individuals that sequences were able to be attained from for each species, and the authority(ies) used in aid of identification. Species highlighted yellow are species sequenced, and species highlighted red are species that sequences were unable to be attained from.

Area:	Species:	Family:	Total No.:	Sequenced:	Authority Used:
Coromandel	*Abarenicola assimilis* Wells 1963	Arenicolidae	8	0	Morton & Miller (1968)
	Eulalia microphylla Schmarda 1861	Phyllodocidae	5	4	S. J. Whitley (1966)
	Lepidonotus polychroma Schmarda	Polynoidae	6	3	Morton & Miller (1968)
	Onoscolex pacificus Moore 1909	Scalibregmatidae	4	4	S. J. Whitley (1966)
	Perinereis novae hollandiae Kinberg	Nereididae	6	6	Morton & Miller (1968)
	Pomatoceros caeruleus Schmarda 1861	Surpulidae	10	0	Morton & Miller (1968)
Tauranga	*Asychis theodori* Colville 1926	Malanidae	5	1	Morton & Miller (1968)
	Aglaophamus macroura (sp3)	Nephytidae	2	1	Morton & Miller (1968)
	Axiothella quadrimaculata Augener	Malanidae	4	0	Morton & Miller (1968)
	Glycera americana (sp2)	Glyceridae	8	4	Morton & Miller (1968)
	Nicon aestuariensis Knox 1951	Nereididae	2	1	Morton & Miller (1968)
	Onoscolex pacificus Moore 1909	Scalibregmatidae	5	5	S. J. Whitley (1966)
	Orbina papillosa Ehlers 1907	Aricidae	5	3	S. J. Whitley and Morton & Miller
	Owenia fusiformis delle Chiaja 1841	Oweniidae	5	2	Morton & Miller (1968)
	Pectinaria australis Ehlers 1905	Pectinariidae	3	0	Morton & Miller (1968)
	Nereid sp2	Nereididae	4	4	Morton & Miller (1968)
	Nereid sp1	Nereididae	2	2	Morton & Miller (1968)
	Pomatoceros caeruleus Schmarda 1861	Surpulidae	12	0	Morton & Miller (1968)
	Scolecolepis sp. Blainville 1828	Spionidae	6	0	S. J. Whitley and Morton & Miller
	Thelepus spectabilis Ehlers 1897	Terebellidae	8	0	Morton & Miller (1968)
Manukau	*Glycera americana* (sp1)	Glyceridae	4	4	Morton & Miller (1968)
	Orbina papillosa Ehlers 1907	Aricidae	1	0	S. J. Whitley and Morton & Miller
	Scolecolepis sp. Blainville 1828	Spionidae	3	2	S. J. Whitley and Morton & Miller

Table 3.2: Continued.

Raglan	*Aglaophamus macroura* (sp2)	Nephytidae	1	1	Morton & Miller (1968)
	Axiothella quadrimaculata Augener	Malanidae	5	0	Morton & Miller (1968)
	Glycera americana (sp1 and sp2)	Glyceridae	13	4	Morton & Miller (1968)
	Orbina papillosa Ehlers 1907	Aricidae	5	0	S. J. Whitley and Morton & Miller
	Scolecolepis sp. Blainville 1828	Spionidae	6	0	S. J. Whitley and Morton & Miller
	Thelepus spectabilis Ehlers1897	Terebellidae	6	0	Morton & Miller (1968)
Napier	*Pomatoceros caeruleus* Schmarda 1861	Surpulidae	10	0	Morton & Miller (1968)
	Scolecolepis sp. Blainville 1828	Spionidae	5	2	S. J. Whitley and Morton & Miller
Wellington	*Axiothella quadrimaculata* Augener	Malanidae	6	6	Morton & Miller (1968)
	Orbina papillosa Ehlers 1907	Aricidae	1	1	S. J. Whitley and Morton & Miller
Nelson	*Aglaophamus macroura* (sp1)	Nephytidae	5	5	Morton & Miller (1968)
	Eulalia microphylla Schmarda 1861	Phyllodocidae	5	5	S. J. Whitley (1966)
	Glycera americana (sp1 and sp2)	Glyceridae	6	6	Morton & Miller (1968)
	Lepidonotus polychroma Schmarda	Polynoidae	7	7	Morton & Miller (1968)
	Nicon aestuariensis Knox 1951	Nereididae	4	4	Morton & Miller (1968)
	Orbina papillosa Ehlers 1907	Aricidae	6	5	S. J. Whitley and Morton & Miller
	Owenia fusiformis delle Chiaja 1841	Oweniidae	3	3	Morton & Miller (1968)
	Pectinaria australis Ehlers 1905	Pectinariidae	4	4	Morton & Miller (1968)
	Perinereis novae hollandiae Kinberg	Nereididae	2	0	Morton & Miller (1968)
	Nereid sp1	Nereididae	6	6	Morton & Miller (1968)
	Nereid sp3	Nereididae	2	0	Morton & Miller (1968)
	Pomatoceros caeruleus Schmarda 1861	Surpulidae	13	0	Morton & Miller (1968)
	Sabellaria kaiparaensis Augener 1926	Sabellariidae	12	2	Morton & Miller (1968)
	Scolecolepis sp. Blainville 1828	Spionidae	6	4	S. J. Whitley and Morton & Miller

3.2 Molecular Analysis:

A 543-bp COI fragment was successfully obtained from 111 individuals from 16 of the 20 morphologically identified species (Table 3.2). Sequences were not able to be gained from 4 out of the 20 morphologically recognised species. These species were: *Pomatoceros caeruleus*, *Thelepus spectabilis*, *Abarenicola assimilis*, and *Nereid* sp3. No insertions or stop codons were detected, although one area of deletion was detected in all individuals of *Orbina papillosa* sequenced (from Tauranga, Wellington, and Nelson), where three successive base pairs were missing from the same location in each individual. The nucleotide composition was A = 25 %, T = 33 %, C = 24 %, G = 18 %, somewhat biased towards A-T. In total 221 characters (sites) were constant, with 229 parsimony-informative characters, and 19 parsimony-uninformative characters. The assumption of homogeneity across base frequencies for all sequences was supported for 3rd - codon positions ($\chi^2_{351} = 16.13$, P = 1.00), but was rejected for all sites ($\chi^2_{351} = 1026.43$, P = <0.01) and parsimony informative sites only ($\chi^2_{351} = 1904.75$, P = <0.01). Bootstrap support of the NJ tree was gained for the outermost branches only, leaving intermediate branches unsupported.

3.2.1 Intraspecific Divergences.

Initial average intraspecific divergence was 3.4 %, with a range of 0 % to 32.7 %. The high average and high maximum were contributed to high intraspecific divergence within two species, *Glyera americana* with a range of 0 – 18.9 %, and *Aglaophamus macroura* with a range of 0.6 – 22.4 %. Neither *Glyera americana* or *Aglaophamus macroura* had any individuals fall near an intermediate value;

rather, individuals were grouped into extremes of high and low divergence for each species. All individuals identified as *Glycera americana* and all individuals identified as *Aglaophamus macroura* appeared morphologically identical; however, closer examination of the data revealed that individuals could be separated into two groups for *Glycera americana*, with maximum divergences of 2.8 % and 3.9 %; and three groups for *Aglaophamus macroura*, with a maximum divergence of 2.4 % - as two of the three groups are represented only by a single individual, average divergence could only be calculated for one of these groups (Table 3.3). These values support the possibility of two cryptic species in *Glycera americana*, with one found in Manukau, and the one in Tauranga, and a mixture of the two species found in both Raglan and Nelson. These two cryptic groups will be treated as separates sub-species in this study and are henceforth labeled "*Glycera americana* sp1" and "*Glycera americana* sp2", and are from the family *Glyceridae*. With *Aglaophamus macroura*, the low divergence (0.6 – 2.4 %) found between all individuals from Nelson made up one group, which contrasted with the high divergence between the Nelson individuals and the other two collected from Raglan and Tauranga. The low divergence between the specimens collected from the Nelson population suggests that the high divergence found between the areas is not a normal level for intraspecific divergence and therefore is indicative of more than one cryptic species. The specimens showed no noticeable difference in morphology, and with such low representation from the Raglan and Tauranga areas it is not possible to state that they are a cryptic species complex conclusively. However, given the molecular evidence, in this study the individuals from Nelson, Raglan, and Tauranga will be treated as separate species and consecutively labeled "*Aglaphamus macroura* sp1", "*Aglaphamus macroura* sp2", and "*Aglaphamus macroura* sp3", and are from the family *Nephytidae*.

Initially, 20 species were identified morphologically. With these cryptic species, 23 are recorded.

Table 3.3: Intraspecific divergences in Glycera americana and Aglaophamus macroura before and after cryptic species delineation.

Before separation:	AVG	MIN	MAX
Glycera americana	0.096	0.000	0.189
Aglaophamus macroura	0.139	0.006	0.224
After separation:	AVG	MIN	MAX
Glycera americana sp1	0.013	0.000	0.039
Glycera americana sp2	0.010	0.000	0.028
Aglaophamus macroura sp1	0.01336	0.00557	0.02412

After allowing for the cryptic species found in *Glycera americana* and *Aglaophamus macroura*, maximum divergences within separate populations of polychaetes had a minimum of 0 % and a maximum of 3.7 % (Table 3.7 and Table 3.4). The low values are from *Scolecolepis* sp from Manukau (0 %), *Nereid* sp1 from Tauranga (0 %), and *Nereid* sp1 from Nelson (0.2 %). The high values are from *Glycera americana* sp1 from Nelson (3.7 %), *Owenia fusiformis* from Nelson (3 %), and *Glycera americana* sp2 from Raglan (1.9 %). As not all individuals and species were successfully sequenced, only 9 species were able to be used to test the intra- and inter-population divergence level.

Table 3.4: Intraspecific divergences calculated before and after cryptic species delineation of Glycera americana and Aglaophamus macroura.

	AVG	MIN	MAX
Intraspecific Before	0.032	0.000	0.327
Intraspecific After	0.008	0.000	0.050

The maximum divergences between separate populations had a minimum of 0.3 % and a maximum of 3.5 % (Table 3.5). The high end range of these values were mainly caused by three species, which showed great diversity between the geographically distant populations. These were *Owenia fusiformis* (3.5 % between Nelson and Tauranga), *Glycera americana* sp1 (1.5 % between Nelson and Raglan, and 1.4 % between Nelson and Manukau), *Glycera* sp2 (1.3 % between Raglan and Tauranga and 1.1 % between Raglan and Nelson), and *Nereid* sp1 (1.3 % between Nelson and Tauranga). The other 5 species tested for inter-population divergences in Table 3.5 showed diversity of <1 %, with *Onoscolex pacificus* (0.3 % between Coromandel and Tauranga), *Scolecolepis* sp (0.3 % between Napier and Manukau, and 0.4 % between Nelson and Manukau), and *Lepidonotus polychroma* (0.5 % between Nelson and Coromandel). The overall maximum sequences divergence values within species (Table 3.6) ranged from 0.2 – 5.0 % with an average maximum value of 1.8 %. The low end values are represented by *Axiothella quadrimaculata* (0.2 %), *Nereid* sp2 (0.4 %), *Perinereis novae hollandiae* (0.4 %), and *Sabellaria kaiparaensis* (0.7 %). The high end values are represented by *Owenia fusiformis* (5 %), *Nicon aestuariensis* (4.1 %), and *Glycera americana* sp1 (3.9 %).

The divergences between the *Owenia fusiformis* collected in New Zealand and the sequences gained from GenBank were high (19.1 % between Nelson and GenBank, and 17.9 % between Tauranga and GenBank). The GenBank sequences were taken from worms found in the Northeast Atlantic (information from GenBank), and so this population is therefore more geographically separated from the New Zealand populations more than any other species compared here. With the evidence of high genetic divergence and geographic separation of these

populations, the *Owenia fusiformis* from New Zealand will be treated as a separate species to those sequences gained from GenBank.

Table 3.5: Inter-population variation in sequences divergences:

Glycera americana sp1	Manukau	Raglan
Raglan	0.008	
Nelson	0.014	0.015

Glycera americana sp2	Raglan	Nelson
Nelson	0.011	
Tauranga	0.013	0.007

Scolecolepis sp.	Napier	Nelson
Nelson	0.007	
Manukau	0.003	0.004

Orbina papillosa	Nelson	Wellington
Wellington	0.006	
Tauranga	0.005	0.007

Owenia fusiformis	Tauranga	GenBank
GenBank	0.179	
Nelson	0.035	0.191

Onoscolex pacificus	Coromandel
Tauranga	0.003

Lepidonotus polychroma	Coromandel
Nelson	0.005

Nereid sp1	Nelson
Tauranga	0.013

Eulalia microphylla	Nelson
Coromandel	0.008

Table 3.6: Overall Intraspecific variation in sequences divergences.

	AVG	MIN	MAX
Glycera americana sp1	0.013	0.000	0.039
Glycera americana sp2	0.010	0.000	0.028
Orbina papillosa	0.005	0.000	0.010
Pectinaria australis	0.008	0.002	0.012
Eulalia microphylla	0.007	0.000	0.017
Nereid sp1	0.006	0.000	0.015
Axiothella quadrimaculata	0.001	0.000	0.002
Scolecolepis sp	0.005	0.000	0.017
Sabellaria kaiparaensis	0.007	0.007	0.007
Lepidonotus polychroma	0.004	0.000	0.009
Nicon aestuariensis	0.016	0.000	0.041
Aglaophamus macroura sp1	0.013	0.006	0.024
Perinereis novae hollandiae	0.001	0.000	0.004
Nereid sp2	0.002	0.000	0.004
Onoscolex pacificus	0.003	0.000	0.013
Owenia fusiformis	0.029	0.015	0.050
Marenzelleria arctica (GenBank)	0.167	0.167	0.167
Owenia fusiformis (GenBank)	0.003	0.002	0.004
Pectinaria koreni (GenBank)	0.006	0.006	0.006

Table 3.7: Intra-population sequences divergences of different species.

	AVG	MIN	MAX
Glycera americana sp1 Manukau	0.007	0.002	0.011
Glycera americana sp1 Nelson	0.018	0.004	0.037
Glycera americana sp2 Tauranga	0.005	0.000	0.009
Glycera americana sp2 Raglan	0.014	0.004	0.019
Glycera americana sp2 Nelson	0.004	0.004	0.004
Orbina papillosa Nelson	0.005	0.000	0.010
Orbina papillosa Tauranga	0.006	0.006	0.007
Eulalia microphylla Coromandel	0.005	0.000	0.009
Eulalia microphylla Nelson	0.009	0.004	0.015
Nereid sp1 Tauranga	0.000	0.000	0.000
Nereid sp1 Nelson	0.001	0.000	0.002
Owenia fusiformis Nelson	0.022	0.015	0.030
Owenia fusiformis Tauranga	0.015	0.015	0.015
Owenia fusiformis (GenBank)	0.003	0.002	0.004
Scolecolepis sp Napier	0.006	0.006	0.006
Scolecolepis sp Nelson	0.008	0.002	0.017
Scolecolepis sp Manukau	0.000	0.000	0.000
Lepidonotus polychroma Coromandel	0.005	0.004	0.006
Lepidonotus polychroma Nelson	0.003	0.000	0.009
Onoscolex pacificus Coromandel	0.004	0.000	0.007
Onoscolex pacificus Tauranga	0.003	0.000	0.007

3.2.2 Interspecific Divergences.

Average divergences between polychaete species ranged from 13.8 – 36.8 % with an average of 26.4 %. The lowest divergences occurred between *Nereid* sp1 and *Nereid* sp2 (13.8 %), *Glycera americana* sp1 and *Glycera americana* sp2 (17.2 %), *Marenzelleria arctica* and *Scolecolepis* sp (17.9 %), *Owenia fusiformis* and *Owenia fusiformis* from GenBank (18.6 %), *Nereid* sp2 and *Perinereis novae hollandiae* (19.2 %), and *Aglaophamus macroura* sp1 and *Aglaophamus macroura* sp2 (19.9 %). All other interspecific divergences were over 20 %. The highest divergences occurred between *Owenia fusiformis* and *Onoscolex pacificus* (36.8 %), *Owenia fusiformis* and *Orbina papillosa* (35.4 %), and *Onoscolex pacificus* and *Nereid* sp2 (35.2 %) (Table 3.8).

Three NJ trees were constructed in PAUP*, using uncorrected "p" distances and the parameters for the Akaike model GTR + G + I (as selected using Modeltest 3.5), Figure 3.4 shows a NJ tree of tree length 1831, calculated using all individuals. The CI (consistency index) excluding uninformative sites was 0.3085, and the RI (retention index) was 0.4269. A NJ tree was also constructed using parsimony-informative sites only, which resulted in a tree identical to the one constructed using all sites (Figure 3.6). Figure 3.5 shows a NJ tree constructed using representatives of each species and divergent areas. In Figure 3.4, all individuals from the same morphologically described species were grouped together, even in cases where individuals were collected from different locations. There was no difference in structure between the trees calculated using all site and parsimony-informative sites only. The tree calculated using representative species only showed more optimal familial grouping of the *Malanidae* species, and some

reorganisation of some clades, for example *Onoscolex pacificus* is grouped with *Asychis theodori* in Figure 3.4, while in Figure 3.5 these two have moved and are grouped with *Axiothella quadrimaculata*, a species of the same family as *Asychis theodori*. *Sabellaria kaiparaensis* has also moved, and its intermediate node joins all others species below *Sabellaria kaiparaensis*, except for the two *Spionidae* species. The three *Nereididae* species and their join to *Lepidonotus polycroma* has moved to a lower branching position as well. *Nicon aestuariensis* is still separated from the other *Nereidiae* species, instead joined with the *Aglaophamus macroura* species.

The six families *Nereididae*, *Glyceridae*, *Nephytidae*, *Malanidae*, *Pectinariidae*, and *Spionidae*, were not all grouped together very favourably, with both of the two species in the *Malanidae* and the *Spionidae* well separated and grouped with species from other families. The *Nereididae* showed better results, with all but one of the four species sequenced grouped. The species not grouped with the others, *Nicon aestuariensis*, is grouped with the *Aglaophamus macroura* species of the *Nephytidae* family. The *Glyceridae*, *Pectinariidae*, and *Nephytidae* grouped well, with all species together in their respective families.

The maximum divergences between the species of the same families varied, with the *Spionidae* (*Scolecolepis* sp and *Marenzelleria arctica*) at 17.9 %, the *Malanidae* (*Axiothella quadrimaculata* and *Asychis theodori*) at 24.8 %, the *Glyceridae* (*Glycera americana* sp1 and *Glycera americana* sp2) at 17.2 %, the *Nephytidae* (*Aglaophamus macroura* sp1, *Aglaophamus macroura* sp2, and *Aglaophamus* sp3) at 20.8 %, the *Pectinariidae* (*Pectinaria australis* and

Pectinaria Koreni) at 22.7 %, and the *Nereididae* (*Nicon aestuariensis*, *Nereid* sp1, *Nereid* sp2, and *Perinereis novae hollandiae*) at 24 % (Table 3.8).

Table 3.8: Interspecific divergence matrix for all polychaete species sequenced.

Species:	*G-* Sp1	*G-* Sp2	*O-papil*	*P-austr*	*E-micro*	*N-* Sp1	*A-quad*	*Scol-Sp*	*S-kaip*	*L-poly*	*N-aest*
Glycera americana sp1											
Glycera americana sp2	0.172										
Orbina papillosa	0.269	0.284									
Pectinaria australis	0.277	0.263	0.279								
Eulalia microphylla	0.282	0.273	0.298	0.287							
Nereid sp1	0.225	0.260	0.280	0.256	0.294						
Axiothella quadrimaculata	0.269	0.283	0.270	0.261	0.274	0.253					
Scolecolepis sp	0.257	0.248	0.258	0.258	0.301	0.214	0.244				
Sabellaria kaiparaensis	0.253	0.272	0.304	0.257	0.269	0.255	0.235	0.224			
Lepidonotus polychroma	0.242	0.235	0.285	0.237	0.276	0.221	0.271	0.201	0.237		
Nicon aestuariensis	0.229	0.253	0.255	0.250	0.289	0.219	0.274	0.247	0.253	0.261	
Aglaophamus macroura sp1	0.260	0.249	0.263	0.241	0.270	0.226	0.239	0.244	0.253	0.240	0.234
Aglaophamus macroura sp2	0.257	0.275	0.261	0.255	0.264	0.236	0.258	0.248	0.244	0.248	0.225
Aglaophamus macroura sp3	0.257	0.257	0.267	0.271	0.241	0.240	0.237	0.236	0.241	0.249	0.222
Perinereis novae hollandiae	0.255	0.251	0.285	0.262	0.288	0.214	0.254	0.239	0.262	0.215	0.240
Nereid sp2	0.243	0.241	0.280	0.255	0.296	0.138	0.250	0.224	0.263	0.224	0.219
Onoscolex pacificus	0.294	0.275	0.310	0.292	0.330	0.277	0.269	0.257	0.297	0.301	0.288
Owenia fusiformis	0.305	0.319	0.354	0.337	0.284	0.338	0.318	0.330	0.300	0.328	0.322
Asychis theodori	0.252	0.262	0.276	0.260	0.285	0.283	0.248	0.238	0.246	0.249	0.277
Marenzelleria arctica (Genbank)	0.239	0.248	0.280	0.255	0.285	0.229	0.235	0.179	0.228	0.233	0.250
Owenia fusiformis (Genbank)	0.349	0.333	0.343	0.356	0.271	0.347	0.309	0.334	0.316	0.338	0.312
Pectinaria koreni (Genbank)	0.280	0.283	0.285	0.227	0.301	0.251	0.273	0.239	0.287	0.257	0.273

Minimum interspecific divergences
Maximum interspecific divergences
Intra-family divergences

Table 3.8 Continued.

Species:	*Ag-* Sp1	*Ag-* Sp2	*Ag-* Sp3	*P-n-hol*	*N-* Sp2	*O-pacif*	*O-fusi*	*A-theo*	*M-a(G)*	*O-f(G)*	*P-k(G)*
Glycera americana sp1											
Glycera americana sp2											
Orbina papillosa											
Pectinaria australis											
Eulalia microphylla											
Nereid sp1											
Axiothella quadrimaculata											
Scolecolepis sp											
Sabellaria kaiparaensis											
Lepidonotus polychroma											
Nicon aestuariensis											
Aglaophamus macroura sp1											
Aglaophamus macroura sp2	0.199										
Aglaophamus macroura sp3	0.207	0.208									
Perinereis novae hollandiae	0.266	0.251	0.249								
Nereid sp2	0.236	0.226	0.231	0.192							
Onoscolex pacificus	0.282	0.289	0.297	0.325	0.286						
Owenia fusiformis	0.329	0.333	0.295	0.320	0.352	0.368					
Asychis theodori	0.247	0.276	0.246	0.277	0.283	0.272	0.318				
Marenzelleria arctica (GenBank)	0.239	0.258	0.256	0.245	0.240	0.280	0.318	0.249			
Owenia fusiformis (GenBank)	0.321	0.310	0.291	0.317	0.346	0.379	0.186	0.343	0.317		
Pectinaria koreni (GenBank)	0.261	0.266	0.255	0.284	0.266	0.278	0.312	0.279	0.243	0.336	

Minimum interspecific divergences
Maximum interspecific divergences
Intra-family divergences

3.2.3 Comparing Intra- and interspecific divergences.

Intraspecific divergences were low compared to interspecific divergences, with the maximum intraspecific divergence of 5 % falling well below the minimum interspecific divergence of 13.8 % (Table 3.9). The gap between these values is presented in Figure 3.3, where a histogram shows the clear delineation between the intra- and interspecific divergences. The majority of interspecific divergences were greater than 20 %, - only 2.34 % of interspecific divergences were less than 20 %.

Table 3.9: Summary Table of total average divergences of COI sequences.

	AVG	MIN	MAX
Interspecific Divergences (within)	0.264	0.138	0.368
Intraspecific Divergences (betweer	0.008	0.000	0.050

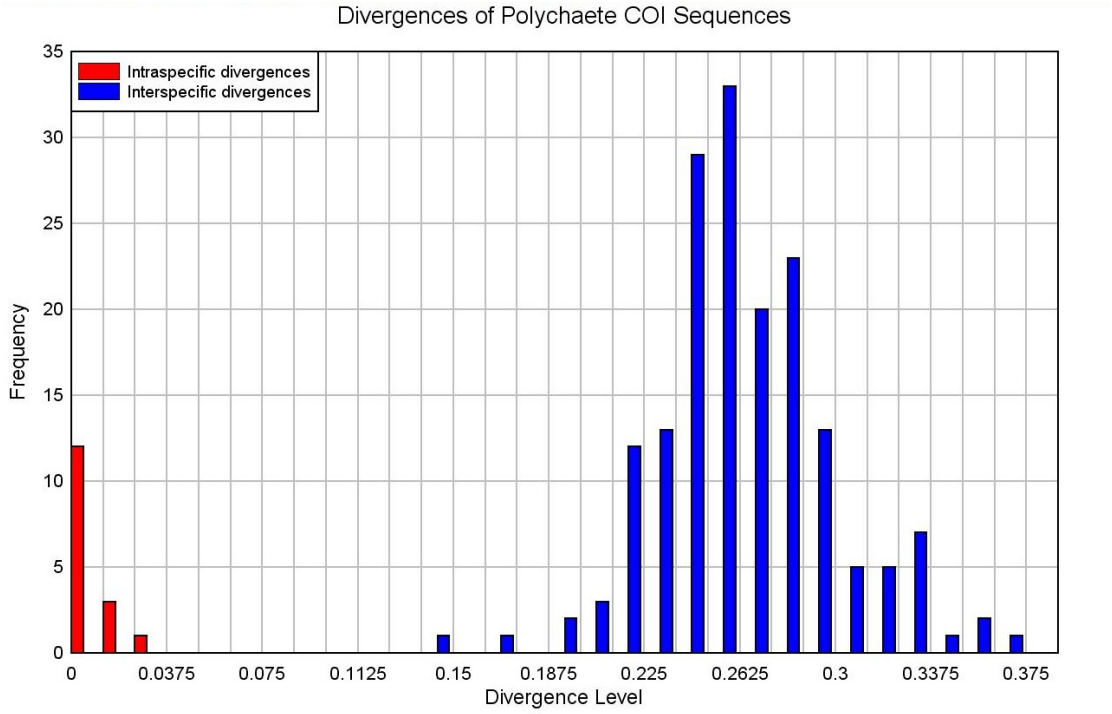

Figure 3.3 Intra- and Interspecific divergences in COI sequences among individuals of polychaete worms.

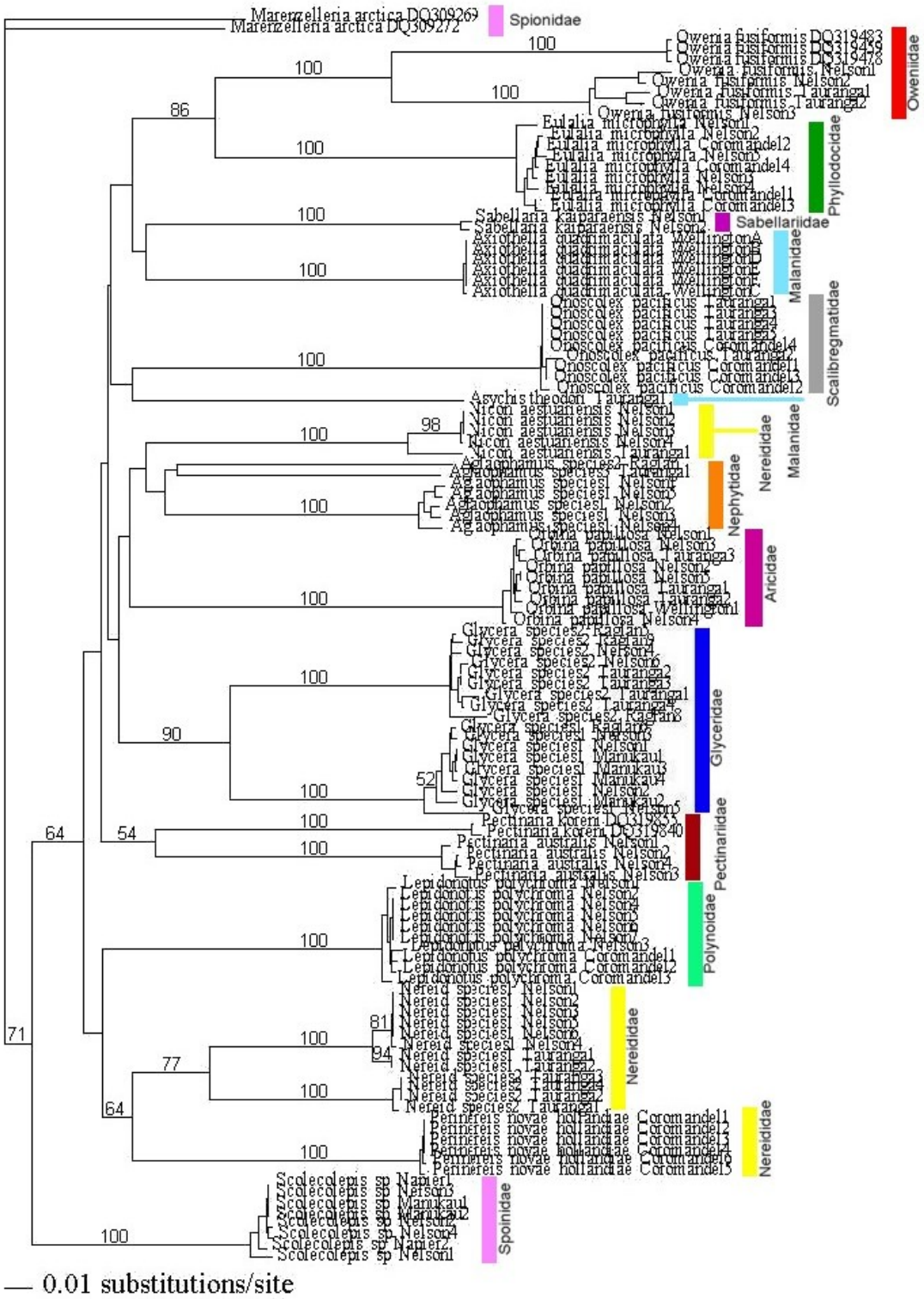

Figure 3.4: Neighbour-Joining tree calculated using uncorrected "p" distances, showing bootstrap values and family groupings.

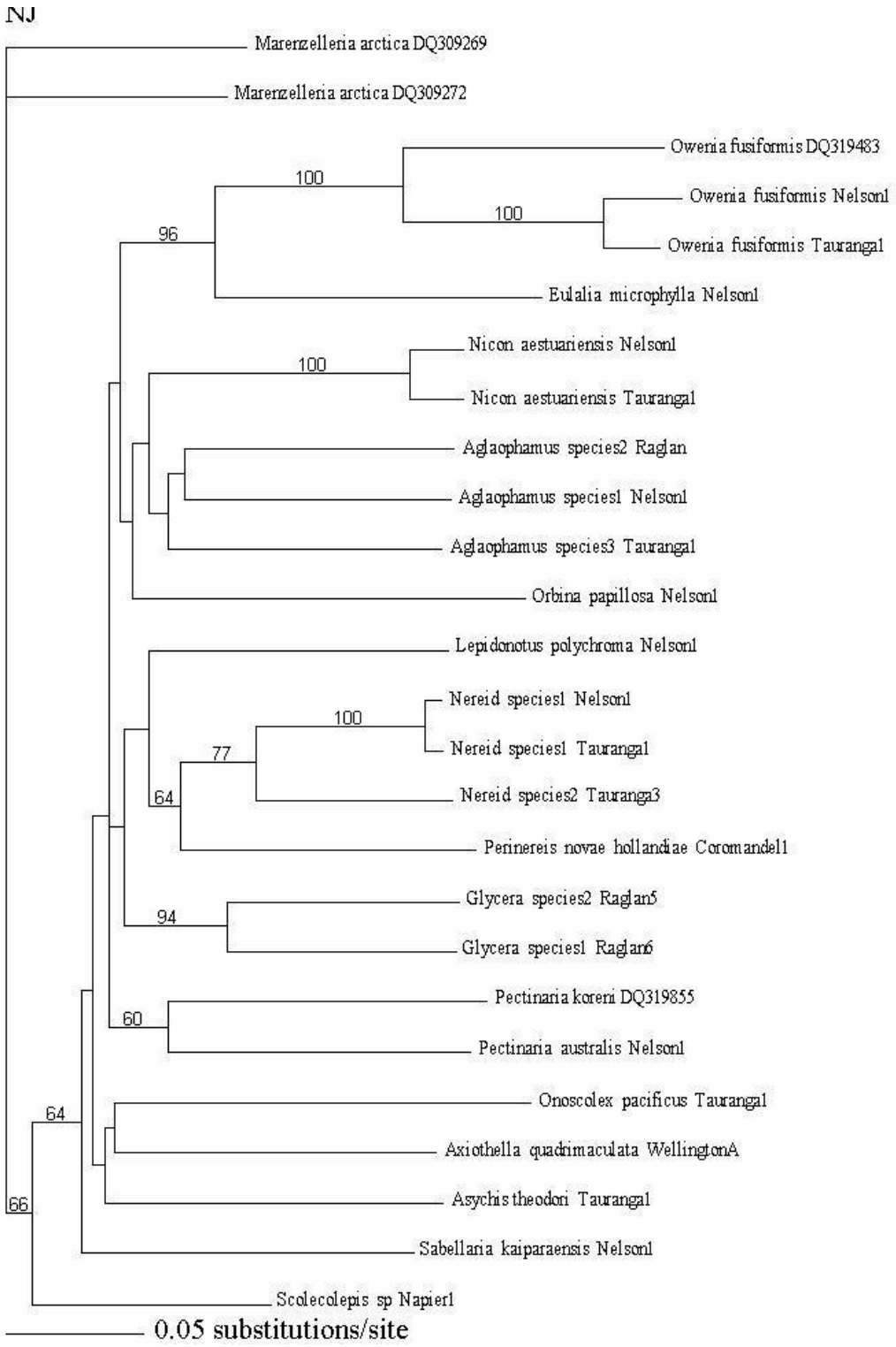

Figure 3.5: Neighbour-Joining (NJ) tree calculated using uncorrected "p" distances, with representative species only and showing bootstrap values.

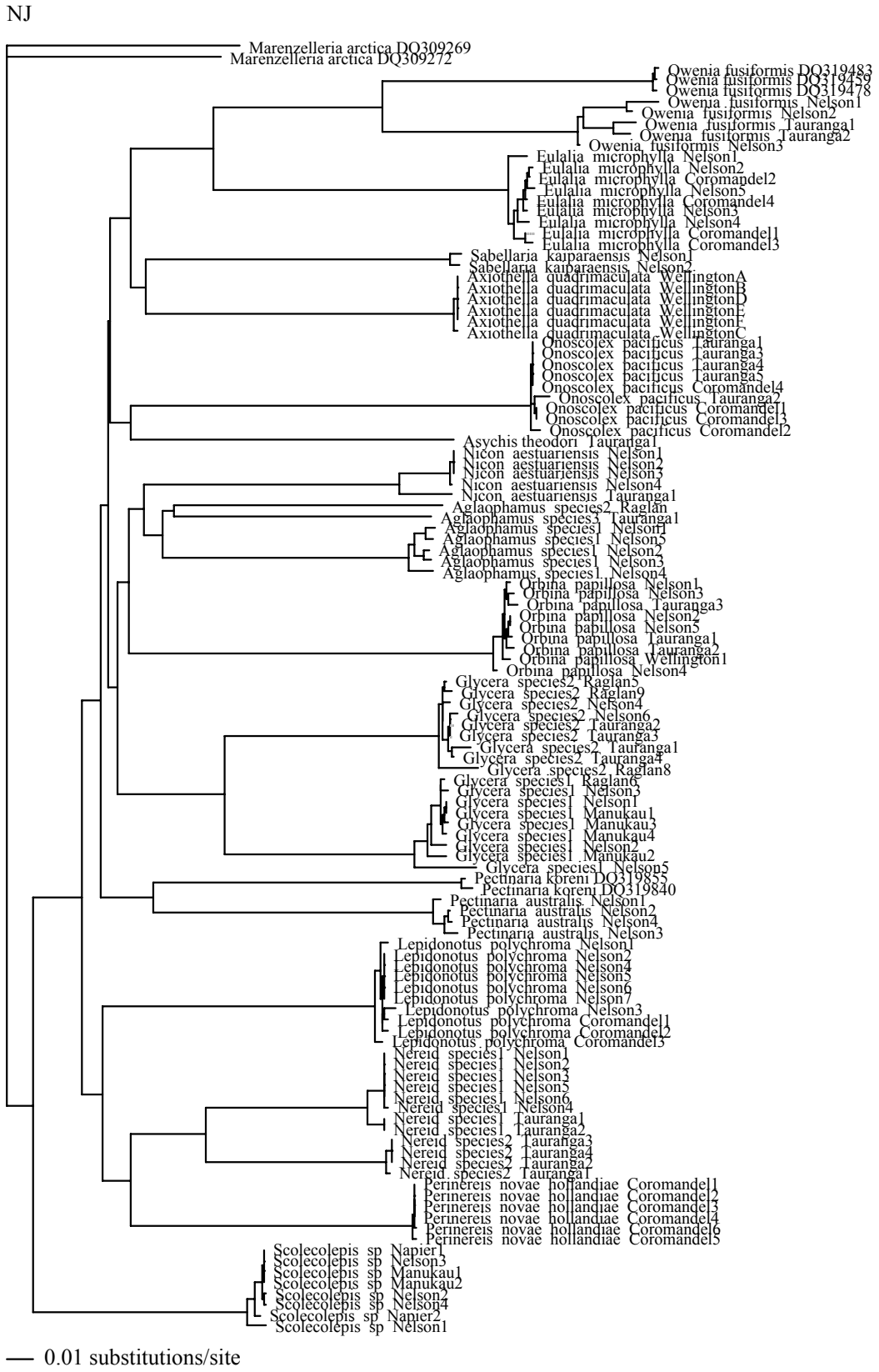

Figure 3.6: NJ tree constructed using uncorrected "p" distances, and using parsimony-informative sites only.

4 Discussion

4.1 Morphological Analysis:

Morphological identifications of species of Polychaete worms was time consuming and somewhat inaccurate, with molecular analysis showing possible misidentifications of at least two different cryptic species. The amount of material available to assist morphological identifications of Polychaetes in New Zealand was limited, and at times unhelpful, with three species from the Nereididae unable to be identified any further than the family level.

4.2 Molecular Analysis:

4.2.1 Intraspecific Divergences:

Intraspecific sequence divergences showed an average of 0.8 % with a maximum of 5 %. Of the 19 species sequenced, the highest divergence was from *Owenia fusiformis* (5 %), collected from two locations of Nelson and Tauranga, and may be the result of the geographic separation between the two populations. However, both populations also show intraspecific divergences of ≤ 3 %. This maximum value is large compared to divergences found in other invertebrates. Several studies on a variety of taxa have found intraspecific divergences of < 1 %. For example, these taxa include butterflies (Hajibabaei et al. 2006), springtails (Hogg and Hebert 2004), leeches (Siddall and Budinoff 2005), North American birds (Hebert et al. 2004b), and extinct New Zealand moa (Lambert et al. 2005). Barrett and Hebert (2005) found a threshold of 2 % successfully delineated species of spiders, similar to Monaghan et al. (2005), with a 2 % maximum for neotropical beetles. The maximum intraspecific level of 5 % for species of New Zealand polychaete worms is more comparable to that found by Smith et al.

(2006), with maximum intraspecific divergence of 3.021 % for parasitoid flies (Diptera: Tachinidae); and to Ball et al. (2005), with a 3.4 % maximum divergence within species of North American mayflies (Ephemeroptera).

4.2.2 Interspecific Divergences:

In contrast to the low intraspecific divergences found for New Zealand polychaetes, interspecific sequences divergences showed an average of 26.4 %, ranging from 13.8 % to 36.8 %. Accordingly, the comparatively small intraspecific divergences are unlikely to confound species boundaries. This similar to Ball et al. (2005), who found that levels of variation between geographically separated populations of the same species were not high enough to complicate species identification.

The lowest divergence (13.8 %) was between two morphologically similar Nereid species (*Nereid* sp1 and *Nereid* sp2). Interspecific divergence of 17.2 % was also found between the two species of *Glycera americana*. Both species were morphologically very similar. Such occurrences are not surprising, as Sato (1999) has previously found a complex of *Nereididae* with genetically distinct species - a conclusion based on examination of behaviour and physical egg size.

The minimum value of 13.8 % is comparable to those of other taxa, with several studies finding levels between 4.5 – 20 %, for example, beetles (Monaghan et al. 2005), springtails (Hogg and Hebert 2004), birds (Hebert et al. 2004b), butterflies (Hajibabaei et al. 2006), mayflies (Ball et al. 2005), and parasitoid flies (Smith et al. 2006). In contrast to the New Zealand polychaete worm species,

other studies of COI have found much lower defining divergence levels of < 2 %, such as spiders (Barrett and Hebert 2005), and extinct moa in New Zealand (Lambert et al. 2005). The total range of interspecific divergence shown in other studies ranges from 1.3 – 19 %, therefore a threshold level of ~ 8 – 10 % for New Zealand polychaete worms would not be unusual.

The mean divergence level of 26.4 % found in this study corresponds to Hebert et al. (2003), where most sequences' divergences (70.3 %) were above 16 %, with a maximum divergence of 32 % for species of Annelids, whereas the maximum divergence found in this study was 36.8 %. Hebert et al. (2003) also found divergences between 4 – 8 %, which is contradictory to the minimum value found in this data, although it is not clear what species have been used to obtain these values. It is possible that the authors encountered a species with multiple phenotypes that confounded their data, or that the data presented here is not an accurate representation of the level of variation present in this taxa. These authors suggest that a mean divergence level of >11 % between different species of various invertebrate taxa was appropriate for discriminating between species. Accordingly, in the case of polychaetes collected in New Zealand, such a level would be suitable, as all interspecific divergences are >13 %.

4.3 Deeper Taxonomic Groups:

The Neighbour-Joining (NJ) tree presented in Figure 3.4 shows individuals from the same species grouped together in the same clades. Some species from the same families have been grouped closely such as the Nereids (excepting *Nicon aestuariensis)*, while other families such as the Malanids (*Asychis theodori*

and *Axiothella quadrimaculata*), and the Spoinidids (*Marenzelleria arctica* (from Genbank) and *Scolecolepis* sp) have not.

Some species of polychaete worm have enough variation between their respective populations to differentiate between them, and have been divided as such on the NJ tree. These species include *Nicon aestuariensis*, *Lepidonotus polychroma*, and *Nereid* sp1. In fact, while the three *Aglaophamus macroura* species appear to be genetically distinct, there is a clear delineation between individuals from the North and South Island, and the two North Island species (sp2 and sp3) group more closely to each other than they do to sp1 from the South Island. This may possibly indicate the dissimilation of a common ancestral species northward, with the Tauranga and Raglan species having diverged more recently, after they were founded from the South Island.

In other species such as *Eulalia microphylla*, *Glycera americana* sp1, *Glycera americana* sp2, *Orbina papillosa*, and *Onoscolex pacificus*, the individuals from the different populations are mixed and cannot be separated at all. It is possible that divergences between species of the same family that are not phylogenetically supported in the NJ tree have reached a divergence upper limit, and continued mutations and evolution of the sequences mostly results in pyrimidine or purine transitions (i.e.: A to T or C to G, or vice versa)(Siddall and Budinoff 2005). This usually has little to no effect on the function of proteins encoded from these areas. In fact, it is possible that after having reached this limit of divergence, a species' sequence can become more like those of a totally different taxonomic species. The more recent branches of the NJ tree are supported by bootstrap values obtained from PAUP. However, values for deeper

phylogenetic divergences were low, and so were not given. This effect is also recorded by Nylander et al. (1999), who studied Oligochaete divergences and genetic phylogenetic relationships using COI and 28s. The authors suggested that the fragment of COI gene had evolved too rapidly to enable a phylogenetic assessment at the higher levels of clitellate and other annelid relationships. The section of 28s DNA resolved deeper nodes better than COI. In contrast, 28s may be a better marker to use for investigation into the genetic taxonomy of polychaetes as it evolves more slowly than COI, which may improve support of inner nodes in the phylogenetic trees. The high divergence shown in the NJ tree is also reflected in the low CI obtained, which is a product of the high amount of parsimony informative sites. Ideally, the number of parsimony informative sites needed for resolution of phlogenetic trees is approximately 20 % of the total number of sites. The number of parsimony informative sites in the Polychaete worms from New Zealand is closer to 50 %.

As also stated by Ball et al. (2005), the use of the COI DNA Barcoding and NJ tree in this study were not intended to infer deeper phylogenetic relationships, and such relationships as may be shown by the tree will have no bearing on the ability of COI sequences to distinguish between species.

4.4 Utility of sequence-based identification of NZ Polychaetes:

The New Zealand polychaete fauna has received limited attention in terms of morphological or taxonomic examination (Glasby and Read 1998). Individual species' distributions have not been recorded in much detail, let alone any possible differences between geographically separated populations. A

comprehensive taxonomic study of species and families present in New Zealand would be required before the utility of this method for Polychaetes can be determined. This is particularly relevant as some species collected for this research were unable to be identified in detail, especially when only very few and old keys are available to aid in identification. Also, Meyer and Paulay (2005) state that the use of thresholds does not bode well for delineating closely related species in taxonomically understudied groups. The promise of sequence-based identification will be realized only if based on solid taxonomic foundations, and that barcoding performs poorly in incompletely sampled groups, with some species poorly represented in this data it would be sensible to re-evaluate after more comprehensive sampling has been done.

It should also be noted that polychaetes can reproduce sexually or asexually, in times of optimal conditions, like the sudden availability of an abundant food source (Glasby et al. 2000; Rouse and Pleijel 2001). It is possible then that individuals sampled from a single area be genetically identical, asexually reproduced, clones. However, the data collected from polychaetes in New Zealand show that very few of the sequences gained from individuals of the same species were identical, and while this has little to no effect on the present data, it may be found in future studies.

DeSalle et al. (2005) noted that two main types of errors existed in DNA barcoding inference: 1) mistaking individual variation for species level variation by using too few individuals and a highly variable gene region; or 2) failing to identify true species differences, by using a conserved gene region sequenced for too few individuals to recover sufficient variation. Only a small fraction of one

small gene is used here to identify between species. It is possible that close genetic divergences between groups of morphologically similar or indistinguishable species, such as the *Glycera americana* and *Aglaophamus macroura* species could be the result of single population where more than one clearly distinct haplotype exists (DeSalle et al. 2005). DeSalle et al. (2005) suggests using multiple sections of DNA combined to construct phylogenetic 'total evidence' trees to infer revolutionary history. The authors proposed a framework, requiring corroboration from more than one line of evidence, which is consistent with current taxonomic practices. This would use 'private' differences in sequence bases as defining characters for group delineation, where certain species' groups have unique base differences to all other groups. Most utilization of DNA sequence-based identification uses distance measures to make inference as to species designation, while classical taxonomy uses character-based delineation. Accordingly, changing to character based identifications may prove to be more compatible with classical taxonomy (DeSalle et al. 2005).

It is possible that variation may be better shown in genes other than COI, although these preliminary results demonstrate that this gene locus shows potential for becoming a reliable standard for polychaete species identification. Research by De Lay et al. (2005) illustrated the use of barcode sequence analysis of nematodes on the D2D3 region of large subunit (LSU) from rDNA (ribosomal DNA), in conjunction with video imagery to identify species. Two polychaete species were also included, revealing intraspecific differences of 0 % to 5.3 %, although interspecific differentiation was not investigated.

Sequences were not obtainable from all species collected and identified: for four species out of 22 (*Pomatoceros caeruleus*, *Abarenicola assimilis*, *Nereid* sp3, and *Thelepus spectabilis*) DNA extraction and PCR amplification failed on several attempts. In the cases of *Abarenicola assimilis* and *Thelepus spectabilis*, it is possible that specimen degradation occurred, especially before the preservation process. However, for *Pomatoceros caeruleus*, many specimens were taken from four different geographic areas, and over multiple sampling excursions, therefore specimen degradation is very unlikely. Even with extractions done immediately and samples taken from fresh, live specimens, not a single sequence was obtainable from 45 separate individuals. Accordingly, it is possible that the primers used (Folmer et al. 1994) do not work for this particular species. Unfortunately time restrictions prevented testing alternative primers, although it would clearly warrant further research. Another possibility is the potential presence of PCR inhibitors in the chemical makeup of this particular species. BSA (Bovine Serum Albumin) is used in the PCR recipe and is an anti-inhibitor, although there could potentially be an inhibitor that BSA is ineffective against.

No species of polychaetes tested from New Zealand demonstrated overlap in intra- and interspecific sequence divergences. However, this is not always true for all animals investigated. For example, Hajibabaei et al. (2006) found overlap in some species of Lepidoptera (butterflies) from Costa Rica. The authors suggest that cases of barcode overlap might signal very recent speciation or hybridisation. Both the two *Glycera americana* and two *Nereid* species found in New Zealand show lower variation than any other species tested, which may indicate that they have diverged more recently. Divergences were still large enough for each to be

distinguished as separate species, and did not overlap with conspecific divergences.

A discouraging aspect to DNA-based identification is that a universal set of criteria does not exist and the divergence cut-off limit will have to be revised from group to group (DeSalle et al. 2005). Another potential problem is the fact that mtDNA is inherited matrilineally, so that any new hybridization between species would not be visible as the offspring inherit the mother's DNA, and the offspring would be identified as the mother's species (Hajibabaei et al. 2006). However, a morphological taxonomic investigation can be made if this is suspected.

The popularity of COI DNA 'barcoding' is increasing rapidly, with mass amounts of invertebrates and vertebrates collected in the field inevitably becoming mass amounts of data to be analysed. With so much data needing to be processed, the 'taxonomic impediment' exists just as much for molecular data as it does for traditional collections (Brower 2006). A vital part of this is the need to find an optimum way of processing these data, and DNA barcoding has been used alone in some instances to infer phylogenies (e.g. Moore (1995)). Brower (2006) reassessed the data from Hebert et al. (2004a) using the same style of analysis of characters as DeSalle et al. (2005), and suggested that the number of cryptic species suggested by Hebert and colleagues was too high, and criticizing the methods of data analysis used in the study to identify new species of butterfly. Prendini (2005) comments on Hebert and Barrett's (2005) use of DNA-based identification of new species of spiders. Many scientists are open to the idea of a universal barcode gene, even while being understandably cautious, with many articles discussing the potential advantages and disadvantages of DNA-based

identification, such as Moritz and Cicero (2004). If DNA-based identifications were to be used in the inference of deeper phylogenetic relationships, it is suggested that it be used in conjunction with traditional taxonomic methods (Tautz et al. 2003; Dayrat 2005; DeSalle et al. 2005).

4.5 Conclusions

The differences found between intra- and interspecific divergences supports the hypothesis that COI sequence divergences could be used to identify species of Polychaete worm. However, more research is needed on these invertebrates in New Zealand. A broader, comprehensive study would assess more fully the viability of sequence-based identification. It is not expected that additional data will cloud or confuse the present statistics and their overall result. It is also essential that the reason why some species are unable to be sequenced be found and resolved, as this method will not work as an effective identification tool if not all species can be sequenced.

Its must also be cautioned that the results found here from COI locus sequence analyses are not intended to indicate that DNA-based identification is meant to replace traditional taxonomic approaches. In fact, DNA-based identification cannot be accomplished without the involvement and expertise of taxonomists who can identify specimens from which reference sequences are obtained. These taxonomists are needed to deal with taxonomic issues resulting from the discovery of provisional species based on significant genetic divergence (Ball et al. 2005). COI locus sequence analysis is only proposed here as a means in which to make identifications of already taxonomically proven species easier. It is an

economically viable option, with prices of sequencing at present at $8 per reaction. Time can be better spent by professional taxonomists in defining and identifying new species than sorting previously established species.

4.6 Future Research

A broader spectrum analysis of species COI sequences to support these findings is desirable, as well as research into why some species were not able to be sequenced, and a possible solution.

An analysis of a different fraction of gene, such as the suggested 28s, should be undertaken to determine deeper phylogenetic relationships that could be revealed by DNA. There is a noticeable lack of morphological information on the New Zealand polychaete fauna.

A broader spectrum COI sequence analysis may also aid in identifying those species that have possible cryptic subspecies requiring more study, such as *Glycera americana*, the Nereids, and *Aglaophamus macroura* in this study.

A comprehensive study on the taxonomy of species present in New Zealand is required, with the compilation of a reliable polychaete taxonomic key. This could be undertaken species by species, and should be done in conjunction with mtDNA COI sequence analysis to help define species boundaries accurately.

Research following this study should start with a detailed investigation of the morphology and distribution of the possible cryptic species of *Glycera americana*, and *Aglaophamus macroura*, as observed in these preliminary results.

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Glossary

Anaerobic
No Air

Anoxic
No oxygen

Ballast
The water taken on by an empty cargo ship to equalize balance during travel

Benthos
Deep sea at or near the sea floor (benthic)

Biomass
The weight of organisms present in a unit of area

Brackish
Water in estuarine environments that is a mixture of fresh and salt

Chaeta
The chitinous bristles of the parapodia of polychaete worms

Chitin
The substance fingernails and hair are made of

COI
Cytochrome c oxidase subunit 1

Conspecific
An organism belonging to the same species as another organism

Congeneric
A species belonging to the same genus as another species

Cryptic
Hard to tell between, specifically between species

Detrimental
Bad

Endemic
Introduced, but so long ago it's almost native

Estuarine
From an estuary environment, in the area of fluctuating salinity when freshwater discharges meet the sea

Euryhaline
Able to tolerate a wide range of salinities

Evert
To turn inside out; turn the inner surface of outward (pertaining to proboscis becoming "everted") (see proboscis below)

Haploid
An organism or cell having only one complete set of chromosomes, like a sperm or ova

Inter-population
Arising or occurring between populations of the same species

Interspecific
Arising or occurring between species

Intra-population
Arising or occurring within a population of the same species

Intraspecific
Arising or occurring within a species; involving the members of one species

Morphological
Pertaining to the physical structure of an organism

Paragnaths
Chitinous teeth (see chitin above)

Parapodia
A paddle like limb

Pelagic
Open waters of the ocean (specifically where an organism lives: "pelagic")

Proboscus
Extendable mouth part that folds in on itself

Appendices

Appendix 1: Laboratory Protocols.

Salting Out DNA Extraction Method:

1. Put pieces of tissue of each sample into correspondingly labeled 1.5mL eppendorf tubes (size and which piece of tissue is dependant on species and size of specimen, E.G. antennae or parapodia or body tissue)
2. Add 25µL of Protienase K (10mg/ml) and 600µL of TNES Extraction Buffer (see below for recipe).
3. Incubate overnight at 37 °C, oscillating at 50 rpm.
4. (Next day) mix briefly using a vortex mixer, then add 350µL of 5M NaCl and shake hard for 15 seconds.
5. Microfuge for 5 minutes at full speed (approx. 14000 rpm).
6. Take liquid off and put into new labeled eppendorf tubes.
7. Add 700µL of cold 100% Ethanol (the ethanol can be added first for ease), then invert the tubes and back again a couple of times to mix.
8. Microfuge for 5 minutes at full speed.
9. Pour the liquid (salt/ethanol mix) out and then microfuge again briefly (10 -20 seconds) to get the remaining liquid to the bottom of the tubes. Remove this with a pipette (50 – 60µL usually).
10. Wash the pellet in the tubes by adding 700µL of 70% Ethanol and mixing round by pumping the ethanol in and out of the pipette.
11. Microfuge for 5 minutes at full speed.
12. Repeat step 9 to remove all ethanol.

13. Air-dry the pellet in the tubes using a speed-vac centrifuge, spinning for 15 minutes at medium speed.
14. Dissolve the DNA pellet in 40µL of Milli-Q distilled water.
15. Store Frozen.

TNES Extraction Buffer:

Tris 1Mol (pH 7.5) (Tris (hydroxymethyl) aminomethane): NaCl 5 Mol: EDTA 500mMol (Ethylenediaminetetraacetic acid): SDS 5% (Sodim Dodecyl Sulfate): Milli-Q distilled water = 1.25:2:1:2.5:18.25.

5x TBE:

108g Tris (buffer grade)

55g Boric acid

8.3g EDTA (di-sodium for everyday use, not molecular grade)

1500 ml distilled water to dissolve in, and adjust pH to 8, and then top up to 2L with distilled water.

Appendix 2: Table of Representative COI sequences of polychaetes from New Zealand.

Marenzelleria arctica DQ309269	- - C - - - - - - - - - - - C - - - - - - - - - - - - - - - - A - - - - - - -
Marenzelleria arctica DQ309272	- - T - T - - - - - - - - - T - - - - - - - - - - - - - - - - C - - C - - - -
Owenia fusiformis DQ319478	- - G G A T - - T - - G T - - - - - - - T - - - - - - - - G - - - - - - - - T -
Pectinaria koreni DQ319855	- - G - - - - - - - - G T - - - - C - - T - - A G - - - - - G - - - - - - A - - - -
Pectinaria australis Nelson 1	- - - - - - - - - - - - - T - - - - - - - T - - - - - - - - - A - - C - - A - - - -
Lepidonotus polycroma Nelson 1	- - - - - - T - - T - - - - - T - - - - - - - - - - - C - - - - - - - - C - - - -
Scolecolepis sp Napier 1	- - - - - - - - - - - G - - T -
Owenia fusiformis Nelson 1	- - G G A T - - - - - - G T - G - - C - - T - - A - - - - - - - - - - - - - - T -
Owenia fusiformis Tauranga 1	- - G G A T - - - - - - G T - G - - C - - T - - A - - - - - - - - - - - - - - T -
Nereid sp1 Nelson 1	- - T - - C - - - - - - - - - - - - - - - - - - C - - C - - - - - A - - C - - - -
Nereid sp1 Tauranga 1	- - T - - C - - - - - - - - - - - - - - - - - - C - - C - - - - - A - - C - - - -
Nereid sp2 Tauranga 1	- - C - - C - - T - - - - - T - - - - - - - - - C - - - - - - - - A - - C - - T -
Nicon aestuariensis Nelson 1	- - C - - C - - T - - G T - - - - C - - T - - C - - - - - - - - - A - - C - - - -
Nicon aestuariensis Tauranga 1	- - C - - - - - T - - G T - - - - C - - T - - C - - - - - - - - - A - - C - - - -
Perinereis novae hollandiae Coromandel 1	- - - - - T - - - - - - - - T - - C - - - - - C - - - - - - - - - C - - - - - T -
Sabellaria kaiparaensis Nelson 1	- - T - - - - - - - - G - - G - - - - - T - - - - - - - - - - - A - - A - - T -
Onoscolex pacificus Tauranga 1	- - G - - G - - - - - C - - - - - C - - - G T - - - - - - G - - - - - C - - - -
Aglaophamus sp2 Raglan 1	- - - - - - - - T - - - T - G - - - - - - - - A - - - - - - A - - - - - - - - - -
Aglaophamus sp1 Nelson 1	- - - - - - - - - - - - - - - T - - - - - - - - A - - - - - - A - - A - - C - - - -
Aglaophamus sp3 Tauranga 1	- - T - - - - - T - - - T - - - - - - - T - - C - - - - - - - - - - - - - - - - -
Orbina papillosa Nelson 1	- - - - - - - - T - - G T - - - - C - - - - - C - - - - - - A - - - - - - - - - -
Glycera sp2 Raglan 5	- - C - - - - - - - - - - - T - - C - - - - - A - - - - - - A - - C - - - - - T -
Glycera sp1 Raglan 6	- - C - - - - - - - - - - T - - - - C - - - - - A - - - - - - - - - A - - - - - T -
Eulalia microphylla Nelson 1	- - - - - G - - T - - G - - G - A - - T -
Axiothella quadrimaculata Wellington A	- - T - - - - - - - - G - - C - - - - - T - - - - - - - - - G - - C - - G - - - -
Asychis theodori Tauranga 1	- - - - - G - - - - - - - T - - - - C - - - - - A - - C - - - - - A - - C - - T -

Marenzelleria arctica DQ309269	- - C - - - - - - - - - - - C - - - - - - - - - - - - - - - - - A - - - - - - -
Marenzelleria arctica DQ309272	- - T - T - - - - - - - - - T - - - - - - - - - - - - - - - - - C - - C - - - -
Owenia fusiformis DQ319478	- - G G A T - - T - - G T - - - - - - - T - - - - - - - - G - - - - - - - - T -
Pectinaria koreni DQ319855	- - G - - - - - - - - G T - - - - C - - T - - A G - - - - - G - - - - - - A - - - -
Pectinaria australis Nelson 1	- - - - - - - - - - - - T - - - - - - - T - - - - - - - - A - - C - - A - - - -
Lepidonotus polycroma Nelson 1	- - - - - T - - T - - - - - T - - - - - - - - - - - - C - - - - - - - - C - - - -
Scolecolepis sp Napier 1	- - - - - - - - - - - - G - - T -
Owenia fusiformis Nelson 1	- - G G A T - - - - - G T - G - - C - - T - - A - - - - - - - - - - - - - - T -
Owenia fusiformis Tauranga 1	- - G G A T - - - - - G T - G - - C - - T - - A - - - - - - - - - - - - - - T -
Nereid sp1 Nelson 1	- - T - - C - - - - - - - - - - - - - - - - - - C - - C - - - - - A - - C - - - -
Nereid sp1 Tauranga 1	- - T - - C - - - - - - - - - - - - - - - - - - C - - C - - - - - A - - C - - - -
Nereid sp2 Tauranga 1	- - C - - C - - T - - - - - T - - - - - - - - - C - - - - - - - - - A - - C - - T -
Nicon aestuariensis Nelson 1	- - C - - C - - T - - G T - - - - C - - T - - C - - - - - - - - - A - - C - - - -
Nicon aestuariensis Tauranga 1	- - C - - - - - T - - G T - - - - C - - T - - C - - - - - - - - - A - - C - - - -
Perinereis novae hollandiae Coromandel 1	- - - - - T - - - - - - - - T - - C - - - - - C - - - - - - - - - C - - - - - T -
Sabellaria kaiparaensis Nelson 1	- - T - - - - - - - - G - - G - - - - - T - - - - - - - - - - - A - - A - - T -
Onoscolex pacificus Tauranga 1	- - G - - G - - - - - C - - - - - C - - - G T - - - - - - G - - - - - - C - - - -
Aglaophamus sp2 Raglan 1	- - - - - - - - - T - - - T - G - - - - - - - - - A - - - - - - A - - - - - - - - -
Aglaophamus sp1 Nelson 1	- - - - - - - - - - - - - - - T - - - - - - - - - A - - - - - - A - - A - - C - - - -
Aglaophamus sp3 Tauranga 1	- - T - - - - - - T - - - T - - - - - - - - T - - C - - - - - - - - - - - - - - - -
Orbina papillosa Nelson 1	- - - - - - - - - T - - G T - - - - C - - - - - - C - - - - - - A - - - - - - - - - -
Glycera sp2 Raglan 5	- - C - - - - - - - - - - - - T - - C - - - - - - A - - - - - - A - - C - - - - - T -
Glycera sp1 Raglan 6	- - C - - - - - - - - - - - T - - - - C - - - - - - A - - - - - - - - - A - - - - - T -
Eulalia microphylla Nelson 1	- - - - - G - - T - - G - - G - A - - T -
Axiothella quadrimaculata Wellington A	- - T - - - - - - - - G - - C - - - - - - T - - - - - - - - - G - - C - - G - - - -
Asychis theodori Tauranga 1	- - - - - G - - - - - - - T - - - - C - - - - - - A - - C - - - - - - A - - C - - T -
Reference bases	G G A A G A G A C C A A C T A T A T A A C A C T A T T G T T A C T G C T C A C G

Marenzelleria arctica DQ309269	T - - T - - - - - - - - - - - - G - - T - - - - - G - - - - - C - - A T - G C - T
Marenzelleria arctica DQ309272	T - - - - - - - - - - - - - - - - T - - - - C - - - - - T - - C - - - T - G C - -
Owenia fusiformis DQ319478	G T - G - - - - - - - - - - - - G T - G - - G - - G - - T - - G A G A - - G T - G
Pectinaria koreni DQ319855	- - - T - - - - - - A T T - - G - - T - - C - - - - - C - - - - - - T - - T - -
Pectinaria australis Nelson 1	C - - - - - - - - G - T C - - - - - - - - T - - - - - - - - - - - G T - - C - C
Lepidonotus polycroma Nelson 1	T - - T - - C - - T - - - - - - - - - - T - - C - - - - - - - - - - - - - - T - -
Scolecolepis sp Napier 1	C - - G - - - - - - - - - - - - - - - - - - - T - - - - - T - - - - - - - - C T - -
Owenia fusiformis Nelson 1	- - - T - - - - - - T - - - - - - - T A G - - G - - G - - T - - - A G - T - G T - G
Owenia fusiformis Tauranga 1	- - - T - - - - - - T - - - - - - - A - T - - G - - G G - T - - G A G - T - G T - G
Nereid sp1 Nelson 1	T - - G - - - - - - T - C - - C T - -
Nereid sp1 Tauranga 1	T - - G - - - - - - T - C - - C T - -
Nereid sp2 Tauranga 1	T - - - - - C - - C - - T - - - - - - - - - - - - - - - T - - C - - C - - - - C - -
Nicon aestuariensis Nelson 1	- - - T - - - - - C - - T - - - - - - - - G - - - - - - - - - - - - - - G C - T
Nicon aestuariensis Tauranga 1	- T - T - - - - - - - - T - - - - - - - - G - - - - - - - - - - - - - - C T - -
Perinereis novae hollandiae Coromandel 1	T - - C - - C - - T - - G - - - T - G - - T - - - - - - G - - - - - - - - - - C - -
Sabellaria kaiparaensis Nelson 1	- - - - - - - - - - T - - T - - - - - - T - - G - - - - - - G - - G - - A - - C C - -
Onoscolex pacificus Tauranga 1	- G - - - - C - - - A T C - - - - - - T - - C - - - - - - - - - - G - - C T - C C - C
Aglaophamus sp2 Raglan 1	- T - - - - - - - - - - C - - - - - - G - - G - - - - - - - - - G G - C - - - C - T
Aglaophamus sp1 Nelson 1	- T - - - - - - - - - - C - - - - - - - - - C - - G - - C - - C - - A - - - - C - -
Aglaophamus sp3 Tauranga 1	- T - T - - - - - - - - - T - G - - - - - C T - -
Orbina papillosa Nelson 1	C - - - G - A - T - - T - - A T - A T - -
Glycera sp2 Raglan 5	- G - - - - - - - - T A - T - A C - -
Glycera sp1 Raglan 6	- - - - - - - - - - T A - T - A C - -
Eulalia microphylla Nelson 1	- T - G - - - - - G - - T - - - - - T - - T - - G - - T - - G G - - T - A T - G
Axiothella quadrimaculata Wellington A	- - - - - - - C - - G A T C - - - T - - - - - - - - G - - C - - G C - - T - - T - -
Asychis theodori Tauranga 1	C - - - - - - - - - - A T - - - - - - T - - C - - - - - - - - - C - - - - - - - C - G
Reference bases	A C T A A T T C G A G C A G A A C T A G G A C A A C C A G G A T C T C T T Y T A

Marenzelleria arctica DQ309269	- - C - - - - - - - - - - - C - - - - - - - - - - - - - - - - - A - - - - - - -
Marenzelleria arctica DQ309272	- - T - T - - - - - - - - - T - - - - - - - - - - - - - - - - - C - - C - - - -
Owenia fusiformis DQ319478	- - G G A T - - T - - G T - - - - - - - T - - - - - - - - G - - - - - - - - T -
Pectinaria koreni DQ319855	- - G - - - - - - - - G T - - - - C - - T - - A G - - - - - G - - - - - - A - - - -
Pectinaria australis Nelson 1	- - - - - - - - - - - - T - - - - - - - T - - - - - - - - A - - C - - A - - - -
Lepidonotus polycroma Nelson 1	- - - - - T - - T - - - - - T - - - - - - - - - - - - C - - - - - - - - C - - - -
Scolecolepis sp Napier 1	- - - - - - - - - - - - G - - T -
Owenia fusiformis Nelson 1	- - G G A T - - - - - G T - G - - C - - T - - A - - - - - - - - - - - - - - T -
Owenia fusiformis Tauranga 1	- - G G A T - - - - - G T - G - - C - - T - - A - - - - - - - - - - - - - - T -
Nereid sp1 Nelson 1	- - T - - C - - - - - - - - - - - - - - - - - - C - - C - - - - - A - - C - - - -
Nereid sp1 Tauranga 1	- - T - - C - - - - - - - - - - - - - - - - - - C - - C - - - - - A - - C - - - -
Nereid sp2 Tauranga 1	- - C - - C - - T - - - - - T - - - - - - - - - C - - - - - - - - - A - - C - - T -
Nicon aestuariensis Nelson 1	- - C - - C - - T - - G T - - - - C - - T - - C - - - - - - - - - A - - C - - - -
Nicon aestuariensis Tauranga 1	- - C - - - - - T - - G T - - - - C - - T - - C - - - - - - - - - A - - C - - - -
Perinereis novae hollandiae Coromandel 1	- - - - - T - - - - - - - - T - - C - - - - - C - - - - - - - - - C - - - - - T -
Sabellaria kaiparaensis Nelson 1	- - T - - - - - - - - G - - G - - - - - T - - - - - - - - - - - A - - A - - T -
Onoscolex pacificus Tauranga 1	- - G - - G - - - - - C - - - - - C - - - G T - - - - - - G - - - - - - C - - - -
Aglaophamus sp2 Raglan 1	- - - - - - - - - T - - - T - G - - - - - - - - - A - - - - - - A - - - - - - - - -
Aglaophamus sp1 Nelson 1	- - - - - - - - - - - - - - - T - - - - - - - - - A - - - - - - A - - A - - C - - - -
Aglaophamus sp3 Tauranga 1	- - T - - - - - - T - - - T - - - - - - - - T - - C - - - - - - - - - - - - - - - -
Orbina papillosa Nelson 1	- - - - - - - - - T - - G T - - - - C - - - - - - C - - - - - - A - - - - - - - - - -
Glycera sp2 Raglan 5	- - C - - - - - - - - - - - - T - - C - - - - - - A - - - - - - A - - C - - - - - T -
Glycera sp1 Raglan 6	- - C - - - - - - - - - - T - - - - C - - - - - - A - - - - - - - - - A - - - - - T -
Eulalia microphylla Nelson 1	- - - - - G - - T - - G - - G - A - - T -
Axiothella quadrimaculata Wellington A	- - T - - - - - - - - G - - C - - - - - - T - - - - - - - - - G - - C - - G - - - -
Asychis theodori Tauranga 1	- - - - - G - - - - - - - T - - - - C - - - - - - A - - C - - - - - - A - - C - - T -
Reference bases	G G A A G A G A C C A A C T A T A T A A C A C T A T T G T T A C T G C T C A C G

Marenzelleria arctica DQ309269	- - - - - - C - - C - - G - - T - - - - - T C - T - - - - - T - - - - - C - - A
Marenzelleria arctica DQ309272	- - - C - - C - - C - - - - - - - - - - - T C - T - - - T - - - - - - - T - - -
Owenia fusiformis DQ319478	- - - - - - - - - C - - T - - T - - - - - T T - A - - - T - G - - - T - G - - G
Pectinaria koreni DQ319855	- - - - - - G - - - - - T - - - - - - - - - - - T - - C T - - - - - - - C - C -
Pectinaria australis Nelson 1	- - - - - - - - - C - - T - - - - - - T - G - - - - - G - - T - - - - - T - C C
Lepidonotus polycroma Nelson 1	- - - A - - - - - - - - - T - - - - - - T - - A - - - - - - - - - - - T - G - - -
Scolecolepis sp Napier 1	- - - C - - G - - - - - T - - - - - - - - C C - A - - C - - - - - G - - T - - -
Owenia fusiformis Nelson 1	- - - T - - T - - - - - - - - T - - G - - T T A A - - G T - G - - - - - - - - -
Owenia fusiformis Tauranga 1	- - - T - - T - - - - - - - - T - - G - - T T - A - - G T - G - - - - - - - - -
Nereid sp1 Nelson 1	- - - A - - - - - - - - - - - - - - - - G - - - - - C - - T - - - - - C - - C
Nereid sp1 Tauranga 1	- - - A - - - - - - - - - - - - - - - - G - - - - - C - - T - - - - - G - - C
Nereid sp2 Tauranga 1	C - - - - - - - - - - - - - - - - - - - T - G - - - - - - - - T - - - T - G - - G
Nicon aestuariensis Nelson 1	C - - - - - - - - - - - T - - - - - - - - T - - A - - - - - T - - - T - - - - A
Nicon aestuariensis Tauranga 1	C - - - - - - - - - - - T - - - - - - - - - - - A - - A - - T - - - T - - - - A
Perinereis novae hollandiae Coromandel 1	- - - - - - - - - C - - T - - T - - - T - - - - - - - - - - T - - - T - G - - -
Sabellaria kaiparaensis Nelson 1	- - - T - - - - - - - - - - T - - G T - - C - - - - A - - T - - - - - T - - G
Onoscolex pacificus Tauranga 1	C - - C - - T - - - - - C - - - - - G - - G - - A - - C T - - - - G - - - - - C
Aglaophamus sp2 Raglan 1	- - - - - - - - - - - - - - - - T - - - - - - - - T - - C - - - - - - - - - - C
Aglaophamus sp1 Nelson 1	- - - T - - - - - C - - - - - T - - - - - - - - - - - - - - - - - G - - - - - G
Aglaophamus sp3 Tauranga 1	- - - T - - G - - - - - - - - - - - - - T - - - - - T - - A T - - - - - - T - - - - - A
Orbina papillosa Nelson 1	- - - A - - - - - - - - - T - - - - - - - - - - - - A - - C - - - - - - - - - - C -
Glycera sp2 Raglan 5	- - - A - - - - - C - - - - - - - - - - - T A - - - - - - - - - - - - - - - - - A
Glycera sp1 Raglan 6	C - - A - - - - - - - - - - - - - - - - - - T A - T - - - T - - - - - - - - - - G
Eulalia microphylla Nelson 1	- - - A - - T - - C - - T - - T - - G T - - - - T - - - T - - - - - G T - - - - - G
Axiothella quadrimaculata Wellington A	- - - C - - G - - - - - G - - T - - - - - T - - - - - A T - - - - - - - - - - - G
Asychis theodori Tauranga 1	- - - T - - C - - - - - G - - - - - - T - - A - - - - C T - - - - - - - - - - - A
Reference bases	T G G G G G A T T T G G A A A C T G A C T A G T C C C T C T A A T A C T A G G T

Marenzelleria arctica DQ309269	- - G - - A - - - - - G - - - - - T - - C - - T - - T - - C - - - - - - - - - - -
Marenzelleria arctica DQ309272	- - - - - A - - C - - G - - - - - - - - G - - T - - - - - - - - C - - - - - - - -
Owenia fusiformis DQ319478	- - - - - T - - - - - - - - - G - - T - - T - - - A - - - - - C - - - - - - G - - - -
Pectinaria koreni DQ319855	- - C - - A - - C - - G - - - - - T - - C - - - A - - - - - - - C - - - - - - G -
Pectinaria australis Nelson 1	- - C - - G - - - - - G - - T - - T - - - - - T A - - - - - - - - - - - - - - - -
Lepidonotus polycroma Nelson 1	- - C - - T - - - - - - - - - T - - - - - - - - - C - - T - - - - - - - - - - - -
Scolecolepis sp Napier 1	- - - - - T - - C - - - - - - C - - - - - - - - - G - - - - - - - - - C - - - - - - - -
Owenia fusiformis Nelson 1	- - - - - G - - - - - - - - - T - - T - - G - - G A - G - - - - - - - - - - - T -
Owenia fusiformis Tauranga 1	- - - - - G - - - - - - - - - T - - T - - G - - - A - G - - - - - - - - - - - T -
Nereid sp1 Nelson 1	- - C - - T - - - - - G - - - - - - - - - - - G T - - - - C - - - - - - - - - C -
Nereid sp1 Tauranga 1	- - C - - T - - - - - G - - - - - - - - - - - G T - - - - C - - - - - - - - - C -
Nereid sp2 Tauranga 1	- - C - - T - - - - - - - - - - - - - - - - - C T - - - - - - - - - - - - - - - -
Nicon aestuariensis Nelson 1	- - - - - T - - - - - - - - - - - - - - C - - - - - - - C - - C - - - - - - - -
Nicon aestuariensis Tauranga 1	- - A - - T - - - - - - - - - - - - - - C - - - - - - - C - - C - - - - C - -
Perinereis novae hollandiae Coromandel 1	- - C - - T - - - - - - - - - - - - - - T - - T - - T - - - - - C - - - - - - - -
Sabellaria kaiparaensis Nelson 1	- - - - - C - - - - - - - - - - - T - - T - - - T - - - - C - - - - - - - - - -
Onoscolex pacificus Tauranga 1	C T - - - A - - C - - - - - C - - - - - - - - - A - - - - C - - C - - - - - C -
Aglaophamus sp2 Raglan 1	- - A - - T - - - - - G - - C - - - - - C - - C T - - - - - - - - - - - T C T -
Aglaophamus sp1 Nelson 1	- - A - - A - - - - - G - - T - - - - - C - - - - - - - - C - - - - - - T C C -
Aglaophamus sp3 Tauranga 1	- - A - - A - - - - - - - - - - - T - - T - - - - - - - - C - - - - - - T C G -
Orbina papillosa Nelson 1	- - G - - A - - - - - - - - C - - - - - C - - T A - - - - - - - C - - - - - - - -
Glycera sp2 Raglan 5	- - C - - A - - - - - - - - - - - T - - T - - T - - C - - C - - C - - - - - - - -
Glycera sp1 Raglan 6	- - - - - A - - C - - - - - - - - - - - T - - T - - - - - - - - - - - - - - - - -
Eulalia microphylla Nelson 1	- - - - - T - - - - - G - - T - - T - - T - - T - - T - - - - - - - - - - - - - -
Axiothella quadrimaculata Wellington A	- - G - - A - - - - - - - - C - - - - - - - - - A - - - - C - - - - - - - - - - -
Asychis theodori Tauranga 1	- T - - - A - - - - - - - - - - - - - - - - - - A - - - - - - - - - - - - - C -
Reference bases	G C T C C W G A T A T A G C A T T C C C A C G A C T A A A T A A T A T A A G A T

Marenzelleria arctica DQ309269	- C - - - - - T - - T - - - - - A - - - - - T - - - - - C - - G G - T T - A - -
Marenzelleria arctica DQ309272	- C - - - - - T - - T - - - - - C - - T T - - - - A - - T - - C G - C T - C - -
Owenia fusiformis DQ319478	- - - - - T - G T - G - - G - - - G - T T - - T T G T - - T - G T - G - G A - -
Pectinaria koreni DQ319855	- C - - - - - T - - G - - - - - G G - T T - - - T T - - T - - - - - G A G C - -
Pectinaria australis Nelson 1	- - - - - - - C - - T - - - - - - G - - - - T - T - - - C - - - - - - T A G A - -
Lepidonotus polycroma Nelson 1	- - - - - - - - - - T - - - - - A - - C - - T - T T - - C - - - - - - T T - C - -
Scolecolepis sp Napier 1	- C - - - - - - - - - T - - - - - - - - C - - T - - - - - T - - C G - C T - C - -
Owenia fusiformis Nelson 1	- - G - G T - - T - - - - - - - - A G - G T - G T T G T - G - - - - - - T - G - - -
Owenia fusiformis Tauranga 1	- - - - G T - - T - - - - - - - - A G - G T - G T T G T - G - - - - - - T - G - - -
Nereid sp1 Nelson 1	- - - - - - - - - - - - - - C - - C - - - - - - - - - - - C - - - T - - T - - A G
Nereid sp1 Tauranga 1	- - - - G - - - - - - - - - C - - C - - - - - - - - - - - C - - - T - - T - - A G
Nereid sp2 Tauranga 1	- - - - - - - - - T - - - - C - - C - - - T - - - - - - - T - - C T - - T - C A G
Nicon aestuariensis Nelson 1	- C - - - T - G - - - - - - - - - C - - - - - - - - - - - - - - - - - T - - G - -
Nicon aestuariensis Tauranga 1	- C - - - - - G - - - - - - - - - C - - - - - - - - - - - - - - - - - T - - G - -
Perinereis novae hollandiae Coromandel 1	- C - - - T - - - - T - - C - - A - - C T - - - - T - - T - - - - - - - T - C A G
Sabellaria kaiparaensis Nelson 1	- C - - G - - T - - G - - - - - - - - C - - T - - A - - C - - - A - T - - - - - -
Onoscolex pacificus Tauranga 1	- C - - - - - G - - - - - - - - - A - - - - - - T - - - - - - - - A G - - - T A - -
Aglaophamus sp2 Raglan 1	- C - - - T - - - - - - - - A - - - - - - - - - - - - T T - - - - A G - T A T G - -
Aglaophamus sp1 Nelson 1	- - - - - - - C - - T - - - - - - - - T - - - T T A - - T T - A G - T A T A - -
Aglaophamus sp3 Tauranga 1	- - - - - - - - T - - - - C - - - - - - T - - - - T - - - - - A G - - A T G - -
Orbina papillosa Nelson 1	- C - - - T - G - - - - - C - - - G - T - - - - T T - - T - - A G - - A T A - -
Glycera sp2 Raglan 5	- - - - G T - - - - C - - - - - - - T - - C - - T A - - - - - - - - - - - C - -
Glycera sp1 Raglan 6	- - - - - - - - T - - - - - C - - C - - C - - T - - A A - - - - - - - - - - - - -
Eulalia microphylla Nelson 1	- - - - - T - G - - T - - - - - - - - G T - - - T T A - - T - A T - - - G G - -
Axiothella quadrimaculata Wellington A	- - - - - - - T - - T - - C - - - - - - - - - - - - A - - T - - - - - - - - - - A G
Asychis theodori Tauranga 1	- - - - G - - T - - T - - - - - - - - T - - T - - A - - - - - A G - - A G - - -
Reference bases	T T T G A C T A C T A C C T C C T T C A C T A A C C C T A C T T C T A G C T T C

Marenzelleria arctica DQ309269	- - - A - - - - - T - - - - - - - - - G - - G - - A - - - - - - - - - - - C - - -
Marenzelleria arctica DQ309272	- - - A - - C - - - - - - - - - - - - - - - G - - A - - - - - - - - - - - A - - C
Owenia fusiformis DQ319478	G - - A - - T - - T - - - G G G - - G - C T - - - - - T - - - - - - - - - - - G
Pectinaria koreni DQ319855	- - - - - - - - - - T - - - - - - - - - - - - T - - T - - - - - C - - - - - A - - C
Pectinaria australis Nelson 1	- - - - - - - C - - G - - - - - - - - - C - - T - - T - - T - - - - - - - - - - -
Lepidonotus polycroma Nelson 1	- A G - - - - - - - T - - - - - - - - - G - - T - - - - - - - - C - - - - - A - - -
Scolecolepis sp Napier 1	A - - C - - - - - - - - - - - - - G - - G - - A - - C - - T - - - - - - - - C - - -
Owenia fusiformis Nelson 1	G - - - - - T - - G - - G G G G - - G - C G - - - - - T - - T - - - - - - - - - T
Owenia fusiformis Tauranga 1	G - - - - - T - - G - - G G G G - - G - C G - - - - - T - - T - - - - - - - - - T
Nereid sp1 Nelson 1	C - - C - - C - - - - - - - - - - - - - - A - - - - - - - - - - G - - A - - -
Nereid sp1 Tauranga 1	C - - C - - C - - - - - - - - - - - - - - - A - - - - - - - - - - - - A - - -
Nereid sp2 Tauranga 1	A - - C - - - - - - - - - - - - - - - - - - A - - A - - - - - - - - - - A - - -
Nicon aestuariensis Nelson 1	- - - A - - C - - - - - - - - - - - - C - C C - - C - - - - - - - - - - - - - -
Nicon aestuariensis Tauranga 1	- - - A - - C - - - - - - - - G - - C - C C - - C - - - - - - - - - - - - - - -
Perinereis novae hollandiae Coromandel 1	A - - A - - - - - - - - - - - - - - - - - T - - T - - G - - - - - G - - - - - T
Sabellaria kaiparaensis Nelson 1	A A G A - - T - - - - - - - - - - - C - - G - - A - - - - - - - - G - - A - - T
Onoscolex pacificus Tauranga 1	- - - C - - - - - - - - - - G G C - - - - - C - - A - - C - - T - - - - - C - - C
Aglaophamus sp2 Raglan 1	- - - A - - - - - - - - - - - - - - - G - - A - - A - - - - - G - - - - - A - - C
Aglaophamus sp1 Nelson 1	G - - C - - - - - T - - - - - - - - - - - - G - - C - - - - - T - - - - - A - - C
Aglaophamus sp3 Tauranga 1	A - - - - - - - - - - - - - - - - - - - G - - C - - T - - T - - T - - - - - C - - -
Orbina papillosa Nelson 1	C - - - - - T - - - - - - - - - - - - - - C C - - - - - C - - - - - - - - - - -
Glycera sp2 Raglan 5	- - - - A - - - - - - - - - - - - - - - - C T - - T - - - - - - - - - - - C - - -
Glycera sp1 Raglan 6	A - - - A - - - - - - - - - - - - - - - T - C G - - - - - C - - - - - - - - - - -
Eulalia microphylla Nelson 1	- - - - - - T - - - - - - C - - - - - - - C A - - - - - - - - G - - G - - - - - -
Axiothella quadrimaculata Wellington A	A - - A - - T - - T - - - - - - - - - - - - T - - C - - - - - - - - - - - - - - C
Asychis theodori Tauranga 1	A - - - - - C - - C - - - - - G - - G - - A - - C - - - - - - - - - - - - - - - G
Reference bases	T G C T G C A G T A G A A A A A G G A G T D G G G A C A G G A T G A A C T G T A

Marenzelleria arctica DQ309269	- - T - - - - - - - - - G - - G - C - - C C - - - - - - - - T - - C - - A - - - -
Marenzelleria arctica DQ309272	- - - - - - - - - - T - - - - - - G - C - - - T - A - - C - - - - - - - - - A - - - -
Owenia fusiformis DQ319478	- - T - - - - - G T - G - - C T C T - - - G - G - - - - - T - - T - - T G G - -
Pectinaria koreni DQ319855	- - - - - - A - - C - - G - - - - - - G - - - C - - - - - G - - - - - - - - - G - - C -
Pectinaria australis Nelson 1	- - - - - - C - - G - - C - - T - - G - - C C - A - - A - - - - - T - - G - - A -
Lepidonotus polycroma Nelson 1	- - - - - - - - - G - - T G - T - - - - - - - - - - - - - - A - - - - - - C - - G - - - -
Scolecolepis sp Napier 1	- - - - - - G - - - - - - - G - - G - C - - - C - - - - - C - - - - - - C - - C - - A -
Owenia fusiformis Nelson 1	- - T - - - - - - - T - G - - G T C T - - - G - G - - G - - T - - T - - G G G G -
Owenia fusiformis Tauranga 1	- - T - - - - - - - T - - - - G T C T - - - G - G - - G - - T - - T - - G G G G -
Nereid sp1 Nelson 1	- - - - - - - - - - - - - G - - - - - - - C - - C - - C - - - - - - - - - G - - C -
Nereid sp1 Tauranga 1	- - - - - - - - - - T - - G - - - - - - - - C - - C - - C - - - - - - G - - A - - C -
Nereid sp2 Tauranga 1	- - - - - - - - - - T - - G - - - - - - - - - - - - - - - A - - T - - - - - - C - - C -
Nicon aestuariensis Nelson 1	- - - - - - C - - - - - - T - - - - - - T - - C - - C - - - - - - T - - C - - C - - A -
Nicon aestuariensis Tauranga 1	- - - - - - C - - - - - - T - - - - - - T - - C - - C - - - - - - T - - C - - C - - A -
Perinereis novae hollandiae Coromandel 1	- - - - - - C - - - - - - C G - - - - C - - C - - - - - - - - - - - - - - - A - - G -
Sabellaria kaiparaensis Nelson 1	- - T - - - - - G T - - - - - G - G - - - T - A - - - - - - - - - G - - A - - - -
Onoscolex pacificus Tauranga 1	- - - - - - - - - - T - - G - - G - - - - C C - G T - C - - - T - C - - G - - A G
Aglaophamus sp2 Raglan 1	- - - - - - G - - G - - C - - T - - - - - - C - - - - - - A - - T - - - - - - A - - A -
Aglaophamus sp1 Nelson 1	- - - - - - C - - - - - - T - - - - - - - - - - - - - - - - C - - - - - - - - - A - - A -
Aglaophamus sp3 Tauranga 1	- - - - - - C - - A - - T - T - - - - - - C - - A -
Orbina papillosa Nelson 1	- - - - - - C - - - - - - - - - C - - - - - - C - - C - - C - - - T - - - - - C - - - -
Glycera sp2 Raglan 5	- - T - - - - - - - T - - G - T - C - - A - - A -
Glycera sp1 Raglan 6	- - - - - - C - - - - - - G G - - - - - T - - C - - - - - - C - - - - - - G - - T - - - -
Eulalia microphylla Nelson 1	- - T - - A - - - A - - T - - C - - - - - - - G - G - - - - - - T - - C - - T - - - -
Axiothella quadrimaculata Wellington A	- - - - - - C - - - - T - - - - - T - - - - - - C C - - - - - A - - T - - - - - - G - - - -
Asychis theodori Tauranga 1	- - T - - A - - - - - - T G - T - A T - - - - - - A - - - - - - - - - - C - - C - - - -
Reference bases	T A C C C T C C T C T A T C A A G A A A T A T T G C T C A C G C A G G R C C T T

Marenzelleria arctica DQ309269	- - - - - - - - - - T - - - - - - - - - - - - - - - - - T C - T - - - - - G - -
Marenzelleria arctica DQ309272	- - - - - - - - - - G - - - - - - - - - C - - A - - - - - T - - - - - - - - - G - -
Owenia fusiformis DQ319478	- A - - T - - - A - - A - C - - - - - - - - - - T - A - - T - - - - - - T - - T - C
Pectinaria koreni DQ319855	- - - - - - - - - T - G - - C - - - - - C - - - - - - - - T C - T - - C - - - A -
Pectinaria australis Nelson 1	- - - - - - - C - - G - - - - - - - - - C - - A - - - - - C C - T - - C - - - A -
Lepidonotus polycroma Nelson 1	- G - - T - - C - - - - - - A - - - - - - - - - - - - C - - C A - T - - - - - - - - -
Scolecolepis sp Napier 1	- C - - - - - C - - G - - A - - - - - - - - - - - - - - - T C - G - - - - - - - - -
Owenia fusiformis Nelson 1	- - - - - - - - - A - - A - - - - - - - - - - - - - - - - T A - - - - - T - - T - C
Owenia fusiformis Tauranga 1	- - - - - - - - - A - - A - - - - - - - - - - - - - - - - T A - - - - - T - - T - C
Nereid sp1 Nelson 1	- C - - C - T - - - - - - - - - - -
Nereid sp1 Tauranga 1	- C - - C - T - - - - - - - - - - -
Nereid sp2 Tauranga 1	- - - - - - - C T - - - - A - - - - - C - - C - - - - - C C - - - - - - - - - - -
Nicon aestuariensis Nelson 1	- A - - G - - C T - - - - - - - - - - - - - C - - A - - C - - G - - G - - G - C
Nicon aestuariensis Tauranga 1	- A - - G - - C T - - - - - - - - - - - - - C - - A - - C - - G - - G - - G - C
Perinereis novae hollandiae Coromandel 1	- A - - T - - C - - C - - - - - - - - - - - - - - - - - T - - - - - - - - T - -
Sabellaria kaiparaensis Nelson 1	- C - - - - - C T - - - - A - - - - - - - - - - - - - T - - - - - T - - - - -
Onoscolex pacificus Tauranga 1	- - - - - - - - C - - T - - A - - - - - - - - - A - - A - - C C - - - - - - - - G - -
Aglaophamus sp2 Raglan 1	- - - - T - - - T - - - - A - - - - - - - - - - - - C - - C - - G - - T - - G - C
Aglaophamus sp1 Nelson 1	- A - - - - - - - - - - - C - - - - - - - - - C - - - - - C - - - - - C - - - - C
Aglaophamus sp3 Tauranga 1	- A - - - - - - - - - T - - - - - - - - - - - - T - A - - C - - G - - T - - T - C
Orbina papillosa Nelson 1	- A - - - - - - - A - - - T A - - - - - C - - C - - C - - C C - - - - - - - - T - -
Glycera sp2 Raglan 5	- - - - - - - C - - G - - - - - - - - - C - - - - - A - - C C - - - - - T - - - - -
Glycera sp1 Raglan 6	- C - C - - - - - - T - - - - - - - - - - -
Eulalia microphylla Nelson 1	- - - - - - - - - T - T - - - - - - - - - - - - - T - A - - C - - G - - T - - - - - -
Axiothella quadrimaculata Wellington A	- A - - - - - - C - - T - - A - - - - - - - - - A T - A - - C - - - - - - - - - G - C
Asychis theodori Tauranga 1	- - - - - - - - C - - T - - C - - - - - - - - - G - - C - - T C - T - - C - - T - C
Reference bases	C T G T A G A T C T A G C T A T T T T T T C T C T T C A Y T T A G C A G G A G T

Marenzelleria arctica DQ309269	- - - - - - A - - - - - - T - - T - - C - - T - - C - - - - - - - - - C - - - - - - -
Marenzelleria arctica DQ309272	- - - T - - - - - - - T - - - - G - - - - - - - - - - - - - - - - - - A - - - A - -
Owenia fusiformis DQ319478	- - - T - - - - - - - T - G - - T - - A G - T - - - - - - - - - - - - T - - - - - C
Pectinaria koreni DQ319855	- - - - - - - - - - - - - C - - - - - - - A - C - - C - - - - - - - - - A - - - - - C
Pectinaria australis Nelson 1	C - - - - - C - - C T - - - - - - - C A - C - - - - - - - - - C - - A - - T - - -
Lepidonotus polycroma Nelson 1	C - - - - - - - - - - T - - - - - T - - C - - T - - C - - - - - - - - - A - - C - - -
Scolecolepis sp Napier 1	- - - - - - - - - - - T - - - - - - - - - - - T - - C - - - - - - - - - C - - G - - A
Owenia fusiformis Nelson 1	- - - T - - - - - - - A - - - - G - - G G - - - - - - - A - - - - - - G - - T - - -
Owenia fusiformis Tauranga 1	- - - T - - - - - - A T - - - - G - - G G - - - - - - - - - - - - - - G - - T - - A
Nereid sp1 Nelson 1	- - - T - - C - - - A - - - - - - - - - - T - - - - - C - - - - - - A - - - - - - -
Nereid sp1 Tauranga 1	- - - T - - C - - - A - - - - - - - - - - T - - - - - C - - - - - - A - - - - - - -
Nereid sp2 Tauranga 1	- - - T - - C - - - A - - - - - - - - - - C - - C - - - - - - - - - A - - - - - - -
Nicon aestuariensis Nelson 1	C - - - - - A - - - A - - - - - - - C T - - - - - - - C - - C - - T - - - - - - A
Nicon aestuariensis Tauranga 1	C - - - - - A - - - A - - - - - - - C T - - - - - - - C - - C - - T - - - - - - A
Perinereis novae hollandiae Coromandel 1	- - - C - - - - - - C A - - - - T - - A - - C - - - - - - - - - C - - T - - - - - - -
Sabellaria kaiparaensis Nelson 1	C - - - - - - - - - - - - - - - G - - - T - - - - - - - - - - - - - A - - T - - A
Onoscolex pacificus Tauranga 1	- - - C - - - - - - C - - - - - G - - - A - T - - C - - C - - C T - A - - - - - - C
Aglaophamus sp2 Raglan 1	C - - - - - - - - - - - - - - - - C - - - G - - - - - - - C - - C - - T - - C - - -
Aglaophamus sp1 Nelson 1	C - - T - - A - - - T - - - - C - - C - - - - - - C - - C - - - - - - T - - T - - A
Aglaophamus sp3 Tauranga 1	- - - - - - - - - - - - - - - - G - - A G - - - - - - - - - - - - - - C - - T - - A
Orbina papillosa Nelson 1	A A G - - - A - - - - - - T - C C T - A A - T - - - - - - - - - - - - T - - - - C A
Glycera sp2 Raglan 5	- - - T - - - - - - T - - - - - - - A T - - - - C - - - - - - C - - T - - - A - C
Glycera sp1 Raglan 6	C - - - - - - A - - - - - - - - - T - - C - - - - - - - - - C - - - - - - T - - C A - -
Eulalia microphylla Nelson 1	- - - T - - - - - - - T - G - C G T - A - - G - - - - - - C - - - - - - T - - T - C -
Axiothella quadrimaculata Wellington A	- - - T A G - - - - - - - - - - G - - - A - T - - C - - - - - - - - - - A - - - - - C A
Asychis theodori Tauranga 1	C - - - - - - A - - - - - - T - - - - - - - A - T - - - - - - - - - - T - C - - - - A - -
Reference bases	T T C A T C T A T T C T A G G A G C T C T A A A T T T T A T T A C W A C A G T T

Marenzelleria arctica DQ309269	- - - - - - - - G - - - - G - - - - - - C T - - - - - - - - - - - - - T A - C -
Marenzelleria arctica DQ309272	- - - - - - C - - - - - - - - G - - - - - - - - - - - - - T - - - - - - G - - T A - C -
Owenia fusiformis DQ319478	- - - - - - - - - G - - - - G - T - T - - C A - - T T G T - T - - - - - - T C - C -
Pectinaria koreni DQ319855	- - - - - - - - - G - - - - - C - - - - - C - - T - - G T - - - - G - - G - - - -
Pectinaria australis Nelson 1	- - - - - - - - - - - - - C - - C
Lepidonotus polycroma Nelson 1	- - - - - - C - - - - - - - - A T - - - - - - - - - - - - - - - - - C - - - - - - T - - - -
Scolecolepis sp Napier 1	- - C - - - - - - - - - - - - G - - - - - - - C T - - - - G - - - - - - - - - - A - - -
Owenia fusiformis Nelson 1	- - - - - - C - - G - - - - G - T - C - - T A - T G T T G - T - - G - - G C - - -
Owenia fusiformis Tauranga 1	- - - - - - C - - G - - - - G - T - C - - T A - G T T G T - T - - G - - G C - - -
Nereid sp1 Nelson 1	- G - - - - - T T - - - - - - - - - - C -
Nereid sp1 Tauranga 1	- G - - - - - C T - - - - - - - - - - C -
Nereid sp2 Tauranga 1	- - C - - - - - - - - C - - T - - - - - - G - - T - - G - - - - - - - - - - - - - A -
Nicon aestuariensis Nelson 1	- - - - - - - - G - - - - - - - - G G - - - - - T - - C - - - - - - - - - - - - - A -
Nicon aestuariensis Tauranga 1	- - - - - - - - - - - - - - - - - - G G - - - - - T - - C - - - - - - - - - - - - - A -
Perinereis novae hollandiae Coromandel 1	- - C - - C - - - - - - - - - T - - - - - T - - T - - C T - - - - - - - - T - - - -
Sabellaria kaiparaensis Nelson 1	- - - - - C - - - - - - - - G - - C - - C - T - - - - - - - T - - - - - - - - - - -
Onoscolex pacificus Tauranga 1	G C - - - - - - - - - - C C - T - - - A C - T A C - - C - - T - - - - - - C - - - -
Aglaophamus sp2 Raglan 1	- - A - - C - - - - - - - - G - - - - - - G - - - - - - T - - - - - - - - - - - A -
Aglaophamus sp1 Nelson 1	- - A - - C - - - - - - C - G - - - - - - - - - - - - T T - - - - - - - - - - - A -
Aglaophamus sp3 Tauranga 1	- - A - - - - - - - - - G - G - - - - - - - - - - - - G - - - - - - - - - - - - - -
Orbina papillosa Nelson 1	- A A - G - C - T - - - - G T G : : : - - A - - A C - - - - - - T - - - A - A -
Glycera sp2 Raglan 5	- - A - - - - - - - - - C - - T G C C - - - A - - - A - - - T - - - - - - - - - - -
Glycera sp1 Raglan 6	- - A - - C - - - - - - T - - T G C - - - T A - - - A - - - T - - - - - - - - - G -
Eulalia microphylla Nelson 1	- - A - - - - - - - - - T - - T - G - - - G T - - - - T - - G - - - - - - G - - A -
Axiothella quadrimaculata Wellington A	- - C - - C - - - - - - G - - - C - - - - - T T - - - A - T - - - - G - - T A - A -
Asychis theodori Tauranga 1	C - A - - - - - - - - - C A G - - G - - - T T - C - A - - - G - - - - - - A - - -
Reference bases	A T T A A T A T A C G A T C A A A A G G A C T A C G A C T A G A A C G A G T T C

Marenzelleria arctica DQ309269	- - T - - - - - - - - - G - - G - C - - C C - - - - - - - - T - - C - - A - - - -
Marenzelleria arctica DQ309272	- - - - - - - - - - T - - - - - - G - C - - - T - A - - C - - - - - - - - - A - - - -
Owenia fusiformis DQ319478	- - T - - - - - G T - G - - C T C T - - - G - G - - - - - - T - - T - - T G G - -
Pectinaria koreni DQ319855	- - - - - - A - - C - - G - - - - - - G - - - C - - - - - G - - - - - - - - - G - - C -
Pectinaria australis Nelson 1	- - - - - - C - - G - - C - - T - - G - - C C - A - - A - - - - - - T - - G - - A -
Lepidonotus polycroma Nelson 1	- - - - - - - - - - G - - T G - T - - - - - - - - - - - - - - - A - - - - - - C - - G - - - -
Scolecolepis sp Napier 1	- - - - - - G - - - - - - - G - - G - C - - - C - - - - - C - - - - - - C - - C - - A -
Owenia fusiformis Nelson 1	- - T - - - - - - - T - G - - G T C T - - - G - G - - G - - T - - T - - G G G G -
Owenia fusiformis Tauranga 1	- - T - - - - - - - T - - - - - G T C T - - - G - G - - G - - T - - T - - G G G G -
Nereid sp1 Nelson 1	- - - - - - - - - - - - - - G - - - - - - - C - - C - - C - - - - - - - - - G - - C -
Nereid sp1 Tauranga 1	- - - - - - - - - - - T - - G - - - - - - - - C - - C - - C - - - - - - G - - A - - C -
Nereid sp2 Tauranga 1	- - - - - - - - - - - T - - G - - - - - - - - - - - - - - - A - - T - - - - - - C - - C -
Nicon aestuariensis Nelson 1	- - - - - - C - - - - - - T - - - - - - T - - C - - C - - - - - - T - - C - - C - - A -
Nicon aestuariensis Tauranga 1	- - - - - - C - - - - - - T - - - - - - T - - C - - C - - - - - - T - - C - - C - - A -
Perinereis novae hollandiae Coromandel 1	- - - - - - C - - - - - - C G - - - - - C - - C - - - - - - - - - - - - - - - A - - G -
Sabellaria kaiparaensis Nelson 1	- - T - - - - - - G T - - - - - - G - G - - - T - A - - - - - - - - - G - - A - - - -
Onoscolex pacificus Tauranga 1	- - - - - - - - - - - T - - G - - G - - - - C C - G T - C - - - T - C - - G - - A G
Aglaophamus sp2 Raglan 1	- - - - - - G - - G - - C - - T - - - - - - C - - - - - - A - - T - - - - - - A - - A -
Aglaophamus sp1 Nelson 1	- - - - - - C - - - - - - T - - - - - - - - - - - - - - - - C - - - - - - - - - A - - A -
Aglaophamus sp3 Tauranga 1	- - - - - - C - - A - - T - T - - - - - - C - - A -
Orbina papillosa Nelson 1	- - - - - - C - - - - - - - - C - - - - - C - - C - - C - - - T - - - - - C - - - -
Glycera sp2 Raglan 5	- - T - - - - - - - T - - G - T - C - - A - - A -
Glycera sp1 Raglan 6	- - - - - - C - - - - - - G G - - - - - T - - C - - - - - - C - - - - - - G - - T - - - -
Eulalia microphylla Nelson 1	- - T - - A - - A - - T - - C - - - - - - - G - G - - - - - - T - - C - - T - - - -
Axiothella quadrimaculata Wellington A	- - - - - - C - - - T - - - - - T - - - - - - C C - - - - - A - - T - - - - - - - G - - - -
Asychis theodori Tauranga 1	- - T - - A - - - - - - T G - T - A T - - - - - - A - - - - - - - - - - C - - C - - - -
Reference bases	T A C C C T C C T C T A T C A A G A A A T A T T G C T C A C G C A G G R C C T T

Marenzelleria arctica DQ309269	- - - - - - - - - - - - - T - - T - A - - G
Marenzelleria arctica DQ309272	- - - - - - - - - - A - - T - C - - - - - - A - - -
Owenia fusiformis DQ319478	- T - A T - G - - A T - - - -
Pectinaria koreni DQ319855	- - - - - - - - - - - - - G - - T - - - T - - - - - C - - - - - - C - - - - - - - - - - G
Pectinaria australis Nelson 1	
Lepidonotus polycroma Nelson 1	C - - - - - - T - - C - - T - - - - - - - - - - T - - C - - - - - - - - - - - - - - A - - -
Scolecolepis sp Napier 1	- - - - - - - C - - - - - - T - - - - - - - - - - T - - - - - - - - - - - - - - - - - A - - -
Owenia fusiformis Nelson 1	- T - G T - G - - A T - - - - T - - A T - G - - G - - - - - G A - - - - - - - - - - G
Owenia fusiformis Tauranga 1	- A - G T - G - - A T - - - - T - - A T - G - - G - - - - - G A - - - - - - - - - - G
Nereid sp1 Nelson 1	A - - - - - - - A G - - - C - - C - - C T - - - - - - - - - - - - - - - - - - C - - -
Nereid sp1 Tauranga 1	A - - - - - - - A G - - - C - - C - - C T - - - - - - - - - - - - - - - - - - C - - -
Nereid sp2 Tauranga 1	- - - - - T - - - A G A - - T - - C - - C T - - - - - - - - - - - - - - - - - C - - - - - -
Nicon aestuariensis Nelson 1	- - - - - T - - - - - C - - G - - - - - - C - - C - - - - - - - - - - A - - - - - - - - -
Nicon aestuariensis Tauranga 1	- - - - - - - - - - C - - G - - - - - - C - - C - - G - - - - - - A - - - - - - - - -
Perinereis novae hollandiae Coromandel 1	- - - - - T - - - A G C T - - - - - - - - - T - - - - - T - - - - - - C - - C - - C - - -
Sabellaria kaiparaensis Nelson 1	G - - - - - - C - - - - - - - - - - - - - - C - - T - - T - - - - - - A - - C - - G - - -
Onoscolex pacificus Tauranga 1	C - - - - - - C - - - - T - - - - - - - - - C - - - - - - - - - - G - - - - - - - - - G - - G
Aglaophamus sp2 Raglan 1	- - - - - T - - - - - A - - C - - - - - - - - - - - - - - T - - G - - C - - - - - - - - - -
Aglaophamus sp1 Nelson 1	- T - A - - C - - C - - - - - - T - - - - - - - - - - - - T - - A - - - - - - C - - G
Aglaophamus sp3 Tauranga 1	- - - C T - - - - - - T - - - - - T - - A - - - - - - C - - - - - - - - - - C - - C - - G
Orbina papillosa Nelson 1	- - - - - - - T - - A - - C - - T - - A - - T - - - - - - - - - - C - - C - - A - - -
Glycera sp2 Raglan 5	A T - A - - C G - C - - T - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - -
Glycera sp1 Raglan 6	A - - - - - - - G - - - - C - - - - - - A T - - - - - G - - C - - A - - - - - - - - - -
Eulalia microphylla Nelson 1	A - - A T - G - - - T - - - - T - - A T - - - - - T - - G - - - - - - - - - - A - - -
Axiothella quadrimaculata Wellington A	A - - - - - - - - - - T - - - - G - - G - - - - - - - - - - - - - - A - - - - - - A - - -
Asychis theodori Tauranga 1	- - - - - - - G - - C T - - - - - - - - - T - - - - - - - - - G - - A - - C - - A - - -
Reference bases	T C T T C T A T C T C T A C C A G T T C T A G C A G G A G C T A T T A C T A T A

Marenzelleria arctica DQ309269	-	-	-	-	-	T	-	-	A	-	-	-	-	-	-	-	-	T	C
Marenzelleria arctica DQ309272	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	C
Owenia fusiformis DQ319478																			
Pectinaria koreni DQ319855	-	-	C	-	-	G	-	-	-	-	-	-	-	-	-	-	-	C	-
Pectinaria australis Nelson 1																			
Lepidonotus polycroma Nelson 1	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	T	C
Scolecolepis sp Napier 1	-	-	-	-	-	-	-	-	A	-	-	-	-	-	G	-	-	T	-
Owenia fusiformis Nelson 1	T	-	A	T	-	G	-	-	-	-	-	T	-	-	-	-	-	T	-
Owenia fusiformis Tauranga 1	-	-	A	T	-	G	-	-	-	-	-	T	-	-	-	-	-	C	C
Nereid sp1 Nelson 1	-	-	-	-	-	G	-	-	-	-	-	-	-	-	A	-	-	C	-
Nereid sp1 Tauranga 1	-	-	-	-	-	G	-	-	-	-	-	-	-	-	A	-	-	C	-
Nereid sp2 Tauranga 1	-	-	-	-	-	-	-	-	C	-	-	-	-	-	A	-	-	T	-
Nicon aestuariensis Nelson 1	T	-	A	-	-	-	-	-	A	-	-	T	-	-	A	-	-	C	C
Nicon aestuariensis Tauranga 1	G	-	-	-	-	-	-	-	A	-	-	T	-	-	A	-	-	C	-
Perinereis novae hollandiae Coromandel 1	-	-	A	T	-	-	-	-	C	-	-	T	-	-	-	-	-	C	-
Sabellaria kaiparaensis Nelson 1	-	-	C	T	-	-	-	-	A	-	-	-	-	-	A	-	-	T	A
Onoscolex pacificus Tauranga 1	-	-	C	-	-	-	-	-	-	-	-	-	-	-	C	-	-	C	C
Aglaophamus sp2 Raglan 1	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	C	-
Aglaophamus sp1 Nelson 1	-	-	-	-	-	G	-	-	G	-	-	T	-	-	A	-	-	C	-
Aglaophamus sp3 Tauranga 1	-	-	G	T	-	G	-	-	C	-	-	T	-	-	A	-	-	T	C
Orbina papillosa Nelson 1	-	-	A	T	-	-	-	-	A	-	-	-	-	-	A	-	-	C	C
Glycera sp2 Raglan 5	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	C
Glycera sp1 Raglan 6	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	C	C
Eulalia microphylla Nelson 1	T	-	A	T	-	G	-	-	A	-	-	T	-	-	-	-	-	T	-
Axiothella quadrimaculata Wellington A	-	-	A	-	-	G	-	-	A	-	-	T	-	-	A	-	-	T	-
Asychis theodori Tauranga 1	-	-	A	T	-	-	-	-	C	-	-	-	-	-	-	-	-	T	C
Reference bases	C	T	T	C	T	A	A	C	T	G	A	C	C	G	T	A	A	Y	T