Evolution of New Zealand's Marine Caddisflies: A Phylogenetic and Phylogeographic Assessment of the Chathamiidae (Insecta: Trichoptera)

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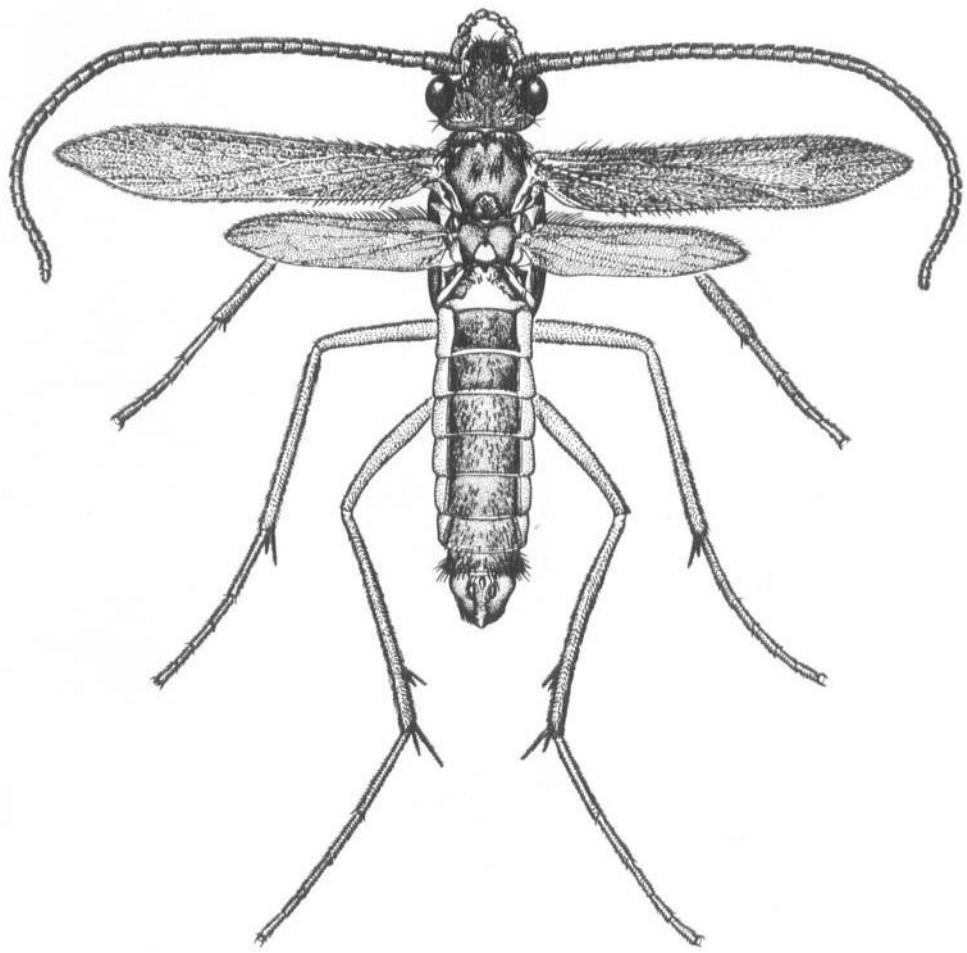

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Image: *Chathamia brevipennis* adult male from Riek (1976)

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Abstract

The Chathamiidae are an interesting family of caddisflies, unusual as all of the five known species are believed to breed entirely within the marine intertidal, comprising one of very few known marine insect groups. Additionally the family approaches almost complete endemicity status in New Zealand, and may represent an ancient lineage representative of ancient vicariance from Gondwana. However one species, the common and widespread *Philanisus plebeius* is also known to have a disjunct population in New South Wales Australia, hypothesised to represent a recent anthropogenic dispersal. This thesis, using DNA information, examined the Chathamiidae at varying phylogenetic levels.

Firstly the species *Philanisus plebeius* was incorporated into a thorough intraspecific phylogeography, including samples from both New Zealand and Australia. The population as a whole was genetically diverse, with the population divisible into two major haplogroups, each restricted to discrete geographic areas with no overlap being observed. One of these groups was restricted to just two localities in the central eastern North Island, whereas the remainder included most remaining samples from both Islands of New Zealand, and also Australia. All Australian samples were found to comprise a single haplotype, differing by a single base pair from the most common haplotype in New Zealand. It was decided that the Australian population therefore represents a recent dispersal event from New Zealand, although unless the Australian haplotype remains undiscovered in New Zealand the level of divergence found is not congruent with a human introduction. One sequence intermediate between the two major haplogroups was identified from a single haplotype from Tauranga. It seemed that much of the population of *Philanisus plebeius* has been affected by recent demographic expansion, likely due to the effects of the last glacial maximum (LGM).

The five species of the Chathamiidae were then analysed in a phylogeny. It was found that the genus *Chathamia* was polyphyletic, with the species *C. integripennis* nested within the genus *Philanisus*. The remaining species, *C. brevipennis* from the Chatham Islands, was basal to all the remaining members of the family. A strict molecular clock found a recent Pleistocene age (roughly 0.5 Ma) for divergence of the Kermadec Island species *Philanisus fasciatus*, and a Pliocene-Pleistocene age (roughly 3 Ma) for the Chatham Island species *Chathamia brevipennis*. For a comparison with the species *C. brevipennis*, the other Chatham Island caddisfly taxa *Oecetis chathamensis*, and *Hydrobiosis lindsayi* were compared with New Zealand relatives; indicated to have late and early Pleistocene ages respectively. A short sequence of the gene COI was amplified for the species *Philanisus mataua*, however this was found to contain two sequences reflecting either heteroplasmy or sample contamination, inhibiting confident phylogenetic placement. Additionally a larval sample from Sydney was demonstrated to represent *C. integripennis*, recorded outside of Northern New Zealand for the first time. Finally the Chathamiidae was included in a higher level phylogeny with related families, and was show to comprise a monophyletic group, sister to the Australasian family of the Conoesucidae. A relaxed molecular clock estimated a Cretaceous (roughly 90 Ma) age for the Chathamiidae, congruent with a vicariant age in New Zealand.

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Chapter One: General Introduction

1.1 The Chathamiidae, the world's only marine caddisflies.

New Zealand is well known for its unique and apparently ancient biota, and several lineages of its flora and fauna have undergone evolutionary trajectories not seen anywhere else in the world. One particularly interesting and little known group, are a small family of case-making caddisflies, the Chathamiidae. Chathamiid caddisflies are especially unusual as almost uniquely among all insects they develop entirely within the intertidal zone of the marine environment, thus representing a rare case of a marine insect. Importantly, the Chathamiidae are also one of very few insect families that approach endemicity status to New Zealand, and thus potentially represent one of the oldest members of the native fauna. As the assumed antiquity or Gondwanan element of New Zealand's biota is now being questioned, groups such as this are of particular importance in contemporary debates. Thus this group represents an especially unique and interesting case for a study in evolutionary biology in two distinct ways. Breeding in seawater, the family is unusually biogeographically and ecologically among insects; and also may perhaps represent a small group of significant or even Gondwanan age in New Zealand.

Chathamiids are medium sized (10-5mm wing length), pale-brown caddisflies. They can be recognised by characteristic 5 jointed maxillary palpi and a hairy facial protrusion of the males, and also distinctive large ovipositors found on the females (Riek 1976, Ward 1994). To date five species of Chathamiid caddisflies have been identified; *Chathamia brevipennis*, *C. integripennis*, *Philanisus fasciatus*, *P. plebeius* and *P. mataua* (Tillyard 1925, Riek 1976, Ward 1995). All species are native to the greater New Zealand region. However the common species *Philanisus plebeius* has also been known to inhabit the coasts of New South Wales, Australia since identification by Hudson (1904) and has been confirmed as the same species from New Zealand (Riek 1970). This is the only known incidence of any of New Zealand caddisfly species being also found in Australia (out of over 240 species); as far as known all remaining species are fully endemic (Collier 1993). This, combined with

its unusual marine habitat as well as the comparatively localised population in Australia (none are known from Victoria or Tasmania for example) has raised the possibility that the Australian population is an accidental human introduction (Gibbs 2005), although an Australian origin has also been suggested (Winterbourne & Anderson 1980).

Philanisus plebeius Walker was first described from Christchurch (Riek 1976) in 1858. An 1858 record from the Austrian scientific expedition *Novara* 1857-9 is presumably the same sample, first given separate family status in the Philanisidae by Mosely & Kimmins (1953). *Chathamia brevipennis* was described from a single brachypterous (short-winged) male from Kaingaroa, Chatham Island by Tillyard (1925), from which it was assigned to the Rhyacophilidae under the subfamily Chathamiinae. *C. brevipennis* was recognised as sharing characteristics with *P. plebeius* by Wise (1965), who transferred the species to the Philanisidae. Riek (1976) acknowledged insufficient difference between the two genera to warrant separate subfamily status. Chathamiidae has nomenclatural priority over the Philanisidae.

A new species of *Philanisus*; *P. fasicatus* was identified from Raoul Island of the Kermadecs in 1967, morphologically distinct from *P. plebeius.* Another species was also identified from Northern New Zealand, which Riek (1976) described as a new species of *Chathamia*, *C. integripennis*, although assigning the species to the genus based on a 'similarity' to *C. brevipennis*. The most recently described taxon; *Philanisus mataua*, was first recognised from male specimens collected in 1993 (Ward 1994) and described to species by Ward (1995). Female *P. mataua* remain unknown and may be indistinguishable from *P. plebeius*. *C. brevipennis* and *P. fasciatus* to date are only known from Chatham and Raoul Islands respectively, and it is unknown whether they are more broadly distributed in their respective island groups. *C. integripennis* and *P. mataua* appear restricted to the northern North Island (although *P. mataua* is evidently rarer, and likely frequently confused with the similar and much more common *P. plebeius*). *P. plebeius* is widely distributed through most of mainland New Zealand and offshore islands including the Three Kings Islands, although perhaps absent on the South and West Coasts of the South Island, and much of the West coast of the North Island (although

this also likely reflects a lack of sampling). In Australia the species is not known to occur North of Sydney, nor the coasts of Victoria or Tasmania and thus is not indicated to be widespread there.

Marine case-making caddisfly larvae have been identified from Chatham Island and thus likely those of *C. brevipennis* (Riek 1976), otherwise all known Chathamiid larvae and general ontogeny (such as ovipostion, larval habitat and pupation) are only known definitively from *P. plebeius*. Larvae of the remaining three species remain undescribed (larval caddisflies are often impossible to distinguish to exact species) although are still assumed to be marine in nature based on phylogeny and the coastal nature of the adults. Larvae can be found in the intertidal zone towards the low tide mark, most abundantly in sheltered, open rock pools on rocky coasts, although they have also been associated with seagrass beds in soft sediment, foraging us much as 2 m underwater (Riek 1976). This continual existence in full seawater is the highest salinity (35%) tolerated by any caddisfly (Kefford *et al.* 2004, Flint & Giberson 2005). Larvae build cases from nearby material, usually coralline algae, and are most commonly associated with the calcareous alga *Corallina* although they are also found on a variety of algal species and seagrass (Allan 1958, Riek 1976, Winterbourne & Anderson 1980, Taylor & Cole 1994, Taylor & Steinberg 2005). The diet of the larvae is herbivorous, and in spite of a common association with *Corallina*, gut content has been found to comprise primarily of non-calcerous species (Winterbourne & Anderson 1980). Larvae enclose their cases and pupate in algae near the shoreline in late spring, completing their entire development in the sea (Cowley 1976, Riek 1976). Adults emerge presumably mostly from late Spring to Summer and are present year-round, found flying all months of the year except for June-July (Riek 1976, Anderson & Lawson-Kerr 1977, Ward *et al*. 1996).

It was discovered that *P. plebeius* also demonstrate an unsual parasitism on Asterinid starfish, first described in *Parvalustra* (formerly *Patiriella*) *exigua* in Australia (Anderson et al. 1976) and later described in the related *Patiriella regularis* in New Zealand (Winterbourne & Anderson 1980), both the most common starfish in their respective regions. All Chathamiid female adults possess a large and distinct ovipositor (this is not found in any related family), which had been found in *P. plebeius* to forcibly deposit egg masses within the host's body cavity. It is assumed the host is largely

used as a stable environment for completing embryonic development and the first-instar larvae apparently leave with minimal effect (Anderson & Lawson-Kerr 1977). This is almost certainly the only echinoderm-insect symbiosis known, and also a very rare case of parasitic behaviour in Trichoptera excepting a few known Hydroptilidae which are Parasitoids on other caddisfly larvae (Wells 1992, 2005).

It is currently unknown whether this behaviour is obligate or essential for the caddisfly's development, or whether this is simply opportunistic or facultative behaviour. Similarly it is not known whether this behaviour is typical of other Chathamiidae (although since all species have the ovipositor it is certainly plausible), and whether these species are the only starfish exploited. In New Zealand *Patiriella* species are present wherever representatives of Chathamiidae are found including *P. regularis* and *P. mortenseni* throughout New Zealand and the Chatham Islands (Young 1929, McKnight 1968, O'Loughlin *et al.* 2002), and *P. oliveri* in the Kermadec Islands (Pawson 1961), suggesting this behaviour may be commonplace. Evidence supporting oviposition amongst *Corallina* in Northern New Zealand was presented by Leader & Bedford (1979), although this may have been eggs of *C. integripennis* (Winterbourne & Anderson 1980). Repeated washes of algae by Winterbourne & Anderson (1980) also found very low numbers of eggs although this contrasted with the much higher numbers found in starfish, who also found that the hosts apparently ejected undeveloped eggs along with larvae during *in-vitro* trials. It is unclear how an association or dependence on a host might affect Chathamiid biogeography, dispersal and abundance patterns. It was reasoned by Winterbourne & Anderson (1980), as since *Parvalustra exigua* in Australia is more abundant and lies closer to shore than *Patiriella regularis* in New Zealand that this behaviour and thus the species itself are more likely to have evolved there. This conclusion is unlikely considering that most species of the family are restricted to New Zealand, and it is currently more parsimonious to assume a non-Australian origin for *P. plebeius* and certainly for the remaining Chathamiid species.

Aside from their unusual ecology, the Chathamiidae are also morphologically distinct and for this reason have been given full family status, supported by molecular studies. Riek (1976) suggested the family as being closest to the diverse worldwide family Leptoceridae (superfamily Leptoceroidea).

Subsequent phylogenies by Kjer *et al*. (2001, 2002) based on 28S nuclear data however place the Chathamiidae within the superfamily Sericostomatoidea, with some trees showing a probable relationship to the other Australasian families Calocidae, Helicophidae and Conoesucidae in particular. This has been supported by morphology (Frannia & Wiggins 1997, Henderson & Ward 2007). More recent work on 16S mitochondrial data however indicated the Chathamiidae sharing a weakly supported relationship with the Leptoceridae and the Helicopsychidae (Johanson *et al*. 2009, Johanson & Malm 2010). Grimaldi & Engel (2005) also created a phylogenetic tree based on Kjer *et al.* (2001, 2002) and also Frania & Wiggins (1997), and using fossil material as a calibration tentatively dated the Chathamiidae lineage originating roughly 140 Ma in the late Cretaceous, predating New Zealand's rifting by over 60 million years. While this age remains tenacious and may well be a considerable overestimate, it nevertheless does demonstrate the possible evolutionary importance of this taxon.

1.2 An overview of caddisflies and their biogeography in New Zealand.

Caddisflies are generally small, moth like holometabolous insects, which comprise the order Trichoptera. Adults are usually drab, short lived, nocturnal, and display very little morphological diversity between species, although some species are diurnal and brightly coloured (Holzenthal *et al*. 2007). All species with some very rare exceptions (e.g. Anderson 1967, Hayashi et al. 2008) have fully aquatic larvae, the best known of which are the typical "caddis-worms" of the Integripalpia which use silk to produce a wide variety of portable cases. However throughout the order, larvae display number of ecological forms, including free living predators, retreat-dwellers and net-spinners, collectively well known as 'underwater architects' (Wiggins 2004). Comprising some 13,000 known species in 46 described families, caddisflies are among the more ecologically important of all freshwater invertebrates, comprising higher species diversity than the other fully aquatic insect orders of the Ephemeroptera, Odonata, Plecoptera and Megaloptera combined (de Moor & Ivanov 2006,

Holzenthal *et al.* 2007). Among fully aquatic insect taxa only Culicomorphid flies (Diptera, subclass Nematocera) outnumber them (Grimaldi & Engel 2005).

The Trichoptera is also of some evolutionary importance, whose relationship to the butterfly and moth order of the Lepidoptera forming the superorder Amphiesmenoptera, is perhaps among the most widely accepted higher taxonomic groupings in entomology (Kristensen 1975, 1991, 1995, Wheeler *et al*. 2001, Whiting 2002). The relationship between the two groups is well supported by a number of morphological synapomorphies, including modification of salivary glands into silk producing organs in the larvae, heterogametic females, a double looping of the anal veins on the forewings, and dense setae on the wings (modified into scales in the Lepidoptera). The two probably evolved from the Necrotaulidae, an early Mesozoic insect group known from fossils as early as Triassic in age (Willman 1989, Ivanov & Sukatsheva 2002), and the two orders themselves likely diverged between the Triassic and Jurassic periods.

Traditionally the Trichoptera has been divided into at least three suborders, roughly congruent with life-history of the larvae. The Annulipalpia is comprised of retreat making and net spinning larvae and the Integripalpia comprises all the true case making species. A third group the 'Spicipalpipa' has also been proposed, comprising a number of free living and shelter making caddisfly families now recognised as a basal paraphyletic grade of the Integripalpia (Frannia & Wiggins 1997, Kjer et al. 2001, 2002). The earliest definitive fossil Trichopteran is of early Jurassic in age (180-185 Ma, Ansorge 2002) although some fully modern families, especially the Philopotamidae (Annulipalpia) are well represented from the mid Jurassic onwards suggesting the family may have diverged earlier still in the Triassic. The earliest fossil larval cases appear in the early to mid-Jurassic (Sukatsheva 1985, 1994), although remain rare until an apparent radiation in the Cretaceous, comprising a number of taxonomically unidentifiable trace-fossil 'ichnospecies' (Ivanov & Sukatsheva 2002). Few fossil Integripalpia adults or larvae of Mesozoic age are known, although specimens up to early cretaceous in age can be assigned to modern families (Sukatsheva & Jarzembowski 2001, Ivanov & Sukatsheva 2002, Ivanov 2006, Ponomarenko *et al*. 2001). In this regard the Trichoptera represents a deeply divergent and ancient group, significantly more so than the

related Lepidoptera which are not believed to have undergone major radiation until the Late Cretaceous (Grimaldi & Engel 2005).

The old age of the Trichoptera may be represented in their contemporary biogeography, as a number of groups are restricted to modern fragments of Laurasia (North America and Eurasia) or Gondwana (South America, Antarctica, Australia, Africa, India, Madagascar and New Zealand), suggestive of ancient patterns of vicariance. This is perhaps best reflected in the closely related 'Spicipalpian' families of the Rhyacophilidae and the Hydrobiosidae, each almost entirely restricted to Laurasian and Gondwanan fragments respectively. This pattern is also particularly well demonstrated in the Integripalpia, of the 30 established case making families, 13 are mostly Northern in distribution and 14 are mostly Southern (de Moor & Ivanov 2008). Several of these families are found between the regions although remain species (and particularily genera) poor far from their presumed vicariant landmasses. Only the Leptoceridae, Calamoceratidae and Helicopsychidae are well represented in both hemispheres. All Annulipalpian families are essentially found worldwide, which may indicate all families were well established by the time of major continental rifting, or that the groups have since dispersed widely.

New Zealand is characterised by a rich diversity of caddisflies, comprising over 240 species in 15-16 families (Ward 1967), and all species excepting the marine *Philanisus plebeius* are believed endemic (Collier 1993). A number of normally large or important families are species poor or absent, such as the Hydroptilidae which is represented by only 19 species in 3 genera, compared to over 140 species in 15 genera in Australia (Ward & Henderson 2004). Additionally diversity is unusually disproportionate, with one family, the Hydrobiosidae comprising roughly half of all known species (over 100 species in ten genera, with several undescribed, and all genera also being endemic). Other significant families include the Hydropsychidae, Philopotamidae, Leptoceridae, Coneoesucidae and the Oeconesidae although a number of southerly 'gondwanan' families are present in low diversity including the Calocidae, Helicophidae, Philorheithridae, Kokirridae and the Chathamiidae. The Chathamiidae and Oeconesidae are of particular interest as both groups have a predominantly New Zealand biogeography. Five out of six Oeconesidae genera are endemic to New Zealand, excepting

only the monotypic *Tascuna* from Tasmania (Holzenthal *et al*. 2007). Family level endemism of Trichoptera is demonstrated by two families in Australia, one in South America and four in Africa, and may thus also be expected in New Zealand. The Chathamiidae are the only caddisfly family with all species present in New Zealand, although also has the unusual dual distinction of containing the only caddisfly species shared with Australia.

1.3 Marine insects

The insects represent one of evolution's major success stories. Estimated to represent literally millions of species, no other animal group has radiated to such an extreme degree on land or in fresh water (Erwin 1982, Novotny *et al*. 2002). Although the oceans cover two thirds of the planet, and not withstanding over 300 million years of evolution, only a handful of insect groups have colonised any marine environment (Cheng 1976, Grimaldi & Engel 2005). Insects are known to be able to cope with the evolutionary changes in osmotic potential a saline environment presents; inland saline environments even more concentrated than seawater often have thriving insect communities (e.g. Moreno et al. 1997, Herbst 2006, Velasco *et al.* 2006). Why then insects have generally failed to establish themselves in the sea is an interesting and important question in evolutionary entomology.

Many species of insect are found in marine environments and intertidal areas including many species of adult Coleoptera and Hemiptera, although most remain terrestrial and few are truly aquatic. No species of insect, perhaps with the arguable exception of wingless females of the midges *Pontomyia* and *Clunio* spend their entire lives within seawater. Estuarine or brackish areas may or may not be considered to be marine habitats, however here insects can be highly prevalent. For example the larval caddisflies of the genus *Limnephilus* has been in salt marshes surviving in salinities as high as 30% (Flint & Giberson 2005).

Only three insect groups have actively colonised the marine environment with any success, breeding on or within seawater. The sea skater genus *Halobates* comprises some forty species of

wingless true bugs of the Gerromorpha (Hemiptera) which have a pleustonic ecology living on the water surface. All species with one freshwater exception live on marine or estuarine waters, and five species have exceptionally colonised even the open ocean (Polhemus 1982, Cheng 1985). In this instance an ecological transition from the coast to the open sea may require minimal adaptation, and the oceanic lifestyle has evolved at least twice in the genus (Andersen *et al*. 2000). *Halobates* contrast to other marine insects, as all species have essentially adopted a terrestrial existence on the sea water surface.

The other major lineage can be found amongst Culicomorphid flies. Some mosquito species (Culicidae) breed in isolated saline rock pools (Laird 1988) although by far the greatest radiation is represented by non-biting midges of the Chironomidae. At least fifteen genera of Chironomids have marine representatives which together do not comprise a monophyletic group, having evidently invaded the environment several different times in their evolutionary history (Neuman 1976, Colbo 1996). Although most species are restricted to the intertidal, Chironomids can be abundant; for example larvae of the genus *Halocladius* are estimated to be among the most abundant of all macroinvertebrates in some intertidal systems (Grabary *et al*. 2009). Species of *Clunio* and *Pontomyia* live in the subtidal, and although being unrelated, both possess a number of analogous similarities including minute size (a few mm), sinking egg masses, extremely short adult lifespan (as low as ½ an hour), wing reduction in the adult males, and fully wingless larviform adult females (Neumann 1986, Soong *et al.* 1999). As is typical with chironomids, almost all of their ontogeny is spent in the fully aquatic larval phase (Cheng & Collins 1980).

Insects may not be well represented in the seas as a life cycle dominated by a winged adult has no competitive advantage in the marine environment (Cheng 1985). The most specialised of all marine insects, *Halobates*, *Pontomyia* and *Clunio* have totally or partially lost their wings, and likely disperse by means of oceanic diffusion rather than active movement (Ikawa *et al*. 1998, Anderson *et al*. 2000). Other features such as largely immobile larvae (at least for the majority of holometabolous insects), internal fertilisation, and evolution of a watertight cuticle likely only further limit marine colonisation for insects as a whole. Other invertebrate taxa, particularily Crustaceans (likely ancestral

to Insects), already occupy all available niches otherwise filled by insects in land and freshwater. Lacking any competitive potential, it is unlikely insects will re-invade successfully.

1.4 The natural history and biogeography of New Zealand

New Zealand represents an ideal region to explore the processes of biogeography. In spite of its small size and isolation, the island group is geologically of continental Gondwanan origin, and is often referred to in scientific literature as being both reminiscent of a continental landmass and a distant oceanic island (Cooper & Millener 1993, Wallis & Trewick 2009). This statement applies especially well to the flora and fauna; although a number of plants and animals do appear to reflect vicariant (continental or Gondwanan) origins, more consistently groups appear to have more recent origins from nearby landmasses, more congruent with long distance dispersal. New Zealand is a large landmass, and has an active and complex geology, including processes of mountain building, ice age glaciation and volcanism. Additionally New Zealand itself is comprises a vast and diverse archipelago, and includes a large number of distant oceanic islands, in turn with their own geological and biological histories. The natural history of New Zealand is a vast and highly debated subject, and can only be briefly addressed here.

It is undisputed that New Zealand was once part of the ancient supercontinent Gondwana and connected to what is now Antarctica and Australia, as suggested by an ancient terrestrial fossil record and also clearly demonstrated by an extinct mid ocean ridge in the central Tasman Sea formed by sea floor spreading 85 million years ago and ceasing around 20 million years later (Luyendyk 1995, Sutherland 1999 *a*, Trewick *et al*. 2007). This period saw the rifting of the subcontinent of 'Zealandia' of which modern New Zealand forms only a portion today. Zealandia today is mostly observable under the ocean, forming a mass of underwater rises, ridges and plateaus and taken together with the land surface comprises an area of roughly 3 ½ million square kilometres (10 times the size of New Zealand). It is now widely believed that this was once all inactive, flat dry land when still part of

Gondwana (Campbell & Hutching 2007, Trewick *et al.* 2007). Due primarily to erosion and literal tectonic 'stretching' of the crust, most had fallen underwater by the time the Tasman had reached present size roughly 65-60 million years ago. The remainder existed as number of islands, primarily a proto-New Zealand, which continued to sink until the Miocene (23 million years ago), until compressive forces along the plate boundary finally began to uplift new land (Sutherland 1999 *b*). Other areas above sea level, such as New Caledonia, Lord Howe Island and New Zealand's oceanic Islands (such as the Auckland and Chatham Islands) have primarily volcanic origins.

It is currently unclear to what degree the biotas of Zealandia and New Zealand are linked, and some authors have suggested that the two should now be viewed as entirely separate biogeographic entities (Campbell & Hutching 2007, Trewick *et al*. 2007, Landis *et al*. 2008). It is known that during much of the Oligocene (roughly 35-25 Ma) just prior to active tectonism in the Miocene, that most of modern New Zealand was submerged underwater. The event known as the 'Oligocene drowning' has clearly had a considerable effect on the biodiversity of New Zealand, and molecular studies repeatedly display lineages suddenly radiating shortly after this period (Cooper & Cooper 1995, Trewick & Morgan-Richards 2005). However it is now becoming increasingly argued that submergence was total, and for several million years there was no emergent land in the area whatsoever. All New Zealand Oligocene sediments are comprised of marine limestone (Campbell & Hutching 2007, Landis *et al.* 2008), and the New Zealand crust is unusually thin and less buoyant than typical continental crust, which without ongoing geological activity would quickly sink (Trewick *et al.* 2007). Furthermore, there are no geologically old features whatsoever in New Zealand, all known terrestrial geological activity and erosion is Miocene or younger (Campbell & Hutching 2007).

Modern New Zealand clearly does have an unusual and unique biota, and certainly appears to have an old if not Gondwanan origin. Jared Diamond (1990) famously quoted it as being "the nearest approach to life on another planet", reflecting the almost universal scientific opinion of life having been isolated and evolving in the area for tens of millions of years. Among the best known examples include primitive or relicutal taxa representing lineages of probable Mesozoic age, such as the Tuatara (Sphenodontia), Acanthisittid wrens, Leiopelmatid frogs, Ratite birds, the New Zealand parrots

(Strigopidae), Araucarian conifers (only represented by the Kauri *Agathis australis* in New Zealand) and dozens of groups of invertebrates, including insects, crustaceans, arachnids, snails, earthworms and the Peripatus (Gibbs 2006). Nevertheless endemicity is almost entirely restricted to the species and genus level (totally so in terrestrial plant species) and there are only a few endemic animal families (Pole 1994, 2000, Macphail 1997, Gibbs 2006). In many more respects New Zealand has biological characteristics more typical of a recently formed oceanic island, and most New Zealand species are likely to have arrived from long distance dispersal, mostly from the nearby landmasses of Australia and New Caledonia.

Contrasting to 'Zealandia', post-Oligocene New Zealand can be defined as period of dynamic geological activity and land-building, and has been the source of much historical biogeographic study. New Zealand has a distinctly defined biogeography, with many regions supporting high or low levels of local endemism, for example species rich areas in the Southern South Island, North West Nelson, and the Northern North Island (Wardle 1963). There are also a number of important biogeographic disjunctions for several species largely congruent with these regions, for example the well known 'beech-gap' found in the Central-Western South Island, where a large number of species are absent (most obviously the forest forming southern beeches of *Nothofagus*, but also a number of other plants and also animals) in spite of no observable environmental changes (Wardle 1963, Burrows 1965). In the absence of an obvious contemporary cause for these patterns, many are believed to be the result of prior geological or environmental history. Many distributions have been attributed to the movement of the alpine fault since the Miocene, which has had a dramatic effect on the shape and topography of New Zealand. North-South movement of the alpine fault in the South Island has been argued as a recent case of geographic vicariance in New Zealand, as a number of species or related taxa are only present in disjunct regions of North West Nelson and Southern New Zealand, areas that were in close proximity until recently. This pattern is shown in a significant number of species, even in some caddisflies such as the genus *Rakiura*, and has also been proposed as a cause for the beech gap itself (Heads 1988). However this case of local vicariance has also been discredited by some research and this pattern is more likely to represent more recent local extinctions (Wallis & Trewick 2001).

One major geographic event was an extended oceanic submergence of what is now the southern North Island creating the 'Manawatu Straight', an ancient precursor to the Cook Straight, during much of the Pliocene until uplift in the Pleistocene (Lewis *et al*. 1994, Worthy & Holdaway 2002) (The modern Cook Strait by contrast is a more recent Pleistocene feature). This period is likely to have resulted in an early phase of allopatric speciation between the modern North and South Islands and has been proposed as a cause for low biological diversity in the Southern North Island (Gibbs 2006). More significantly intensifying plate movement in the Pliocene saw the early formation of the Southern Alps as a distinct mountain range. The formation of the Alps created not just a formidable geographic boundary for plant and animal dispersal, but also greatly diversified the New Zealand region into a distinct series of alpine environments. Since the late Pliocene to Pleistocene New Zealand has also been affected by the onset of a number of glacial cycles or 'ice ages', the last of which, the last glacial maximum (LGM) ended only roughly 20 ka before present (Suggate & Almond 2005, Denton *et al*. 2010). Coupled with the presence of a new mountain range, these cycles led to a significant level of glaciation, a massively increased alpine zone, and considerable changes in weather and climate. Particularly during the Pleistocene, ice ages led to massively reduced sea levels, and the landmass of New Zealand has repeatedly increased and decreased in size several times over the last few thousand years. These fluctuating sea levels have more importantly repeatedly opened and shut the Cook and Foveaux Straits, significantly affecting dispersal of both marine and terrestrial species (Suggate *et al*. 1978, Fleming 1979, McGlone 1988, Naish 2005). The environmental changes in New Zealand since the Pleistocene in this thesis are especially focal to Chapter Two, and will not be discussed further here.

Another very important geological aspect in New Zealand is the presence of volcanism. New Zealand sits along a plate boundary, which north of the alpine fault comprises an active subduction zone, continuing along the Kermadec and Tonga ridges several thousand kilometers northeast into the Pacific. This region has been the source of active volcanism for just as long as mountain building in the South, since the early Miocene 23 Ma (Graham 2008). The central North Island is considerably affected by volcanism, and the Taupo volcano in particular has resulted in some of the largest of all

worldwide volcanic eruptions in the past several hundred thousand years (Wilson & Walker 1985, Wilson 1993, McDowall 1996, Alloway *et al.* 2007). Eruptions from the central plateau have been implicated in the biogeography of some species in the North Island, and are known to have resulted in a number of local extinctions in the past (McDowall 1996, Alloway *et al.* 2007).

A second significant result of volcanism in the region has been to produce a number of distant oceanic islands, some over 1,000 km from mainland New Zealand. New Zealand is almost completely encircled by distant islands, including Norfolk Island and the Kermadec Islands to the North; the Chatham Islands to the West; and the Antipodes, Campbell, and Auckland Islands to the South. These Islands vary in age and geological origin, although almost all are comprised of volcanic material. The Kermadec Islands represent a continuation of the volcanism directly along the Pacific plate boundary, and are both among the most recent (likely no more than a few thousand years old) and the most active of all New Zealand's volcanic islands. The remaining islands vary in age considerably and have formed typically from ancient basaltic, intra-plate volcanism. Campbell and Auckland Islands are both late Miocene in age (11-7 Ma), Norfolk Pliocene-Miocene (3-2.3 Ma) and the Antipodes late Pleistocene (0.25-0.5 Ma) (Jones & McDougall 1973, Graham 2008). The Chatham Islands formed mostly from volcanism since the past 6 Ma, however do not appear to have emerged until 2 Ma, probably due to tectonic uplift (Campbell & Hutching 2007). All these Islands comprise a number of endemic species of plants and animals, and comprise an important aspect of biogeography in New Zealand; however New Zealand's outer islands are rarely included in contemporary biogeographic studies. Two of New Zealand's oceanic island groups, the Kermadec and Chatham Islands are a focal aspect of Chapter Three of this thesis and are there covered more extensively.

The biological and geological history of New Zealand is long and complex, and here only three areas are to be explored further in any detail in this thesis. The recent Pleistocene glaciations, the age of some of New Zealand's outer Islands, and also the Gondwanan age of New Zealand itself all feature in the Chathamiidae. Thus through use of this small family, this thesis will aim to explore and address the biogeography of New Zealand in a much wider perspective.

1.5 Aims and structure of this thesis.

This thesis aims to focus almost entirely on just a single family of caddisflies, the Chathamiidae, and thoroughly examine it at varying phylogenetic levels. This thesis is divisable into three major areas, each of which is presented in separate chapters as largely independent studies. Each of these three main chapters will also address a theme of a differing biogeographic aspect of the New Zealand region.

Chapter Two will examine a singular species; *Philanisus plebeius*, which will form the basis of a wide interspecific phylogeography. Samples from around New Zealand and Australia will both be used. This chapter will aim to firstly determine the relationship between the New Zealand and Australian populations, and secondly analyse the phylogeographic patterns found within New Zealand specifically. This chapter being based on a single species will be most sensitive to contemporary or very recent historical events. This will include oceanic currents, current geographic boundaries, possible human shipping and also climate and geographic effects during the Pleistocene, particularily the last glacial maximum (LGM).

Chapter Three will aim to examine the phylogeny of all Chathamiid species, and will attempt to improve the known phylogenetic and taxonomic understanding of the family. As two species here are restricted to Islands (the Kermadec and Chatham Islands), this study will also include use of a strict molecular clock to develop age estimates for the Island taxa, and also serve to establish new evidence for the ages of their respective Island groups. This chapter will also include other caddisfly species from the Chatham Islands for use as a comparison with the endemic Chathamiid, *Chathamia brevipennis*. Chapter Four will examine the family level phylogeny of the Chathamiidae and related caddisfly species of several related families, both within and outside of New Zealand. Due to the endemicity status of the Chathamiidae, this chapter will aim to address the Gondwanan nature of New Zealand and the possible vicariant age of this family. Finally Chapter Five will briefly discuss the major conclusions and limitations of this thesis, and will also suggest possible future work that may be undertaken.

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Chapter Two: Genetic structure of an intertidal insect: phylogeography and cryptic speciation of the marine caddisfly *Philanisus plebeius* (Trichoptera: Chathamiidae) from New Zealand and Australia.

2.1 Introduction

The Chathamiidae are a small family of five species of caddisfly (Trichoptera) restricted to the coasts of New Zealand and South Western Australia, unusual as all species known or suspected to breed entirely within the marine intertidal and shallow subtidal environments. As a result the family has received a degree of scientific interest, and the most common and widespread species *Philanisus plebeius* has been the target of ecological and physiological study (Anderson & Lawson-Ker 1976, Anderson *et al*. 1976, Winterbourne & Anderson 1980). However the species still represents a unique opportunity for phylogeographic analysis. The genetic structure of marine insects has been the subject of little research in spite of their evolutionary rarity, and representing a unique link between marine and terrestrial ecosystems.

Very few insects are widely accepted as being marine in nature. A number of species of Chironomid midge (Chironomidae) breed in both the intertidal and subtidal, easily representing the most successful radiation of marine insects. Fifteen genera of chironomids are have marine representatives; most are restricted to brackish or intertidal areas, although tropical species of the flightless genus *Pontomyia* breed well into the subtidal zone as deep as 30 meters in coral reefs (Hashitomo 1976, Neumann 1976, Bretschko 1981, Colbo 1994). Although there has been work on microsatellite variation in the indertidal genus *Clunio* (Kaiser & Heckel 2009), there have been no phylogeographic studies. Flightless Sea skaters of the genus *Halobates* by adopting a pleustonic lifestyle on the water surface are the only insects to have colonised the open ocean, and have been subject to some phylogenetic and phylogeographic study (Anderson *et al*. 2000, Damgaard *et al.* 2000). Chathamiid caddisflies from Australasia arguably represent the only other radiation of fully marine insects, however remain perhaps the least well known.

New Zealand is an ideal locality in which to explore the processes of biogeography, especially regarding the effects of mountain building, volcanism and climate changes since the Pliocene (Cooper & Millener 1993, Markgraf *et al*. 1995, Worthy and Holdaway 2002). Most recently climate and geographical changes due to glacial cycles (roughly 20 in total) during the Pleistocene are well known to have had a considerable environmental effect in New Zealand, with the last such cycle ending 34-18 ka during the last glacial maxiumum (LGM) and modern temperatures appearing around 12 ka (Suggate & Almond 2005, Denton *et al*. 2010). During the LGM temperatures dropped up to 5°C and led to increases of glacial cover and freshwater outwash, principally in the South Island and also localised areas in the North Island (Fleming 1979, Suggate 1990, Pillans 1991, Brook *et al*. 2008, Shakun & Carlson 2010). Lower temperatures greatly increased the alpine zone which also fell to lower altitudes, and forest cover diminished largely to the upper North Island and small localised refugia (McGlone 1988, McGlone *et al*. 1993, Alloway *et al*. 2007). Increased ice caps also caused sea levels to lower by around 120-130 m connecting most of New Zealand into a single landmass; thus marine channels such as Cook and Foveaux Straights were largely closed by land bridges dramatically affecting immigration patterns of marine and terrestrial species (e.g. Suggate *et al*. 1978, Fleming 1979, McGlone 1988, Naish 2005).

The effects of the LGM and past glacial cycles have left a lasting impact still observable in the biogeography and genetic structure of native plants and animals; in terrestrial, freshwater and marine environments. Genetic studies have been conducted on a wide number of freshwater and terrestrial species, finding marked degrees of genetic bottlenecks and postglacial radiations from one or more refugia; including freshwater fish (Wallis *et al*. 2001, King et al. 2003, Waters *et al*. 2007*b*, Waters & Craw 2008, McDowall 2010), stick insects (Trewick *et al*. 2005, Buckley *et al*. 2009, O'Neill *et al*. 2009), freshwater invertebrates (Neiman & Lively 2004, Smith & Smith 2009), bats (Lloyd 2003 *a, b*), *Metrosideros* (Gardner *et al*. 2004), beetles (Leschen *et al*. 2008, Marske *et al*. 2009), cicadas (Marshall *et al*. 2009), skinks (Hare *et al*. 2008) and frogs (Fouquet *et al*. 2010). Most of these examples have a South Island focus, North Island studies by contrast often refer to volcanism from the central plateau as an important factor (McDowall 1996, Gardner *et al*. 2004, Smith *et al*.

2006 *a*, *b*, Shepherd *et al.* 2007, McDowall 2010). However in spite of extensive research, most studies are focused to those most likely to be sensitive to these environmental changes and are thus are usually biased to inland or habitat-specific species, and do not often include both islands.

The subtidal and intertidal biota of New Zealand has also been the subject of similar study. Observed genetic structuring is generally indicative of contemporary geographical or oceanographic boundaries, most importantly a North-South divide roughly congruent with the North and South Islands (Apte & Gardner 2002, Sponer & Roy 2002, Waters & Roy 2004, Ayers & Waters 2005, Goldstein *et al*. 2006, Jones *et al*. 2008, Shears *et al*. 2008, Ross *et al.* 2009). This is usually argued either due to the presence of the Cook Straight since the LGM (Apte & Gardner 2002), or an older separation from prolonged upwelling off the North-Eastern coast of the South Island acting as a barrier for dispersal (Waters & Roy 2004, Ayers & Waters 2005, Goldstein *et al.* 2006). Evidence for postglacial radiations southwards from northerly refugia have also found been for some marine species, presumably due to intolerance of climate, water current or coastline changes associated with glacial periods (Stevens & Hogg 2003, Fraser *et al.* 2009, Hickey *et al*. 2009).

2.1.1 The study organism: *Philanisus plebeius*

The Chathamiidae are of special interest to New Zealand in particular, as the family comes close to full endemicity status. Four of the five species of the Chathamiidae are endemic to New Zealand and have comparatively restricted distributions; one is restricted to the Kermadec Islands, one to the Chatham Islands, and two in the Northern North Island and nearby Islands. The fifth species *Philanisus plebeius* has a wider distribution and is found throughout New Zealand, from the Three Kings Islands in the north to Fiordland in the south, and possibly as far south as Northern Stewart Island. This species has also been known to exist in New South Wales since 1904 (Hudson 1904), and has been confirmed to be the same species as that in New Zealand (Riek 1970). Despite its wide range, *P. plebeius* does have a number of apparent disjunctions in its distribution (see Fig 2.1), and has not been collected from large areas of New Zealand's coastline, including most of the west and

southern coasts of the South Island; the coasts between Canterbury and Dunedin in the South Island, from East Cape to Hawke's Bay in the North Island, and most of west coast of the North Island (with the exception of records from the Taranaki region). There is one record from Stewart Island, a large gravid female collected in 1980 and labelled as 100 m altitude far from the coast (Ward 1994). This may be a misidentification or a recording error; there are no other records of *P. plebeius* from the Island. The distribution in Australia is less well known; however appears to extend roughly from Sydney (roughly equal in latitude for the species northernmost distribution in New Zealand at the Three Kings Islands) for about 200km of coastline southward. The species does not appear to occur further south in Victoria or Tasmania in spite of these areas being closer to in latitude to most of the species' distribution in New Zealand, and thus likely suitable for colonisation.

P. plebeius breeds exclusively in seawater and most commonly in the rocky intertidal associated with coralline algae, but can be found in variety of habitats such as the shallow subtidal and even in seagrass beds in soft substrate; where the cryptic, herbivorous case-making larvae forage underwater as deep as 2 m (Riek 1976).This existence in full seawater is the highest salinity (35%) tolerated by any caddisfly (Kefford *et al.* 2004, Flint & Giberson 2005). Pupation also occurs within seawater and the adults emerge late spring early summer, although can be found almost year-round indicating a long adult lifespan (Cowley 1976, Riek 1976, Anderson & Lawson-Kerr 1977, Ward *et al*. 1996). The species also demonstrates the extremely unusual behaviour of ovipositing its eggs within the coelom of starfish of the genera *Patiriella* and *Parvalustra*, whereupon the first instar larvae leave shortly after hatching (Anderson *et al*. 1976, Winterbourn & Anderson 1980). This behaviour is likely more commensal than parasitic, and the starfish is believed to provide a stable environment in which development takes place, and appears more or less unaffected (Anderson & Lawson-Kerr 1977). It is unclear whether this behaviour is obligate (essential for development) or facultative (opportunistic). How this symbiosis may affect or restrict biogeography or dispersal of *P. plebeius* is unknown, although species of *Patiriella* and *Parvalustra* are abundant and widespread in various subtidal and intertidal habitats New Zealand and Australia.

The trans-Tasman distribution of *P. plebeius* is exceptional; so far all remaining species of caddisfly in New Zealand are fully endemic (Collier 1993). It has been suggested that the Australian population is an accidental human introduction due to shipping (Gibbs 2006), which would make the Chathamiidae as a whole naturally endemic to New Zealand. Human shipping is commonly implicated in the transportation of otherwise poor dispersing marine species, and has been implicated in at least one introduction of a marine chironomid in Western Europe (Brodin & Andersson 2009, Raunio *et al*. 2009). By contrast Winterbourn and Anderson (1980) suggested the Australian population is ancestral as the Australian species *Parvalustra exigua* (formerly *Patiriella exigua*) are more abundant and found closer to shore than *Patiriella regularis* from New Zealand, and thus a more likely candidate for the original host species. However since *Philanisus plebeius* is more widespread in New Zealand and all the remaining species of the Chathamiidae are endemic, it is more parsimonious to consider a New Zealand origin for the species.

Philanisus plebeius has the unique distinction of being an organism found nation-wide with the ecological requirements of a marine species and is thus open to passive transport by marine processes, including currents, rafting of algal wrack or even human shipping. Being fully flighted at adulthood it also behaves as a typical terrestrial insect for a significant portion of its lifespan. Being a member of a largely endemic family, this species is also likely to represent a lineage present in New Zealand for some time, and fully exposed to historical environmental changes. As a result this species has no comparative ecological or biogeographical analogy in New Zealand, and thus presents a unique species for phylogeographic study.

2.1.2 Aims

This study will aim to determine the phylogeography of *Philanisus plebeius*, with two aims. Firstly to establish the genetic relationship between the Australian and New Zealand populations; to determine which region was ancestral, and whether the trans-Tasman distribution is due to natural dispersal or a recent human introduction. Secondly, phylogeographic structure of the population in in

New Zealand will be investigated. Correlations with historical processes, especially Pleistocene climate and geographic changes will be tested and compared with patterns found of other species representative of the freshwater, marine and terrestrial.

2.2 Materials and Methods

2.2.1 Sample Collection.

146 Samples of *Philanisus plebeius* were used in this study from specimens collected specifically for this study from November 2008 to February 2010 from several sites in Australia and both main Islands of New Zealand (see Table 2.1). All New Zealand material was collected by the Author or Dr Ian Henderson (Massey University), whereas Dr Alice Bell (Australian Biological Resources Study, Canberra, Australia) supplied Australian specimens. Adults were primarily collected during dusk and night hours during late summer to early autumn by means of a basic UV light trap. The trap consisted of a 12 v black light powered by a standard 12 v 7.2Ah battery, suspended over a water tray with a few drops of detergent. The trap was situated that it would be visible over a wide area of coastline, close to rocky intertidal habitat if possible, and left for typically under an hour. Adults were also occasionally collected by hand during daylight when possible. Larval and pupal material was also collected year round by hand-searching through quantities of coralline algae from the rocky intertidal. Since the larval stages of the Chathamiidae are either unknown or largely indistinguishable morphologically, larval material was collected from areas only inhabited by *P. plebeius* (outside of the Northern North Island).

Samples were placed directly into 70-80% ethanol in the field and then transferred to 95% ethanol and refrigerated at 4°C for laboratory work. Adults were identified to species in laboratory, with *P. plebeius* differentiated from the sympatric *Philanisus mataua* and *Chathamia integripennis* based on descriptions by Riek (1976) and Ward (1994, 1995). Samples of *Chathamia integripennis*

(from Northern New Zealand) and *Philanisus fasciatus* (Kermadec Islands) were also used as outgroups for phylogenetic analysis (Refer to chapter three of thesis further regarding these species).

2.2.2 DNA sequencing and alignment.

DNA was extracted using a standard phenol-chloroform method, using 1-3 whole legs from adults, mature pupae or late-instar larvae. A small fragment of abdominal tissue was used from very small, pupating or damaged larvae only. The remainder of specimen was then left intact and stored in ethanol for future reference.

A 618 base pair fragment of the protein-coding mitochondrial gene Cytochrome oxidase I (COI) was amplified using the primers LCO1490 and HCO2190 (Folmer et al. 1994, see Table. 2.2). Each reaction template was run in a thermocycler for a 95°C hot-start for 5min; 40 cycles of 30s at 95°C, 30s at 48-50°C and 30s at 72°C; followed by a final extension phase for 10min at 72°C. Products were visualised through gel electrophoresis, purified using 0.5µl ExoSAP-IT DNA purification kit (Global Science) and sequenced using the primer HCO2198 only.

Sequences were imported into Clustal X algorithm in MEGA 4.0 (Kumar *et al.* 2007, 2008) and aligned using default parameters. COI sequences of *P. plebeius* and *C. integripennis* from published studies (Hogg *et al*. 2009, Johanson *et al*. 2009) were imported to facilitate alignment and confirm mitochondrial origin although were not included for analysis.

2.2.3 Genetic Analyses

The COI dataset statistics were generated using the MEGA data explorer tool and DnaSP v5 (Librado & Rozas 2009). TCS 1.21 (Clement *et al*. 2000) was used to construct a parsimony network, sorting the data into observable haplotypes and to visualise the primary phylogenetic structure and distance. The analysis grouped the sequences into two major haplogroups (“A”, and “C”) with a third

minor, intermediate haplogroup "B" identified from a single sample. All identified haplotypes were named firstly by their respective grouping and then numbered respective to total abundance (e.g. haplotype "A1") with "1" representing the most numerous. Pairwise distance statistics were performed in DnaSP and used to construct a mismatch distribution chart as to display genetic distances and population structure. Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) statistics were calculated in DnaSP for the complete dataset and independently for both of the two major haplogroups in order to test for recent demographic expansions.

Analyses of molecular variance (AMOVA) were performed in Arlequin 3.11 to observe genetic relationships at varying geographical hierarchies using data from New Zealand samples of haplogroup A only. Analyses were run using a distance matrix model with 10,000 permutations. Sequences from Australia were omitted from this analysis as they comprised a single unique haplotype, as well as the large geographic distance involved. Also not included were samples from Tauranga and New Plymouth which had less than 6 samples and were considered too geographically isolated from other collection sites to be grouped together. Groupings were based on assumption of a geographic and genetic break South of Kaikora, commonly observed in marine species. Groups from the upper North Island through to Kaikoura were assessed independently (using the geographic groups "Wellington", "Wairarapa", "Auckland" and "Upper South Island"). These groups were then combined (Upper New Zealand) and compared to the further groups "Dunedin" and "Christchurch". In order to improve statistical and geographical robustness, areas in close proximity with small sample sizes were combined into singular 'populations' (distinct from the AMOVA groupings), this included 'Wellington' which combined Pukerua Bay (6), Makara (3), Lyall Bay (1), Pauatahanui (3) and Breaker Bay (12); and 'Auckland' which merged Auckland Harbour (14) with Waiwera (3) (see map in Fig 2.7).

Phylogenetic analyses were also performed on the dataset to further explore the relationship between the three haplogroups using the software packages MrBayes 3.1 (Heulsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) and PAUP* 4.0 (Swofford 1999). Model selection was implemented with MODELTEST 3.7 (Posada & Crandall 1998). The dataset was reduced into single

sequences for each of the identified haplotypes irrespective of locality to minimise computation time. *Philanisus fasciatus* and *Chathamia integripennis* were used as outgroup taxa.

Maximum parsimony (MP, Farris 1970) and Maximum Likelihood (ML, Felsenstein 1981) trees were estimated using PAUP*. Maximum parsimony analysis was performed using a heuristic search model, and bootstrapped using 10,000 replicates. MODELTEST was used to identify the most suitable evolutionary model using the Akiake information criterion (AIC), selecting an HKY + I model. The same model was then implemented for the ML estimates, which was run using 10,000 replicates for bootstrap support with a heuristic search model. In addition a Bayesian analysis was run in MrBayes, once again using the HKY + I model. One cold and three heated Markov chains were run for a total of 50,000,000 generations sampled every 10,000 to obtain a total of 5,000 trees. The first 1,000 trees (25%) were discarded as a burn-in phase, with the last 4,000 trees used to estimate the posterior probabilities.

2.3 Results

2.3.1 Sample Collection

Light trapping only worked during warm, calm nights and some areas yielded no samples (Kaipara Harbour, Muriwai beach, Oamaru, Kaka Point and Curio Bay; see Fig 2.7), or very few (New Plymouth, Mt. Maunganui or Tauranga estuary had only 2 samples each). The remainder of the localities however yielded a larger number of samples, including from rocky, sandy and estuarine beaches. A small number of specimens were also collected from Inland Pukerua Bay, well above sea level and almost a km from the coast. All specimens used for sequencing are listed in Table 2.1.

2.3.2 Sequence data

The dataset comprised 146 sequences of *Philanisus plebeius* (not including outgroups), with a total 22 recognisable haplotypes (see Tables 2.3 and 2.4). Distance analysis strongly indicated a divergence into three identifiable haplogroups (A, B & C). Group 'A' comprised 14 haploytpes, 'B' only one, and 'C' seven. Haplogroup C was found in only Napier and Mangakuri beach, and comprised all sequences from these regions. In total the dataset was 618 base pairs long, 23 sites were variable and 14 parsimony informative. Within the two major groupings 'A' and 'C', 12 and 6 were variable and 2 and 4 were parsimony informative respectively. Base pair frequencies were unequal, averaging 40% T, 14.8% C, 32.8% A and 12.4% G across all sequences.

2.3.3 Haplotypic and phylogenetic structure.

Pairwise distances between haplotypes, and also within and between groups are shown in Tables 2.5 and 2.6. The uncorrected pairwise distances were then used for the basis of a mismatch distribution chart (Fig 2.2). The mismatch distribution chart shows a distinct bi-modal peaking, although this only visibly represented groups A and C, due to the rarity of haplogroup B. The relationships between the haplotypes are also explored in a network analysis in Fig 2.3. The majority of the dataset fell into the 'A' grouping, with group 'B' assigned to just one sample, and a secondary major clade 'C' restricted to just two closely associated localities near Hawkes Bay (Napier and Mangakuri Beach). Overall, genetic distance within *P. plebeius* was high (a mean estimate of 1.04%, and a maximum divergence of 1.97%, estimated from the Kimura-2 model), although distances between and within the clades varied. Haplogroup A was dominated by a single haplotype (A1) from which most remaining haplotypes of this group differed from by a single base pair, including those from Australia. Only one (A8) differed by two changes. In contrast the C group showed a more complex and divergent haplotype network with a relatively bifurcating structure, with a maximum of three base pair changes from the presumed ancestral haplotype (which was not found). Tajima's D and Fu's F_S statistics are shown in Table 2.7. Tajima's D was significantly negative for haplogroup A,

indicating a population expansion; and was non-significant and weakly positive for haplogroup C (indicating stability). Fu's test by contrast was significantly negative for all cases indicating demographic expansion throughout the species, although this was much more pronounced in haplogroup A than in C.

The bayesian, maximum likelihood (ML) and maximum parsimony (MP) analyses were roughly consistent in all consensus trees and monophyly of *Philanisus plebeius* was supported (see Figs 2.4-2.6). Group A was monophyletic, weakly suggested to nest with the B haplotype to form a sister grouping, although supported only by the MP and ML analyses. However the C haplogroup was not strongly supported as monophyletic, at best supported by 65% consensus in the MP analysis, basal to the other two groupings.

2.3.4 Phylogeographic Structure

Haplotypic structure showed some degree of geographical association (see Fig 2.7). The 14 identified A group haplotypes of *P. plebeius* constituted by in large the majority of all samples and localities from this study, including Australia, most of the North Island and all of the South Island. All samples from Australia represented a single haplotype (A3), which although not found in New Zealand differed by a single base pair from the widespread A1 haplotype. The A1 haplotype dominated most of the genetic structure in populations from Auckland south to Kaikoura, and occurred in one sample from Dunedin indicating an almost nationwide distribution. Haplotypic structuring was more distinctive in the lower South Island, sites in Dunedin (Portobello) and Christchurch (Akaroa) were each dominated (~90%) by haplotypes mostly only found in these areas; haplotypes 'A2' and 'A4' for Dunedin and Christchurch respectively. Wide genetic connectivity was indicated however, haplotype A2 was found in Kaikoura, Akaroa and Wellington and even Auckland in small proportions, and haplotype A4 also occurred in Kaikoura. Another common haplotype 'A5' was restricted to New Plymouth and Wellington suggesting South-Western North Island connectivity. The remainder of the haplotypes were generally rare and localised with one ('A6') being found once

in three localities in the North Island (Tauranga, Wellington and Akitio on the Wairarapa coast). The most divergent 'A' haplotype (A8) was found only in Tauranga.

Of the other two haplogroups, the C group was shown to be restricted to just two single localities near Hawke's bay in the North Island (Napier and Mangakuri beach). Of the seven haplotypes found within this group, all showed a degree of geographical association, roughly correlated with the pattern found in the network analysis. Of all haplotypes, only one (C2) was found in both localities, although evidently more common in Napier (50% of samples as opposed to 12% in Mangakuri). Haplotypes C3 and C5 were restricted to Napier and closely related to the other major Napier haplotype C2. The remaining haplotypes C1, C4, C6 and C7 were restricted to Mangakuri beach, and similarly appear to form a monophyletic grouping. Haplogroup B was found in just a single sample from Tauranga, sympatric with A group haplotypes.

Analysis of molecular variance (AMOVA) results are shown in Table 2.8. P-values were shown to be largely non-significant, suggesting low genetic structuring to be found within New Zealand populations of 'A' type *P. plebeius* in New Zealand. In the upper New Zealand grouping, distance within populations accounted for roughly 95% of the variation found, demonstrating geographic structure to be almost entirely absent. Geographic structure was much more evident when upper New Zealand (Auckland to Kaikoura) was compared to Akaroa and Portobello, with 51% of the variation found between groupings. Overall, clear geographic structuring was not found in haplogroup A; all P values were non-significant with the exception of within populations (FCT) in the North-South grouping. The AMOVA tests did however not include Tauranga, Hawke's bay and Australia (due to small sample size, or in the case of Australia, extreme distance), areas with a more pronounced geographic and genetic structure.

2.4 Discussion

2.4.1 Internal Relationships of *Philanisus plebeius*

The most striking result found in this study was the geographic and genetic division of *Philanisus plebeius* into at least two major identifiable groupings. Whereas samples found throughout Australia and most of New Zealand were genetically similar (haplogroup A), samples from two localities in the central east coast of the North Island (haplogroup C) were found to be hightly genetically divergent from the remainder. Neither of these haplogroups was found to occur sympatrically in any region, indicative of a 'category I' phylogegraphic pattern from Avise *et al.* (1987). A further distinctive haplotype 'B', intermediate between the two major groupings was found in a single specimen from Tauranga. The genetic distance between the two major haplogroups is at least twice that found within them, approaches that found between *P. plebeius* and other Chathamiid species (see Chapter Three), and is considerably higher than typical interspecific diversity found within caddisflies (Hogg *et al*. 2009).

A phylogenetic analysis of the data did not develop clear results, with the exception of supporting the monophyly of haplogroup A. Haplogroup C was inferred to be basal in the MP and ML trees (but this not well supported by the Bayesian tree), with groups A & B forming a sister relationship. However haplogroup C was not strongly indicated as being monophyletic in the phylogeny, and was left largely as a polytomy, paraphyletic to the remainder of the species (monophly was only ever weakly supported in the MP tree). At best then the data is probably best represented in an unrooted network as shown in Fig 2.3. The genetic distance within haplogroup C is demonstrated significantly higher than within the other clades, with two groupings represented, separated by two base pair changes and not joined by an existing intermediate haplotype. Distances between the C haplogroup and other haplotypes are considerably higher than within however, and it does seem parsimonious to assume a monophyly of all 'C' haplotypes. Haplogroup A by contrast appears to reflect almost entirely a single radiation from just one widespread and common haplotype (A1), from which most of the remaining haplotypes differed by a single base pair. One northerly haplotype (A8)

uniquely differed by two base pair changes and was closer to the other haplogroups, and therefore may be ancestral to A1, via another extant haplotype (either A6 or A13, although A6 seems more probable, being comparatively widespread and occurring sympatrically with A8 and also haplotype 'B').

Also importantly, Haplogroups A & C had strong geographical associations. Haplogroup C was found only to occur within just two close localities while haplogroup A apparently constituted the large remainder of the entire species' distribution. The limited distribution of haplogroup C is also surprising when the higher genetic diversity of this grouping is considered. Group C unlike group A shows a deep structure not suggestive of a single radiation, despite its much smaller distribution. This was also supported by Tajima's tests, suggesting a more or less stable demographic structure or history of group C, and a bottleneck or a sudden demographic expansion affecting group A. The evolutionary history of the two major groupings is thus presumed to be widely different.

2.4.2 Phylogeography of Haplogroup A & B localities and origin of the Australian population.

Within all localities, excepting Mangakuri and Napier near Hawke's Bay in the North Island, there was little observable genetic structuring. Diversity within sites was generally not high, and was found to be highest in Wellington (6 haplotypes), the area with the widest collecting (5 closely associated localities) and the largest sample size (25). Samples from Auckland to Kaikoura in the South Island were dominated by one haplotype (A1), while samples from Australia, Akaroa and Portobello were dominated by haplotypes uncommon or absent elsewhere. The observed change between Kaikoura and Dunedin is consistent with a number of marine species in New Zealand, likely due to a zone of upwelling serving as a barrier for oceanic dispersal (Apte & Gardner 2002, Stevens & Hogg 2004, Waters & Roy 2004, Ayers & Waters 2005, Veale 2007, Ross *et al*. 2009, Sutherland *et al*. 2010). However being a marine pattern, this would not affect the migration of adults. The dominant haplotype in Christchurch is also found in Kaikoura, and only 2/14 haplotypes were unique to the South Island (none south of Kaikoura).

Both New Plymouth samples were of one haplotype (A5) only found elsewhere as a common type in Wellington, suggesting a possible South-Western North Island connection. However sampling there was low there, and is predicted to contain other haplotypes, notably haplotypes A1 and A2 (New Plymouth lies between Auckland and other genetically similar populations which would imply a continual connectivity, unless A group haplotypes are able to bypass populations on the east coast). Tauranga was also shown to be dominated by rare haplotypes constituting the only known 'B' haplotype, sympatric with 'A' type samples including the only known location of haplotype A8, and also haplotype A6 (both probably close to the ancestry of haplotype A1 and all other A haplotypes). The small number of samples from New Plymouth and Tauranga (only 2 and 4 respectively) does limit what can be confidently inferred from these observations. The evolutionary diversity exhibited within Tauranga was not repeated elsewhere despite much more thorough sampling, suggesting possible higher diversity in northern areas.

Both Australian localities showed the occurrence of just a single haplotype (A3), with a complete absence of genetic variation indicated. This total of 14 samples from two sites 12 km apart exhibiting no variation is very different to the situation in New Zealand, where samples over 10 in size from a single location typically comprised at least 2-3 haplotypes. Phylogenetically the Australian haplotype was typical for its haplogroup, having most likely originated from haplotype A1 recently. Thus the Australian population is strongly indicated a recent singular dispersal event from New Zealand. No evidence for contemporary connectivity between New Zealand and Australia was shown. However as the Australian haplotype was not found in New Zealand, a human dispersal within the last 100-200 years appears too recent to allow for sufficient genetic drift to have occurred, and must be rejected at present. However there remains a strong possibility that A3 does exist in an unidentified locality in New Zealand, or has been recently lost due to lineage sorting.

It is clear that the 'A' lineage is a radiation event following a very recent genetic bottleneck, strongly supported by Tajima's D and Fu's F_S tests. All of haplogroup A with the probable exception of the rare northerly haplotypes A6 and A8 appear to have radiated from the A1 haplotype. Estimating a divergence of this radiation is complicated by the issue that the ancestral haplotype still occurs

within most localities, although a substitution percentage of 0.16% can be used (1 base pair change). Using a molecular clock such as that of Brower (2.3% per mya 1994) gives an age estimation of roughly 70 ka, whereas another recent molecular clock of 3.59% for insects (Papadopoulou et al. 2010) gives a younger age of roughly 45 ka.

Brower's clock in particular has been known to significantly overestimate the age of divergences however, for example giving a similar date for a radiation in the butterfly species *Parnassius mnemosyne* more likely to have occured ~19 ka (Gratton *et al*. 2008). Estimation of divergences for young dates due to inference from very small numbers of substitutions allows for a very large margin of error. Additionally as the most likely ancestral haplotype is still dominant through most of the distribution, evolution into new haplotypes is still minimal. Thus it seems likely that this divergence is no older than roughly 18-20,000 years corresponding with the rough age of the end of the last glacial maximum (LGM).

It is probable that haplogroup A and all of *P. plebeius* as a whole was reduced to northern New Zealand and has thus spread southwards since this time, which may also explain the largely northern dominance of haplotype A1, as well as the northern restriction of haplotypes not directly linked to the presumed southern expansion (particularily haplotypes A6, A8 and also the single B haplotype). This hypothesis is supported as ten of the fourteen 'A' haplotypes were found only in the North Island, and only two to the South Island (and only to areas north of Kaikoura). This inference is also supported by the complete absence of other haplogroups, and also other Chathamiid species south of the upper North Island. The wide distribution of the other most common haplotype A2 may also represent this expansion rather than current connectivity, and its dominance in Dunedin may simply represent a stochastic founder event rather than having evolved *in situ*.

Outside of New Zealand there have been numerous studies on freshwater trichoptera demonstrating Pleistocene contractions of populations, followed by re-dispersal during the interglacials from refugia (Wilcock *et a*l. 2001, Baker et al. 2003, Pauls *et al*. 2006, Murria & Hughes 2008, Previšić *et al.* 2009, Lehrian *et al*. 2009, 2010, Kubow *et al*. 2010). Postglacial radiations since

the LGM are known in New Zealand from a number of marine, freshwater and terrestrial examples. More specifically, radiations from northern refugia have been implicated; including fungus beetles (Leschen *et al*. 2008, Marske *et al*. 2009), cicadas (Marshall *et al*. 2009), Stick insects (Trewick *et al*. 2005, Buckley *et al*. 2009, Morgan-Richards *et al*. 2010), bats (Lloyd 2003 *a*, *b*), skinks (Hare *et al*. 2008), Frogs (Fouquet et al. 2010), the Rātā genus *Metrosideros* (Gardner *et al*. 2004) and marine tripplefin fish (Hickey *et al*. 2009). Postglacial radiation has even been demonstrated in the starfish *Patiriella regularis* (Waters & Roy 2004), with which *P. plebeius* is commensally associated, or even dependent, which adds further support to a glacial retraction of *P. plebeius*.

Philanisus plebeius does appear to be environmentally sensitive and most of the species' apparent disjunctions can probably be ascribed to environmental limitations. Much of the west coast of both islands seems to be uninhabited by the species, likely due to being fully exposed to the west wind drift and thus subjected to high energy wave action and disturbance, and comprises mostly unstable gravel or sand substrates (e.g. Heath 1984, Ewans & Kibblewhite 1990, Hart & Bryan 2008, King et al. 2009). There are also large amounts of alpine freshwater outflow in the South Island, potentially effecting marine communities (Bradford 1983). Other disjunctions appear to be temperature dependent. Due to the action of the subtropical convergence belt, cold water at near Stewart Island flows up eastern coast of the South Island to near Banks peninsula, thus water temperatures on the east coast are lower than similar latitudes on the west (Heath 1982, Greig et al. 1986, Carter *et al.* 1998, Barrows & Juggins 2005). These areas are characterised only by one single record from Stewart Island, and one confirmed population around Dunedin. Attempted collections on the South coast in this study (Kaka Point and Curio Bay on the Catlins coast) and between Dunedin and Canterbury in the North (Oamaru) found no samples. The Dunedin population may be exceptional as there are no confirmed records outside the Otago harbour. The harbour is likely significantly more sheltered than the surrounding coastline, although surface temperatures there are still known to be low (Greig *et al*. 1988).

It is thus possible to hypothesise the southernmost limit of *P. plebeius*, likely restricted mostly north of the line characterised by 15°C of warmest monthly sea temperature (Barrows & Juggins

2005), (which expands to 13C° if Stewart Island and Dunedin are included) (see Fig. 2.7). Warmest water temperature is a likely determinant, probably being important for completing development and stimulating adult emergence (other annual temperatures still display more or less the same regional structure however). Under these assumptions and using data from Barrows & Juggins (2005), *P. plebeius* would have been restricted during the LGM to what is now the upper North Island, a contraction significantly more pronounced if modern disjunctions are also assumed. Thus *P. plebeius* can most likely be considered still existing in the genetic aftermath of a postglacial radiation. The South Western coast of the South Island and most of Northern New Zealand were not sampled, however on the basis of this analysis these are predicted to show low and high genetic diversity respectively.

2.4.3 Cryptic diversity in *P. plebeius*: the origin and identity of haplogroup C.

The status of haplogroup C is of particular interest due to its significant divergence from haplogroups A and B, combined with a discrete geographic restriction including Hawke's Bay and some of the coastline southwards. Using the molecular clock of Brower (1994), the C group diverged from the other groups anywhere between 265-570 ka before present (using the lower divergence between haplogroups B and C), and itself radiating perhaps as long ago as 350 ka. As stated earlier, these dates are likely considerable overestimates but indicate an origin long before the LGM. Phylogeny appears closely correlated with geography, the haplotypes found closely falling within two genetic lineages, each mostly distinct to either Mangakuri or Napier suggesting at least two reproductively isolated populations. Thus migration even within this small region is shown to be low or absent. The populations comprising haplogroup C are suggested as having reached a relative genetic equilibrium, or at most, affected only by a minor demographic expansion.

Even more significantly, haplotypes were shown to switch from type C abruptly to type A between samples from Mangakuri and Whangaehu beaches, a transitional break of just 54 km. It is unknown whether *P. plebeius* occurs between the areas; however as coastal morphology and habitat is

more or less continuous thus it appears probable. A possible genetic turnover or population gap may occur at the outflow of the Porangahau River providing estuarine habitats and sandy subsrate unsuitable for breeding. Adult samples were collected in much larger estuaries in this study alone (Pauatahanui inlet near Wellington and Tauranga estuary), which does suggest a tolerance of the habitat at least for adults.

Alternatively, a genetic barrier may relate to offshore currents. The circular Wairarapa eddy occurs near offshore, and the Southerly flowing East Cape current and the northerly flowing Wairarapa coastal current both converge near Hawke's bay (Heath 1982, Carter *et al.* 1998). Both or all these may represent a likely considerable barrier for marine coastal species, deflecting immigrants and preventing successful emmigration. Largely basal haplotypes from the same region have been observed in a reproductive brooding fish species, the seahorse *Hippocampus abdominalis* (Nickel 2009). Limpet samples from near Napier were shown to represent an allopatric population of a cryptic species, although this taxon had an apparent disjunct distribution being sympatric with a related species in two sites in northern New Zealand (Nakano & Spencer 2007). As similar to the genetic disjunction in the south Island this once again raises the question of larval over adult dispersal in *P. plebeius*.

The Northern boundary was not identified in this study but *P. plebeius* are uncollected from the coastline north of Napier through to East Cape. The coastline of this region appears to have appropriate habitat, including rocky shores. Therefore this 'disjunction' may reflect sampling bias, and the species may be fully present. However absence in this region is congruent with volcanism from the central plateau. Taupo volcano is among the most active rhyolitic volcanoes in existence; erupting nearly 30 times in the last 30,000 years with the last such event in 186 AD, and the largest (the Oruanui/Kawakawa eruption ~26.5 ka) producing 1200km^3 of material (Wilson & Walker 1985, Wilson 1993, McDowall 1996, Alloway *et al.* 2007). The outflows from each eruption tend to have spread eastwards and centralised tephra layers from the Oruanui/Kawakawa eruption 2m in depth have been found as far eastwards as Hawke's bay (Wilson 2001, Alloway *et al*. 2007, Lowe *et al*. 2008). Volcanism, including the recent 186 AD eruption has been well evidenced in the current

biodiversity of the eastern North Island, especially the freshwater fauna (McDowall 1996). Eastward ashflows from the 186 AD eruption in particular fit with the apparent disjunction of *P. plebeius* in the eastern North Island, although the effects of volcanism not been demonstrated as of yet in any marine species in this region, and the presence or absence of *P. plebieus* in this still region requires confirmation.

During the last glacial maximum and previous glaciations, the area including Hawkes Bay and immediately southwards may have represented the southernmost distribution of *P. plebeius* (see Fig 2.7). If the East Cape region is assumed to be uninhabited by *P. plebeius*, due to volcanism or otherwise then this area could have represented a fully isolated population, at least during glacial cycles. Repeated glacial cycles over time combined with genetic drift may have eventually allowed for a high degree of molecular divergence to develop. However if volcanism is implicated as above, then events such as the Oruanui/Kawakawa eruption which occurred during the LGM would have obliterated populations much further south than the 186 AD eruption, weakening this hypothesis somewhat.

A hypothetical separation of the ancestral populations of each of the two main haplogroups may explain current genetic differentiation, although why gene flow has apparently never resumed, or apparent lack of any dispersal whatsoever, is unclear. Geographic relationship between the A and C groupings appears peripatric rather than allopatric, and contact between adults seems highly probable. Caddisflies of the genus *Gumaga* in California have been found to represent an apparent cryptic species complex, maintaining reproductive isolation from one another in spite of close proximity (Jackson & Resh 1998), suggesting this pattern may be widespread. If genetic difference is sufficiently high then species are likely to exhibit assortative mating and thus not interbreed. Sex pheromones are confirmed to be important in mate recognition in a wide diversity of species in trichoptera (e.g. Wood & Resh 1984, Jackson & Resh 1991, Larsson & Hansson 1998) Although individuals of each genetic group may appear physically indistinguishable, sex pheromones may differ substantially enough to prevent mate recognition; a trait known to occur otherwise physically

similar cryptic insect species (Foster *et al*. 1991, Maingon *et al*. 2003, Watts *et al.* 2005, Cáceres *et al*. 2009).

The high genetic distance, as well as possible assortative mating and geographical conservatism suggest that haplogroup C may represent an unidentified cryptic species. Morphological differences were not clearly observed with the possible exception of decreased wing length (Ian Henderson, personal communication). Confirmation of this however requires a thorough analysis of wing length and shape, a morphological attribute likely to show a high degree of phenotypic plasticity; likely correlated to sex (females are typically larger) and developmental history. More thorough sampling to identify the full distribution of the 'C' haplogroup, or identifiable physical features; need to be identified before separate species status is proposed. For the time being at least, it seems practical to retain *P. plebeius* as a singular species, although the possibility of superspecies or species complex is indicated by this study.

2.4.4 Inferred molecular ecology of *Philanisus plebeius* and conclusions.

From this study it seemed to be probable, at least in some areas, contemporary gene flow and overall dispersal and immigration of *Philanisus plebeius* was fairly low, although this pattern was obscured by low genetic diversity through most of the populations. Adult *P. plebeius* are rare inland; in this study occasional samples roughly 1km from the coast were collected, and maximum distances up to 3km away have been shown in some records. As is generally typical of caddisflies, adults tend not to travel far from suitable breeding habitat and dispersal tends to be along water bodies rather than between them (e.g. Kovats *et al*. 1996, Collier & Smith 1998, Griffith *et al*. 1998, Peterson *et al*. 1999, 2004). As a result, *P. plebeius* likely disperses almost entirely along the coast, requiring flight distances over hundreds of kilometres to link some isolated populations. This seems difficult to accept regarding populations in or around the distribution of the 'C' haplotype grouping, where haplotypes are not co-occurring in distances within tens of kilometres, despite the presumed thousands of years for gene flow to have occurred.

Dispersal capability of adult caddisflies is known to be highly variable, depending on the species concerned. Contemporary molecular studies on caddisflies often do demonstrate occasional gene flow over widely isolated populations such as between the Canary Islands, demonstrating occasional or even regular dispersal over 50-100 km of open ocean (Kelly *et al.* 2001, 2002, Schultheis & Hughes 2005, Wilcock *et al*. 2007). However smaller scale dispersal, generally under 20 km has also been indicated and gene flow is suggested more to occur over several generations in other species (Wilcock *et al*. 2003, 2007). In New Zealand, phylogeographic studies on the caddisfly *Orthopsyche fimbriata* have demonstrated little or no contemporary gene flow between populations in river catchments over 100 km apart, although more closely associated streams do share haplotypes (Smith & Collier 2001, Smith *et al*. 2006 *b*, Smith & Smith 2009).

Of all marine insects, the Chathamiidae appear to be perhaps the least specialised. Flight is assumed to be a major factor in the success of insects on land allowing dispersal between discontinuous habitats, but likely to have no competitive advantage in the marine environment (Cheng 1985). Concerning the most specialised marine insects; all species of *Halobates* are completely wingless, and only the short-lived males of the marine midge genera *Clunio* and *Pontomyia* still possess wings, used for gliding short distances or as literal 'oars' for pleustonic movement during reproductive mass-emergences (Neuman 1976, Cheng & Collins 1980, Cheng 1985). As active movement of caddisly larvae is presumed to be low, distribution in the intertidal is likely restricted to where the larvae emerged from their starfish hosts. It seems probable as with some marine chironomids, the distribution of *P. plebeius* in the intertidal is likely 'clumped' or localised (Garbary et al. 2005). As also suggested by close relationships within populations, recruitment of larvae is probably almost entirely from the source adult population.

However some haplotypes were found distributed widely throughout New Zealand, in spite of some of the massive distances between populations, and some genetic breaks do appear to roughly correlate with marine patterns. This suggests a significant amount of migration may in occur in the passive movement of marine larvae instead of active flight by adults. 'Drift' of dislodged caddisfly larvae is common in stream environments, although similar transport by currents in the sea would

almost invariably be fatal for an intertidal species. Nevertheless passive dispersal seems to be a significant source of dispersal for other marine insect species; for example Schärer and Epler (2007) demonstrated the unusual transport mechanism of *Pontomyia* on the shells of hawksbill turtles as part of the epibiota. Adults of *Pontomyia* and are largely flightless (completely in females) and have lifespans restricted to a few hours (Neuman 1986, Soong *et al*. 1999), and are thus likely completely dependent on dispersal of larvae and egg masses for long distance dispersal. Likewise dispersal of *Halobates* is believed to be largely passive, and mostly relates to oceanic 'diffusion' by water motion than active movement of individuals (Ikawa *et al*. 1998, Anderson *et al*. 2000).

P. plebeius are associated with a large number of algal types, including seagrass in soft sediment (Riek 1976, Taylor & Cole 1994, Taylor & Steinberg 2005). Dispersal or rafting of algal rafts and the associated invertebrate communities is becoming increasingly appreciated as an important vector of rapid dispersal for marine organisms over hundreds of kilometers (Waters 2008, Fraser *et al*. 2009, 2010). Small numbers of *P. plebeius*, especially if in a dormant pupal phase (which also then would directly lead to adulthood, not requiring landfall on suitable habitat), could thus easily thus be transported by rafting. Additionally host starfish containing eggs or larvae may provide a robust refuge within which such rafting may occur (and also would themselves actively seek appropriate habitats). Human shipping may also provide a recent vector for dispersal, for both larvae and adults, which may explain some of the structure observed as it would allow for a bypass of certain geographic areas. Although not definitively demonstrated in *P. plebieus*, human shipping has been implicated in the dispersal of marine chironomids in Europe (Brodin & Andersson 2009, Raunio *et al*. 2009).

Overall it is proposed here that *P. plebeius* is a poor active disperser, and phylogeographic structure can be plausibly explained by marine processes. It is assumed that climatic and environmental changes had a profound impact on the species, and re-dispersal to distant localities has since the LGM has been primarily due to passive dispersal of larvae rather than active flight of adults, although stochastic long-distance dispersal of adults also seems likely. Possible important factors implicating the results found in this study do have to be considered. An important limitation was the

use of a single mitochondrial gene. Mitochondria (with very rare exceptions) are maternally inherited, and since no nuclear genes were used paternal inheritance was completely ignored. Male *P. plebeius* are smaller and presumably more active and prone to migration, constituting the majority of adult samples in most localities in this study and also in Winterbourne & Anderson (1980). Additionally some geographically significant areas, in particular Northern New Zealand and the South-Western South Island were especially under sampled. More sampling may reveal new localities of haplogroups B and C, and may yield more cryptic diversity not shown here. Thus the conclusions drawn here may be confidently confirmed or disproven with some further research. Nevertheless the prospect of *P. plebieus* as a new model organism for phylogeographic exploration in New Zealand is well demonstrated in this study.

2.5 Figures

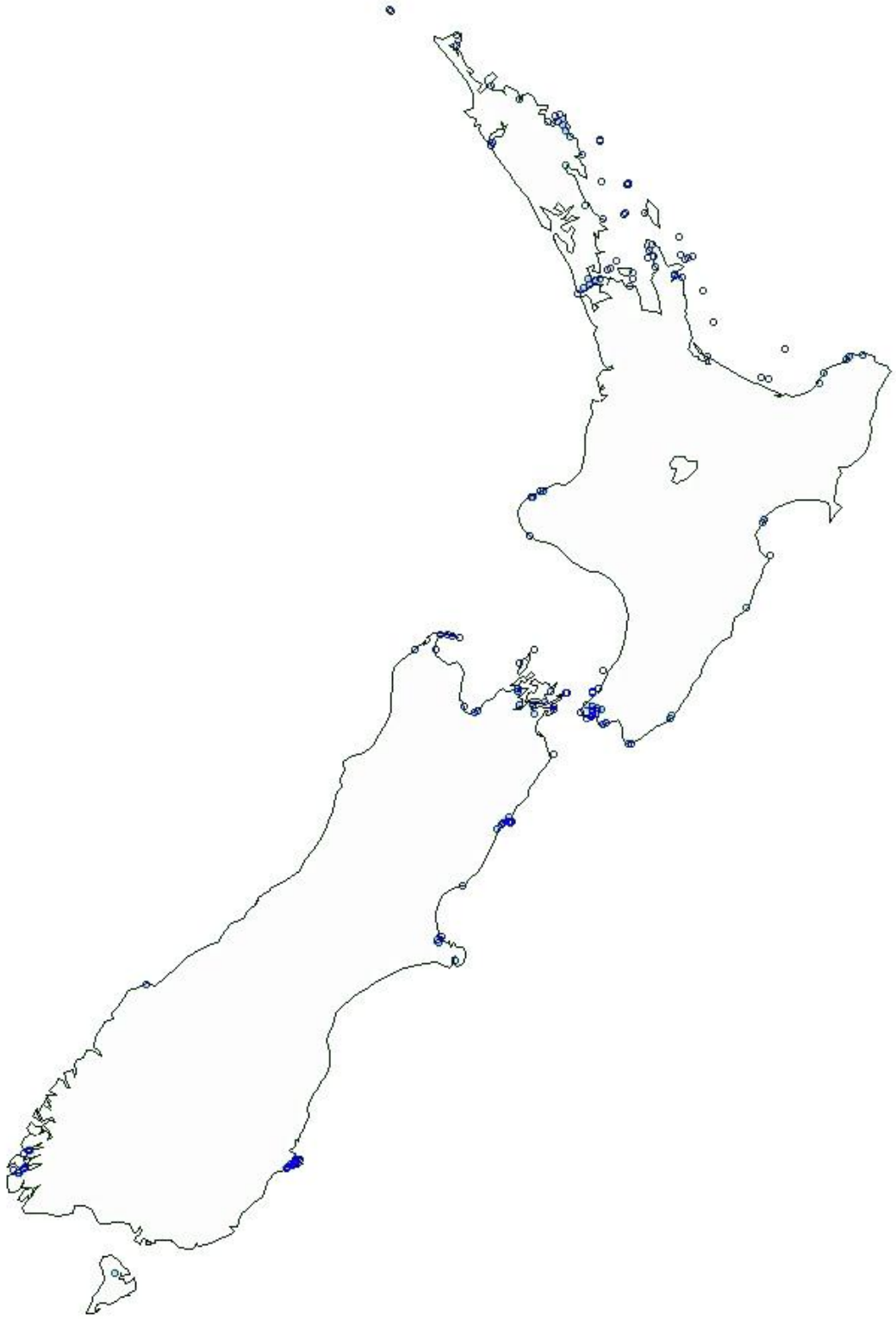

FIG 2.1) Collection records of *Philanisus plebeius* taken direct from the New Zealand trichoptera collection database (http://nzcaddis.massey.ac.nz/). Note apparent disjunctions on the eastern North Island, the South Eastern South Island, and most of the West Coast of both Islands. The collection from Stewart Island is anomalous and possibly an error (unconfirmed). This does not include the Australian distribution of the species (New South Wales only).

TABLE 2.1) List of all samples used for sequencing in this study, and collection details. Brackets in locality designate "greater locality" used due to close association of sites and/or low sample numbers. Note repetitions of localities due to multiple collections.

Species	Specimen Code	Number	Ontogeny	Collector	Locality	Coordinates	Collection Date
Philanisus plebeius	K3	11	Adult	Ian Henderson	Akaroa	43°48'06 S, 172°58'06 E	1/02/2009
Philanisus plebeius	L4	12	Adult	Ian Henderson	AkitioBeach	40°36'21 S, 176°25'14 E	21/02/2010
Philanisus plebeius	B6, B9	14	Adult	Alex Boast	Auckland Harbour (Auckland)	36°50'30 S, 174°51'48 E	6/03/2009
Philanisus plebeius	K10	6	Adult	Alice Wells	Bawley Point (New South Wales)	35°30'50 S, 150°24'03 E	21/11/2008
Philanisus plebeius	L1	1	Larva	Alice Wells	Bawley Point (New South Wales)	35°30'50 S, 150°24'03 E	21/11/2008
Philanisus plebeius	A5, A6	12	Adult	Alex Boast	Breaker Bay (Wellington)	41°20'38 S, 174°49'19 E	12/01/2009
Philanisus plebeius	K1	10	Adult	Ian Henderson	Kaikoura	42°24'50 S, 173°41'07 E	31/01/2009
Philanisus plebeius	K2	1	Adult	Ian Henderson	Kaikoura	42°24'50 S, 173°41'07 E	31/01/2009
Philanisus plebeius	A4	1	Adult	Alex Boast	Lyall Bay (Wellington)	41°20'43 S, 174°47'35 E	8/01/2009
Philanisus plebeius	C1	3	Larvae	Alex Boast	Makara (Wellington)	41°12'55 S, 174°42'15 E	21/07/2009
Philanisus plebeius	B4, B5	4	Adult	Alex Boast	Mangakuri Beach	39°57'59 S, 176°55'14 E	28/02/2009
Philanisus plebeius	B2, B3	7	Adult	Alex Boast	Mangakuri Beach	39°57'59 S, 176°55'14 E	27/02/2009
Philanisus plebeius	C2	6	Adult	Alex Boast	Mangakuri Beach	39°57'59 S, 176°55'14 E	21/07/2009
Philanisus plebeius	L2	6	Adult	Ian Henderson	Te Rua Bay, Marlborough Sounds	41°14'25 S, 174°16'14 E	30/12/2008
Philanisus plebeius	B1	2	Adult	Alex Boast	Mount Maunganui (Tauranga)	37°37'30 S, 174°10'29 E	17/02/2009
Philanisus plebeius	A7	6	Adult	Alex Boast	Napier	39°28'39 S, 176°54'31 E	15/01/2009
Philanisus plebeius	K6	2	Adult	Ian Henderson	New Plymouth	39°03'21 S, 174°01'47 E	25/11/2008
Philanisus plebeius	A9	3	Adult	Alex Boast	Pauatahanui Inlet (Wellington)	41°05'50 S, 174°54'33 E	21/01/2009
Philanisus plebeius	K7, K8	7	Larva / Pupae	Alice Wells	Pebbly Beach (New South Wales)	35°36'33 S, 150°20'09 E	22/11/2008
Philanisus plebeius	K4	9	Adult	Ian Henderson	Portobello	45°50'23 S , 170°39'02 E	3/02/2009
Philanisus plebeius	K5	1	Adult	Ian Henderson	Portobello	45°50'23 S , 170°39'02 E	4/02/2009
Philanisus plebeius	A3	1	Adult	Alex Boast	Pukerua Bay (Wellington)	41°01'39 S, 174°53'15 E	26/12/2008
Philanisus plebeius	A8	4	Adult	Alex Boast	Pukerua Bay (Wellington)	41°01'39 S, 174°53'15 E	21/01/2009
Philanisus plebeius	B10	1	Adult	Alex Boast	Pukerua Bay (Wellington)	41°02'18 S, 174°53'22 E	15/04/2009
Philanisus plebeius	A10	2	Adult	Alex Boast	Tauranga estuary (Tauranga)	37°42'29 S, 174°53'15 E	16/02/2009

TABLE 2.1) Continued.

Species	Specimen Code	Number	Ontogeny	Collector	Locality	Coordinates	Collection Date
Philanisus plebeius	B7, B8	3	Adult	Alex Boast	Waiwera (Auckland)	36°32’56 S, 174°42’32 E	8/03/2009
Philanisus plebeius	L3	11	Adult	Ian Henderson	Whangaehu Beach	40°23'54 S, 176°38'05 E	11/12/2009
Philanisus fasciatus	PF101	1	Adult	Karen Baird	Raoul Island	29°14’56 S, 177°55’14 E	20/10/2009
Chathamia integripennis	CI1	1	Adult	Alex Boast	Waiwera	36°32’56 S, 174°42’32 E	8/03/2009

TABLE 2.2) Primers used for amplification and sequencing.

Gene	Primer Name	Primer sequences (5' - 3')	Reference
COI	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer*et al*. 1994
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer*et al*. 1994

TABLE 2.3) All haplotypes found in this study and frequency in each locality. Specific locality is here used.

Haplotype	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	B	C1	C2	C3	C4	C5	C6	C7	N
Akaroa	0	1	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
Akitio	9	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	12
Auckland	12	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
Bawley Point	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Kaikora	9	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
Lyall Bay	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Makara	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Mangakuri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	2	0	2	0	1	1	17
Marlborough Sounds	5	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	6
Moa Point	7	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	12
Mount Maunganui	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Napier	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	1	0	0	6
New Plymouth	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Pauatahanui	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Pebbly Beach	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Portobello	1	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
Pukerua Bay	3	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Tauranga	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2
Waiwera	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Whangaehu	10	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	11
N	60	16	14	11	8	3	2	2	1	1	1	1	1	1	1	11	5	2	2	1	1	1	146

TABLE 2.4) List of all haplotypes found in this study and base pair composition at all 23 variable sites. Site number corresponds to position on the 618 base pair fragment.

Site	16	49	59	118	184	217	235	271	337	340	343	364	433	448	454	478	484	505	532	538	549	562	586
A1	C	C	T	C	A	A	T	G	A	T	T	T	G	T	T	A	G	C	C	G	T	T	T
A2	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.
A3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
A4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
A5	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
A6	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A7	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A8	.	.	.	.	.	.	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A9	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A10	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
A12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
A13	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
B	T	.	.	.	.	.	C	A	.	C	C	.	.	.	.	.	.	T	T	.	.	A	.
C1	T	.	.	.	.	G	.	.	.	C	C	.	A	C	.	.	.	T	T	.	.	A	.
C2	T	.	.	.	.	.	.	.	.	C	C	C	A	C	.	.	.	T	T	.	.	A	.
C3	T	.	.	T	.	.	.	.	.	C	C	C	A	C	.	.	.	T	T	.	.	A	.
C4	T	.	.	.	.	G	.	.	.	C	C	.	A	C	C	.	.	T	T	.	.	A	.
C5	T	.	.	T	.	.	.	.	.	C	C	C	A	C	.	.	.	T	T	.	.	A	.
C6	T	.	G	.	.	G	.	.	.	C	C	.	A	C	.	.	.	T	T	.	.	A	.
C7	T	.	G	.	.	G	.	.	.	C	C	.	A	C	C	.	.	T	T	.	.	A	.

TABLE 2.5) Pairwise distances between all *Philanisus plebeius* haplotypes (lower left) and standard error (upper right).Calculated in MEGA v 4 with 10,000 replicates using the Kimura-2 parameter model.

	1)	**2)**	**3)**	**4)**	**5)**	**6)**	**7)**	**8)**	**9)**	**10)**	**11)**	**12)**	**13)**	**14)**	**15)**	**16)**	**17)**	**18)**	**19)**	**20)**	**21)**	**22)**
1) A1		0.0016	0.0015	0.0015	0.0016	0.0015	0.0016	0.0023	0.0016	0.0015	0.0016	0.0016	0.0016	0.0016	0.0045	0.0047	0.0047	0.0049	0.0049	0.0044	0.005	0.0053
2) A2	0.0016		0.0022	0.0022	0.0022	0.0022	0.0022	0.0028	0.0022	0.0022	0.0023	0.0021	0.0023	0.0023	0.0043	0.0044	0.0044	0.0047	0.0046	0.0041	0.0047	0.005
3) A3	0.0016	0.0032		0.0021	0.0022	0.0021	0.0022	0.0027	0.0022	0.0021	0.0023	0.0022	0.0023	0.0021	0.0048	0.005	0.0050	0.0053	0.0052	0.0047	0.0053	0.0055
4) A4	0.0016	0.0032	0.0032		0.0021	0.0021	0.0022	0.0027	0.0022	0.0022	0.0022	0.0021	0.0022	0.0022	0.0048	0.0049	0.0049	0.0052	0.0052	0.0047	0.0052	0.0055
5) A5	0.0016	0.0032	0.0032	0.0032		0.0022	0.0022	0.0028	0.0021	0.0022	0.0022	0.0022	0.0024	0.0022	0.0049	0.0044	0.0044	0.0047	0.0046	0.0041	0.0047	0.0050
6) A6	0.0016	0.0032	0.0032	0.0032	0.0032		0.0023	0.0016	0.0022	0.0022	0.0022	0.0021	0.0023	0.0021	0.0042	0.0050	0.0050	0.0052	0.0052	0.0047	0.0052	0.0055
7) A7	0.0016	0.0032	0.0032	0.0032	0.0032	0.0032		0.0028	0.0023	0.0022	0.0023	0.0023	0.0023	0.0022	0.0048	0.005	0.0049	0.0051	0.0052	0.0046	0.0052	0.0055
8) A8	0.0032	0.0049	0.0049	0.0049	0.0049	0.0016	0.0049		0.0028	0.0027	0.0028	0.0027	0.0015	0.0027	0.0038	0.0053	0.0053	0.0056	0.0055	0.0051	0.0056	0.0058
9) A9	0.0016	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0049		0.0022	0.0022	0.0023	0.0023	0.0022	0.0048	0.0049	0.0049	0.0051	0.0051	0.0046	0.0052	0.0054
10) A10	0.0016	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0049	0.0032		0.0021	0.0021	0.0022	0.0022	0.0048	0.0050	0.0050	0.0052	0.0052	0.0047	0.0052	0.0055
11) A11	0.0016	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0049	0.0032	0.0032		0.0022	0.0023	0.0022	0.0048	0.0049	0.0049	0.0052	0.0052	0.0047	0.0052	0.0055
12) A12	0.0016	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0049	0.0032	0.0032	0.0032		0.0023	0.0023	0.0048	0.0049	0.0049	0.0051	0.0052	0.0046	0.0052	0.0055
13) A13	0.0016	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0016	0.0032	0.0032	0.0032	0.0032		0.0023	0.0042	0.005	0.005	0.0053	0.0053	0.0048	0.0053	0.0056
14) A14	0.0016	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0049	0.0032	0.0032	0.0032	0.0032	0.0032		0.0049	0.005	0.0051	0.0053	0.0053	0.0048	0.0053	0.0056
15) B	0.0131	0.0114	0.0148	0.0148	0.0148	0.0114	0.0148	0.0098	0.0148	0.0148	0.0148	0.0147	0.0114	0.0147		0.0035	0.0035	0.0039	0.0039	0.0038	0.0039	0.0043
16) C1	0.0148	0.0131	0.0164	0.0164	0.0131	0.0164	0.0164	0.0181	0.0164	0.0164	0.0164	0.0164	0.0164	0.0164	0.0082		0.0022	0.0027	0.0015	0.0027	0.0015	0.0023
17) C2	0.0148	0.0131	0.0164	0.0164	0.0131	0.0164	0.0164	0.0181	0.0164	0.0164	0.0164	0.0164	0.0164	0.0164	0.0082	0.0032		0.0016	0.0027	0.0016	0.0027	0.0032
18) C3	0.0164	0.0148	0.0181	0.0181	0.0148	0.0181	0.0181	0.0198	0.0181	0.0181	0.0181	0.0181	0.0181	0.0181	0.0098	0.0049	0.0016		0.0032	0.0023	0.0031	0.0036
19) C4	0.0164	0.0148	0.0181	0.0181	0.0148	0.0181	0.0181	0.0198	0.0181	0.0181	0.0181	0.0181	0.0181	0.0181	0.0098	0.0016	0.0049	0.0065		0.0031	0.0023	0.0015
20) C5	0.0131	0.0115	0.0148	0.0148	0.0115	0.0148	0.0148	0.0164	0.0148	0.0148	0.0148	0.0148	0.0148	0.0148	0.0098	0.0049	0.0016	0.0032	0.0065		0.0031	0.0035
21) C6	0.0164	0.0147	0.0181	0.0181	0.0147	0.0181	0.0181	0.0197	0.0181	0.0181	0.0181	0.0181	0.0181	0.0181	0.0098	0.0016	0.0049	0.0065	0.0032	0.0065		0.0015
22) C7	0.0181	0.0164	0.0197	0.0197	0.0164	0.0197	0.0197	0.0214	0.0197	0.0197	0.0197	0.0197	0.0197	0.0197	0.0114	0.0032	0.0065	0.0081	0.0016	0.0081	0.0016	

TABLE 2.6) Mean distances between and within groups, and within all of *Philanisus plebeius*. Calculated in MEGA v 4 with 10,000 replicates using the Kimura-2 parameter model

Grouping	Between/Within Groups	D	SE
Philanisus plebeius	Within	0.01	0.002
Group A	Within	0.003	0.001
Group C	Within	0.009	0.002
Group A-B	Between	0.014	0.004
Group A-C	Between	0.019	0.003
Group B-C	Between	0.011	0.011

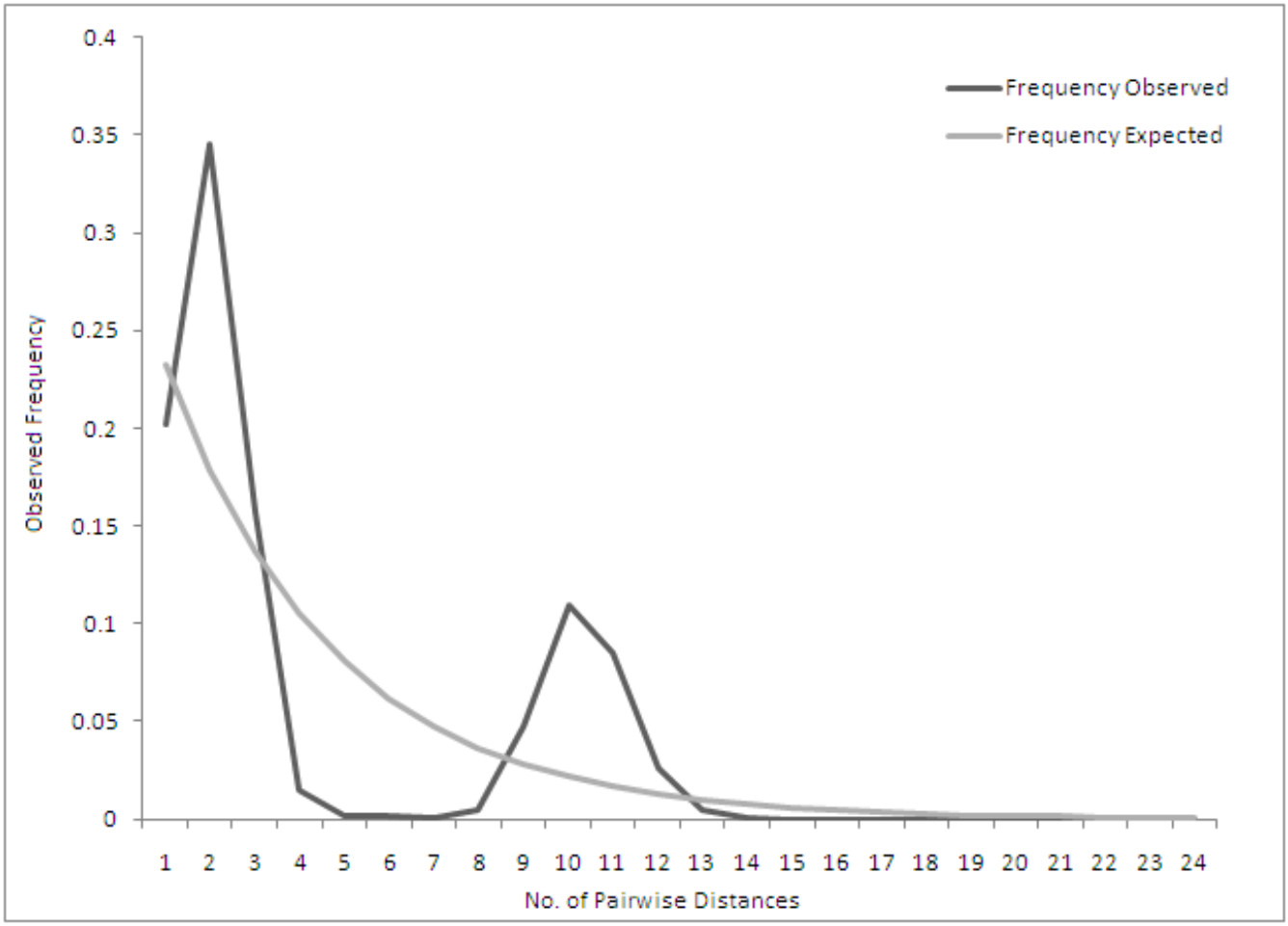

FIG 2.2) Mismatch distribution chart of *P. plebeius* using all 146 sequences. Distance (Pairwise estimate) shown with observed frequency. Expected frequency calculated in DNAsp represents that expected in a stable population.

TABLE 2.7) List of sequence statistics calculated for between haplotype groupings. * indicates a significant P value.

Grouping	Tajimas D	P Value	Fu's F_s	P value
AllHaplotypes	-0.15097	> 0.10	-20.236	< 0.01*
Haplogroup A only	-1.91810	< 0.05*	-18.404	< 0.01*
Haplogroup B only	0.45159	> 0.10	-4.774	< 0.01*

TABLE 2.8) List of all AMOVA statistics calculated in Arequin. * indicates a significant P value

Source of Variation	df	Sum of Squares	Variance components	Percentage Variation	P
Upper North Island Groupings					
Among Groups (a)	3	1.557	0.02056	8.54809	0.10129
Among Populations within Groups (b)	2	0.299	-0.00816	-3.39024	0.5797
Within Populations (c)	76	17.339	0.22814	94.84215	0.02376
Total		19.195	0.24055		
North-South Groupings					
Among Groups (a)	2	8.113	0.20963	51.29439	0.1199
Among Populations within Groups (b)	6	1.885	0.01093	2.67518	0.03931
Within Populations (c)	97	18.248	0.18812	46.03044	< 0.01
Total		28.245	0.40869		

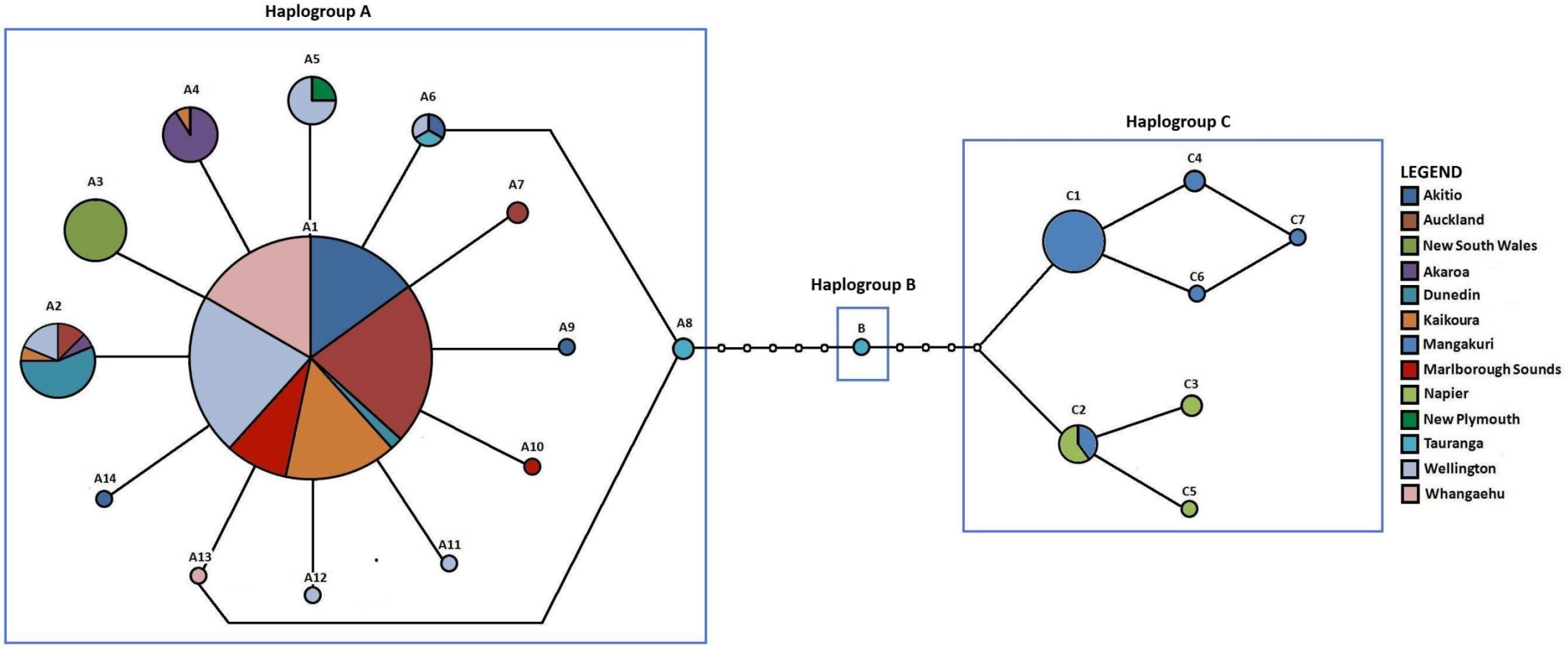

FIG 2.3) Network analysis of full dataset as calculated in TCS, showing all observed haplotypes, number of site changes and locality. Circle size proportionate to sample size. Blue boxes designate genetic scope of each haplogroup

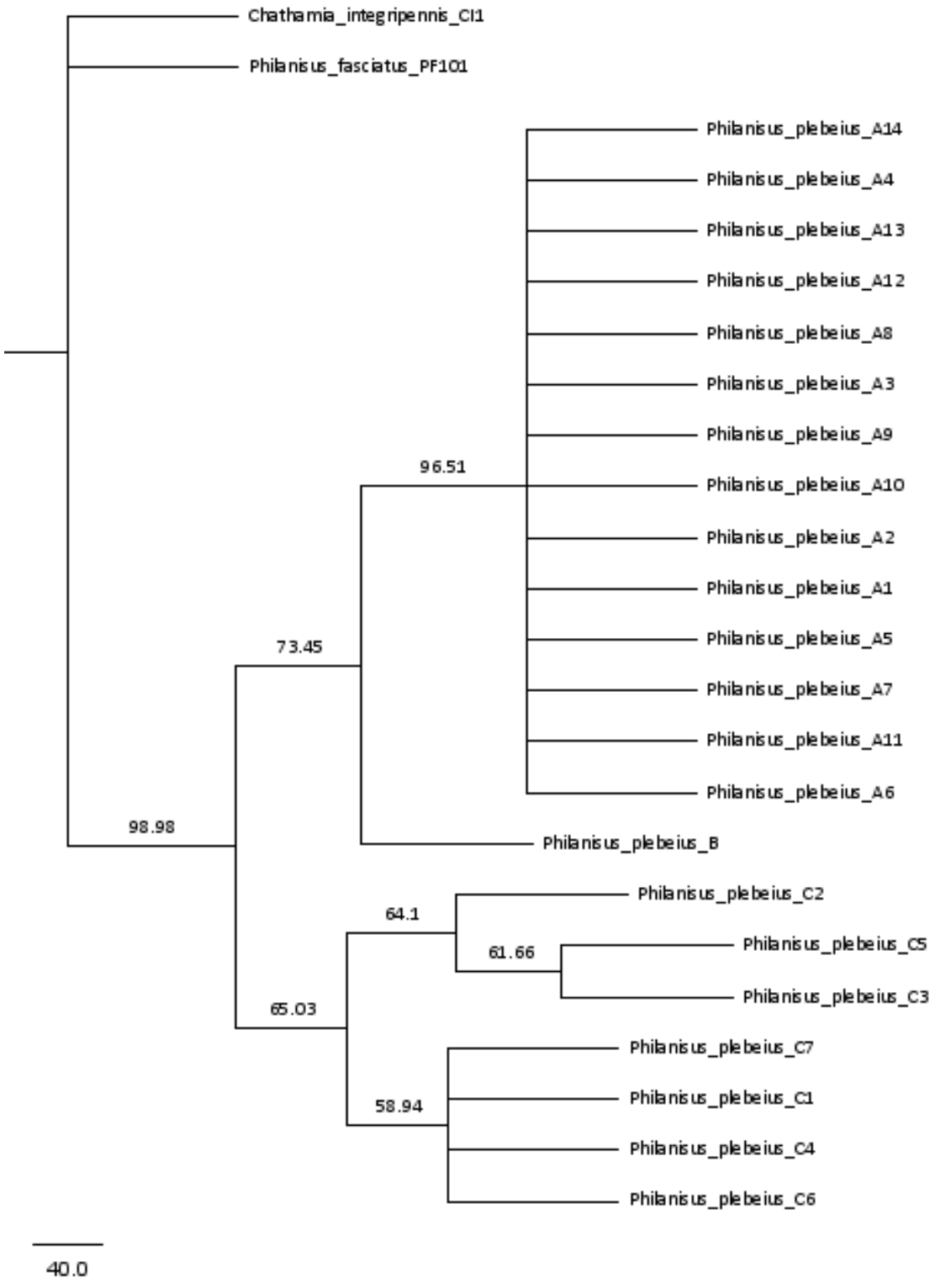

FIG 2.4) Bootstrap consensus Maximum Parsimony (MP) tree of all *Philanisus plebieus* haplotypes (10,000 replicates, Heuristic search logarithm) as inferred in PAUP*. Bootstrap values (%) are shown.

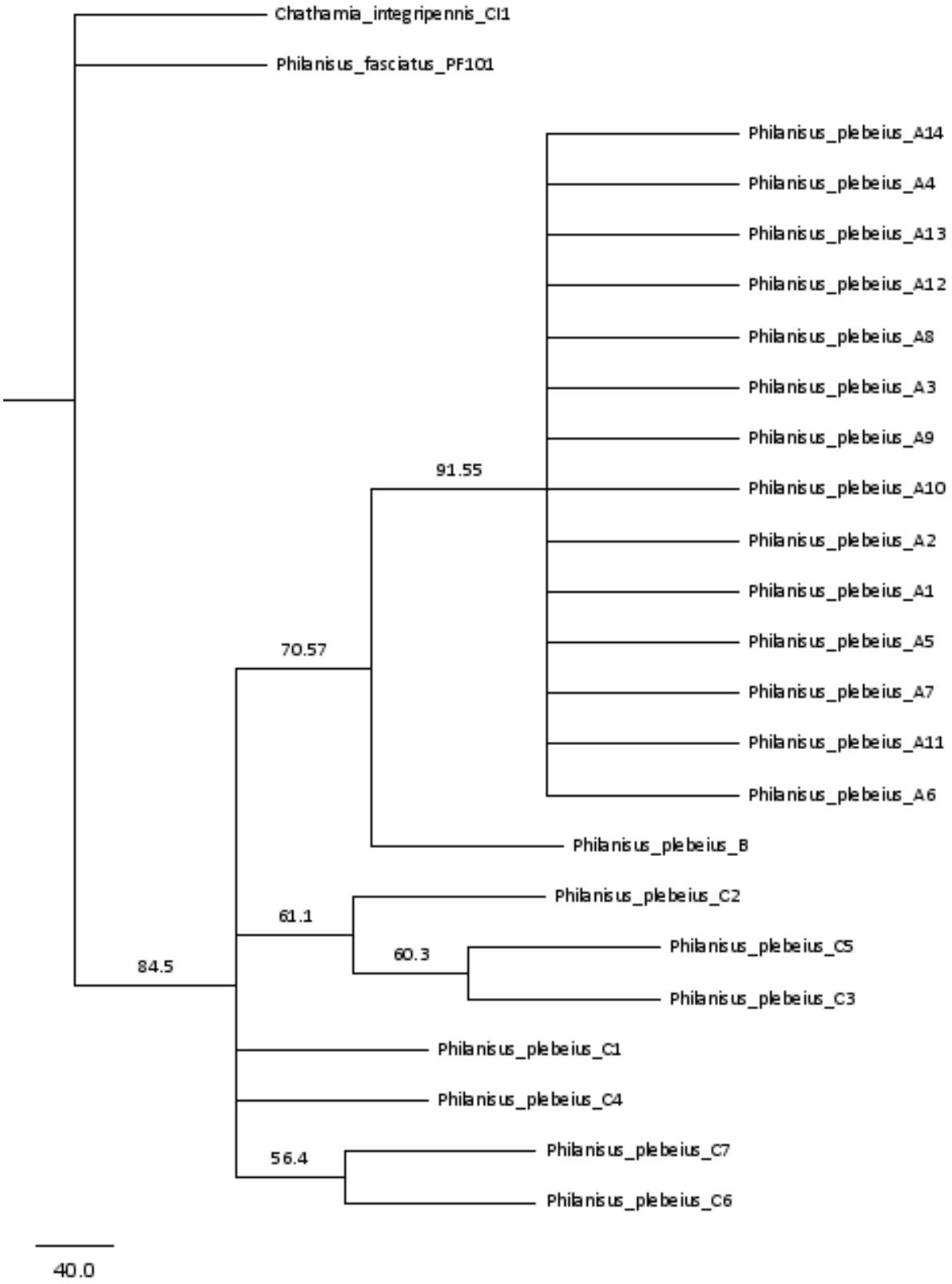

FIG 2.5) Bootstrap consensus Maximum likelihood (ML) tree of all *Philanisus plebeius* haplotypes (10,000 replicates, Heuristic search logarithm) as inferred in PAUP*. Bootstrap values (%) are shown.

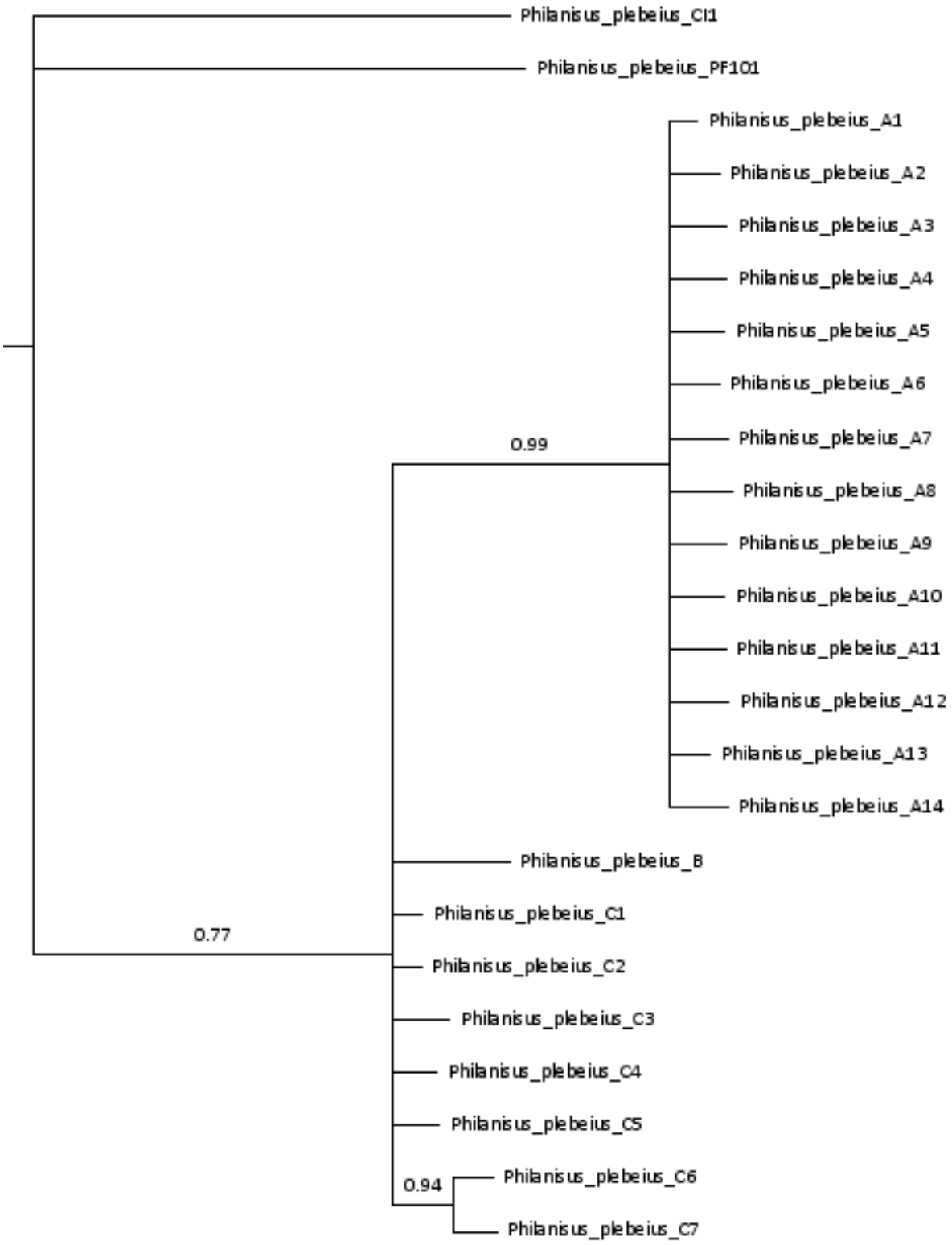

FIG 2.6) Bayesian analysis tree of all *Philanisus plebeius* haplotypes as analysed through MrBayes. Posterior Bayesian probability indices are shown.

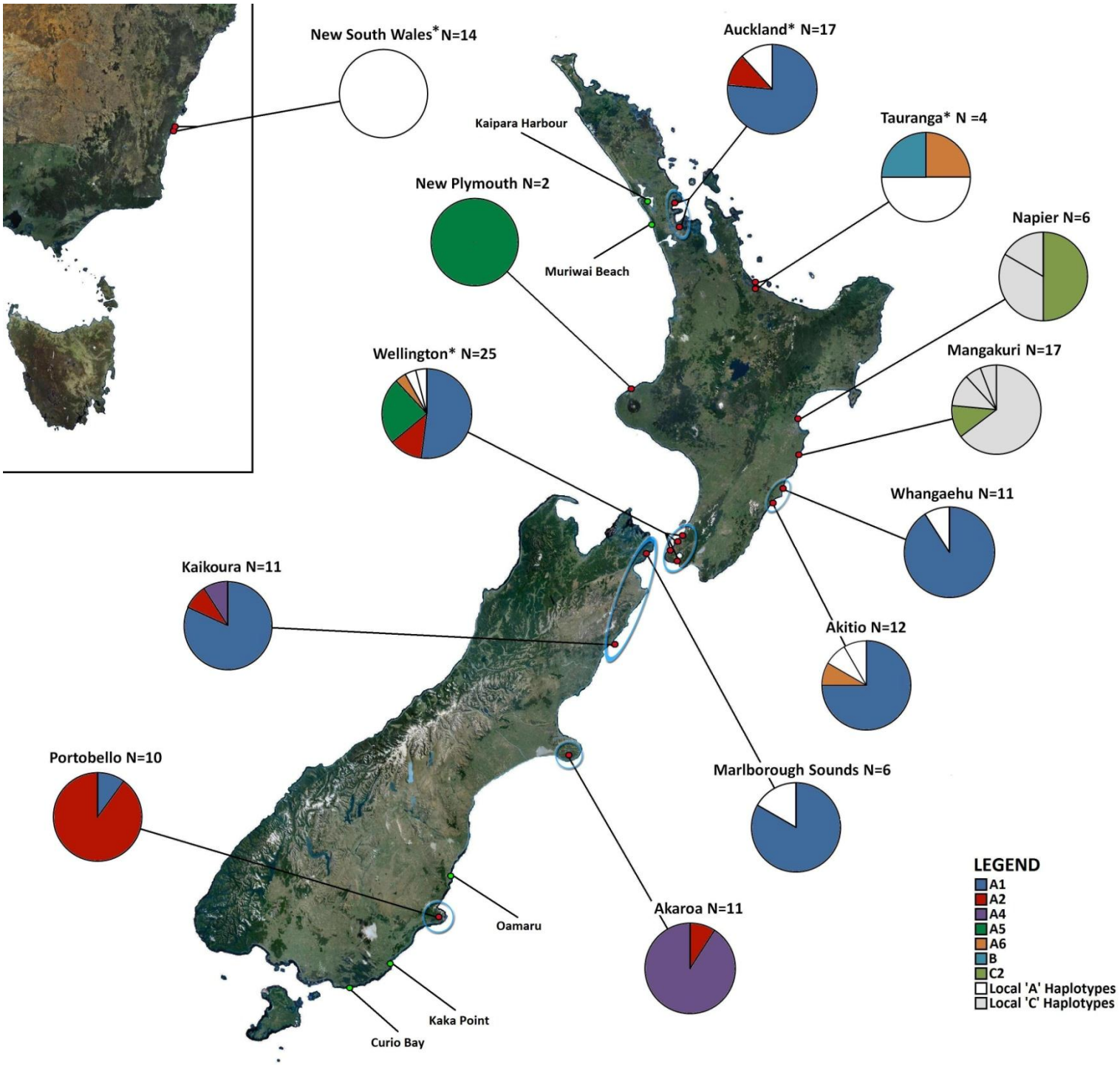

FIG 2.7) Map showing all collection sites and proportion of observed haplotypes. Legend shows haplotype codes. Sample size shown. Sites with* designate combined localities (refer to Table 2.1 for more detail). Circles show areas used for AMOVA analyses (first top four used for first AMOVA only, then combined and compared with the bottom two for the second analysis). Green points show areas where light-trapping was attempted but no samples successfully collected (evidence for population absences in these regions).

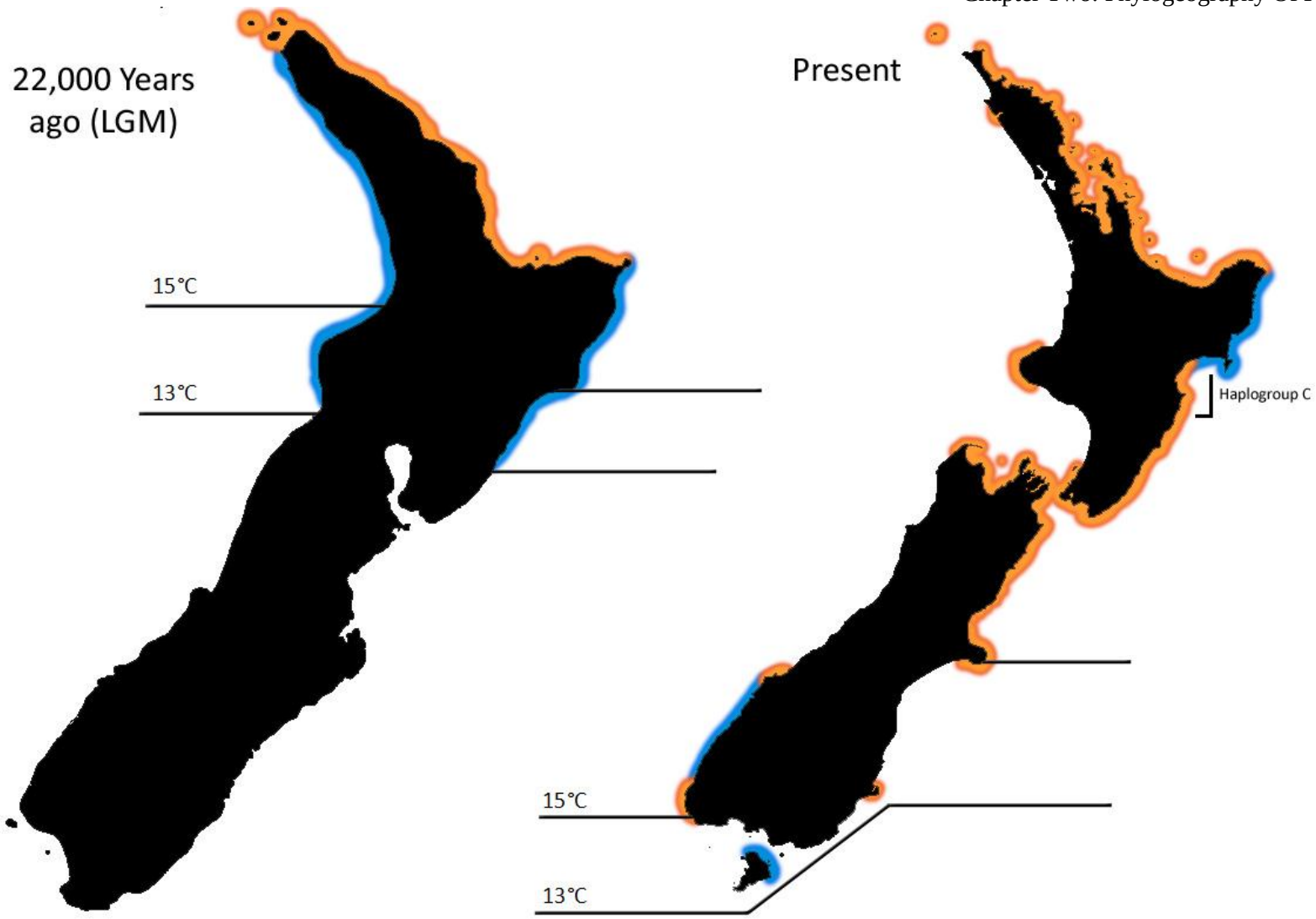

FIG 2.8) Inferred modern and past distributions of *Philanisus plebeius* in New Zealand. Present shows distribution as inferred from collection records, and the past map shows estimated distribution during the last glacial maximum. Bars show sea surface temperature (warmest month) inferred from Barrows & Juggins (2005) and estimated positions during the LGM. Orange represents confirmed or likely distribution, blue equivocal (unconfirmed records, within lower temperature limitation estimates and/or within modern disjunctions). Modern known distribution of haplogroup C shown. Note increased land area during the LGM.

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Chapter Three: Phylogeny of the Chathamiidae and origins of the species *Hydrobiosis lindsayi* and *Oecetis chathamensis* (Insecta: Trichoptera) with regard to the formation of the Kermadec and Chatham Islands.

3.1 Introduction:

Aside from the main islands, New Zealand biogeographic region consists of several groups of isolated oceanic islands as far afield as the Kermadec, Chatham, Auckland, Antipodes and Campbell Islands, comprising an area spanning over 20˚ of latitude from the subantarctic to the subtropics. All these groups share close biological links with New Zealand and to each other in spite of being as far from the main islands as 1,000 km. However, although the biogeographic understanding of New Zealand's mainland is now particularly well documented, New Zealand's oceanic islands are usually ignored in contemporary molecular studies (Heenan *et al*. 2010).

The Chathamiidae are an unusual family of caddisflies that breed in the marine intertidal zone, and are one of a handful of invertebrate families that are often thought of as endemic to New Zealand (Gibbs 2006). Although all five species and two genera are found in New Zealand, uniquely the most common species *Philanisus plebeius* has been found to be resident in New South Wales Australia since 1904 (Hudson 1904, Riek 1970). This is currently the only known New Zealand caddisfly species that is not fully endemic (Collier 1993), although the Australian population is almost certainly a recent dispersal event from New Zealand within the last few thousand years (Refer to Chapter Two of thesis). The family also has the ecological requirements of a marine invertebrate species and therefore does not require any established terrestrial ecology to flourish, yet can disperse as an airbourne winged phase at adulthood. It therefore follows that the Chathamiidae represents a group of evolutionary and biogeographic interest.

There are five established species all having been described in detail by Riek (1976) and Ward (1994, 1995) distributed throughout New Zealand (see Fig 3.1). The abundant and well-studied

Philanisus plebeius is found throughout New Zealand and New South Wales, *P. mataua* is restricted to a few sites in the upper North Island including the three Kings Islands, *P. fasciatus* to the Kermadec Islands, *Chathamia intergripennis* to the upper North Island, and *C. brevipennis* to the Chatham Islands. The island endemic species (*C. brevipennis* and *P. fasciatus*) have only been collected in the largest of the islands in their archipelagos (Chatham Island and Raoul Island respectively) and it is unknown whether they are more widely distributed.

Only the common Chathamiid species *Philanisus plebeius* and *Chathamia integripennis* have been included in any phylogenetic studies to date; although the family is shown to be genetically distinct and monophyly is supported (Kjer *et al.* 2001, 2002, Hogg *et al*. 2009, Johanson *et al*. 2009, Johanson & Malm 2010). In spite of both species being placed in separate genera the genetic distance between the two has been shown to be surprisingly small (a base pair difference of 3.2% for COI, Hogg *et al*. 2009), a distance for caddisflies typically found within genera and smaller than found even within some established species (Hogg *et al*. 2009, Pauls *et al.* 2009, Lehrian *et al*. 2010, Zhou *et al*. 2010). In addition the monophyly of *Chathamia* is unclear, with *C. integripennis* placed in the same genus as the initially described *C. brevipennis* based on 'similarity' with no typical '*Chathamia*' features having been discussed (Riek 1976). It is indicated that the taxonomy of the family may need revision, and as a result any new genetic information should provide important data for resolving the taxonomy of this group.

Both the endemicity status of the family as well as the small number of species, easily allows for a complete phylogenetic analysis. Also importantly the group's archipelagic distribution would mean a phylogeny would also have a significant biogeographic application. *P. fasciatus* for example is likely to have begun diverging since its isolation on the Kermadec Islands. However the island chain is volcanic and has had a dynamic history making a prior estimation of the age of the group, and thus *P. fasciatus*, almost impossible. Although the major islands are estimated a few tens of thousands of years old, the age of continuous land in the island chain itself remains largely unknown (Smith *et al*. 2006). The Chatham Island group has been relatively stable much longer since the early Pleistocene 2Ma and perhaps even earlier (Campbell 1998, Paterson *et al*. 2006, Campbell *et al*. 2006,

Campbell & Hutching 2007, Campbell *et al*. 2008), a characteristic that may be reflected by the relatively divergent morphology of *C. brevipennis*, including brachyptery (wing reduction) in the adults. It is plausible that *C. brevipennis* has been resident on the Chatham Islands shortly since emergence.

Aside from *C. brevipennis* at least four to five other species of Trichoptera are found on the Chatham Islands. *Hydrobiosis lindsayi* (Hydrobiosidae) and *Oecetis chathamensis* (Leptoceridae) are endemic to Chatham Island, although very similar to related species in New Zealand (Tillyard 1925). Two 'micro-caddisfly' species (Hydroptilidae), *Oxyethira albiceps* and *Paraoxyethira hendersonsi* also inhabit the Chathams; however are minute, adaptable and extremely common species also found throughout New Zealand and its subantarctic islands (Wise 1964, 1972, Neboiss 1986, Marris 2000). A final species, *Hudsenoma* species 'X' (Leptoceridae), has been collected on at least two occasions, however remains undescribed as a species. These species present further opportunity for further exploration of caddisfly colonisation and evolution between Chatham Islands and New Zealand and serve as a comparison for any analysis of *C. brevipennis*.

3.1.1 The Chatham Islands

The Chatham Islands are of particular interest due to the large size of the islands (996 km^2) as well as their isolation (roughly 800 km East of New Zealand). The islands are the only emergent region of the extensive continental Chatham rise east of New Zealand, and are known to have once formed part of continental Gondwana; clearly demonstrated for example by dinosaur fossils from the Cretaceous (Stilwell *et al*. 2006, Campbell & Hutching 2007). Ancient vicariance has been hypothesised to explain the origin of the Chatham biota as having a Gondwanan origin (Craw 1988), however the area is now known have remained underwater since the submergence of Zealandia in the Cenozoic (Wood *et al*. 1989, Campbell *et al*. 1994, Trewick et al. 2007). The geological foundations of most of the modern Island group itself only formed from Pliocene-Miocene intra-plate basaltic volcanism dating roughly 6-4 Ma. Despite this, there is evidence suggesting that the region

nevertheless remained submerged and the modern islands only finally emerged due to uplift in the Pleistocene, roughly 2 Ma (Campbell 1998, Paterson *et al*. 2006, Campbell *et al*. 2006, 2008, Campbell & Hutching 2007). Estimating the age of the islands is additionally complicated by the known existence of an emergent volcano now forming Mangere Island (the 'Mangere Volcano') 6-4 Ma, and the modern Island has remains of fossils and a freshwater lake from this period (Campbell & Hutching 2007). However geological evidence indicates the Volcano fully submerged in the mid Pliocene, and there is believed to have been no land in the Chatham region 4-2 Ma. Any of the old 'Mangere biota' is generally assumed long extinct.

The modern Islands today comprises a single large landmass (Chatham Island) and several smaller islands and islets; however during lower sea levels during Pleistocene ice ages the archipelago would have been considerably larger and fully interconnected (Hay *et al*. 1970). The biota of the Chatham Islands is characterised by a high level of endemicity, although is believed entirely of recent New Zealand origin via oceanic dispersal. The endemic biota has been thoroughly investigated through a large number of molecular studies which almost universally point to young Pleistocene origins; in insects (Trewick, Arensburger e*t al.* 2004, Chinn & Gemmell 2004, Trewick *et al.* 2005, Nolan *et al.* 2007, Marshall *et al.* 2008), freshwater crustaceans (Stevens & Hogg 2004, McGaughran *et al.* 2006), Plants (Wagstaff & Garnock-Jones 1998, Heenan *et al.* 2010), Spiders (Vink & Paterson 2003) Galaxiid fish (Waters & McDowall 2005) and Birds (Trewick 1997, Boon *et al*. 2000, Kennedy *et al.* 2000, 2001, Chambers *et al*. 2001, Miller & Lambert 2006, Banks & Paterson 2007). However this general pattern is not always consistent; Pliocene-Micoene ages up to 6 Ma have been inferred in some unusual cases; *Geodorcus* stag beetles (Trewick 2000), Skinks (Liggins *et al.* 2008) and at least four plant species (Heenan *et al.* 2010). Although in the minority, such studies may therefore potentially indicate a link between the modern 'Chatham' and the old 'Mangere' biotas (Heenan *et al.* 2010).

3.1.2 The Kermadec Islands

The Kermadecs comprise a widely separated chain of six islands, all the peaks of large submarine stratovolcanoes 800-1,100 km North-East of New Zealand, terminating with the northernmost Raoul Island, roughly 900 km south of Tonga. Much smaller than the Chatham Islands, the island group only comprises a total of 33 km^2, most of which is comprised of Raoul (29.32 km^2) and Macauley (3 km^2) Islands which also contain most of the biodiversity. Raoul volcano itself is highly active with a complex stratigraphy dating back roughly a million years; however most of the geology indicates submarine formation (such as the old boat cove pillow lavas from 0.6-1.4 Ma). By contrast the modern dacitic caldera dates only from the past 3.7 ka and an age as young as 2 ka has been suggested for the emergent island itself (Brothers & Searle 1970, Kaplin 1981, Smith *et al*. 2006, 2010). The offshore submarine Denham caldera, significantly larger than the emergent Raoul caldera, has a similar age (2.2 ka Worthington *et al*. 1999).

The other main island; the smaller and currently inactive Macauley over 100 km to the South-West of Raoul, is known to have been considerably larger in the past until a major eruptive and caldera collapse event dated 6,310 years ago (producing the sandy bay tephra, SBT), prior to which it apparently approached or even exceeded modern Raoul in size (Brothers & Martin 1970, Lloyd et al. 1996, Smith *et al.* 2003 *a*). Today the caldera remains almost entirely submerged, excepting the small island itself. Curtis Island is the only other island aside from Raoul still showing residual activity, although the island is small, eroded and evidently becoming dormant (Smith *et al*. 1988). None of the other smaller, southerly islands (Cheeseman Island, L'Esperance rock, and L'Havre rock – only exposed during low tide) have been studied nearly as extensively and their ages are unknown, although are dormant and relatively eroded (Smith *et al*. 2006). Due to the dynamic nature of the islands it is currently very difficult estimating the age of the islands as emergent land from geology alone.

The island group does contain a number of endemic plant and animal taxa including intertidal and terrestrial plants, birds and invertebrates, although diversity is low and most species are shared

with Tonga or New Zealand (Dugdale 1973, Watt 1975, Arensburger *et a*l. 2004, Barkla *et al*. 2010). Molecular analysis of the endemic Kermadec pohutukawa (*Metrosideros kermadecensis*) suggests a New Zealand dispersal origin dating into the Pleistocene as much as 0.5-1 Ma before present (Wright et al. 2000, 2003). Molecular studies of Kermadec cicadas (*Kikihia cutora exulis*) show extremely close relationships to relatives in New Zealand, although molecular distances estimate a similar Pleistocene origin of 0.55±0.16 Ma (Arensburger *et al.* 2004). From biological evidence it is indicated that there has been habitable land in the region for as much as 0.5 Ma, although much more molecular information is currently needed.

3.1.3 Aims

This chapter aims to use DNA sequences to construct a phylogeny of the Chathamiidae, and by using a rough molecular clock, to understand the evolution of marine caddisfly species in the Kermadec and Chatham Islands. These dates can then also be used as supportive information in estimations of the island groups themselves. The evolutionary origin of other caddisfly species from the Chatham Islands will also be investigated for comparison with the species *C. brevipennis*.

3.2 Materials and Methods

3.2.1 Sample collection

Samples of *P. plebeius* and *C. integripennis* were collected from around New Zealand and Australia from November 2008 to February 2010 (see Chapter two of thesis). *Philanisus fasciatus* were collected by Karen Baird from Raoul Island in May 2010. Specimens of *C. brevipennis, Hydrobiosisis lindsayi* and *Oecetis chathamensis* were collected from localities in Chatham Island. Samples of *Oecetis unicolor* collected from mainland New Zealand was also used for outgroup analysis. Methodology of collection was largely that as described in Chapter two and included the

collection of larvae, adults and pupae. Adults were collected from near appropriate habitat either being coasts, lakes or streams; either by light-trapping or collected by hand when possible. All larvae or pupae were collected by searching manually through substrate of the rocky intertidal or small running streams, for marine or freshwater species respectively. No samples of *P. mataua* were successfully collected, however a single museum specimen was generously provided for this study. Collection and specimen details are listed in Fig 3.1.

3.2.2 DNA sequencing and alignment

DNA was extracted using a standard phenol-chloroform methodology (refer to Chapter two of thesis), using leg material from a number of specimens (3-5 individuals) of *Oecetis chathamensis*, *O. unicolor*, *Hydrobiosis lindsayi*, *Chathamia brevipennis*, *C. integripennis*, *Philanisus plebeius,* and *P. fasciatus*. For *P. plebeius* only 3 individuals were used; one to represent each of the three genetic haplogroups identified in Chapter Two. Also included was an unidentified larva from near Sydney, originally identified as *P. plebeius*.

A 618 bp fragment of the mitochondrial gene cytochrome oxdidase I (COI) was amplified using the primers HCO2198 and LCO1490 (Folmer *et al*. 1994). Additionally for each of the Chathamiidae species (except *P. mataua*) and each of the three haplogroups of *P. plebeius*; a fragment of the mitochondrial gene 16S was amplified using the primers 16SBRH and 16SARL (Palumbi *et al*. 1991). In the case of the older *P. mataua* specimen, LCO1490 was paired with the primer ChatP12r (new to this study), and was amplified and sequenced twice to counter for possible mis-priming. All primers are listed in Table 3.2. The concentrations and parameters for the PCR template, thermocycler, purification and sequencing have been presented in Chapter Two. All DNA sequencing used one primer only; HCO2198 for COI and 16SBRH for 16S. Also used were a large number of sequences from Genbank from a number of related studies. All sequences are listed with specimen details in Table. 3.1.

Chromatograms were visualised using Chromas software V. 145 (Technelysium Pty Ltd; http://www.technelysium.com.http://au/chromas.html). All sequences were loaded into the Clustal X algorithm in MEGA 4.0 (Kumar *et al.* 2007, 2008). Both COI and 16S were aligned using default parameters, with finer scale editing of 16S by eye.

3.2.3 Genetic analysis and phylogenetic reconstruction

The COI dataset was explored and had sequence statistics determined using the MEGA data explorer tool and DNAsp v 5 (Librado & Rozas 2009). Several separate datasets were used for phylogenetic reconstruction. A dataset of COI for the Chathamiidae was used using all obtained sequences of *P. fasciatus*, *C. brevipennis* and *C. integripennis* with a single sequence used from each of the three haplogroups of *P. plebeius* identified in Chapter Two. A smaller dataset using 16S data was analysed separately. For outgroup taxa, sequences of *Olinga feredayi* and *Zelolessica cheira* were used to represent the Conoesucidae and Helicophidae respectively; two of the closest families to the Chathamiidae (Kjer *et al*. 2001, 2002, Johanson & Keijsner 2008, Johanson et al. 2009, Johanson & Malm 2010, also refer to Chapter four of thesis).

Additionally another dataset comprised entirely of COI was used to explore the origin of the Chatham Island species *Hydrobiosis lindsayi* and the phylogeny of the endemic New Zealand genus *Hydrobiosis* as a whole, with all sequences other than *H. lindsayi* imported from Genbank, constituting over half of all described species (13 out of 24). Additionally another New Zealand genus, *Edpercivalia*, was also included as a possible subtaxon of *Hydrobiosis* as evidenced in another study (Hogg *et al*. 2009). Only two species of *Edpercivalia* were used (out of 12 described species), however if nested within *Hydrobiosis* the genus likely forms a monophyletic crown group. For outgroups, representatives of related Hydrobiosid caddisflies with the Hydrobiosinae were included, using a genetic data from every genus sequenced so far available online. The species *Apsilochorema hwangi* (subfamily Apsilochoreminae) was used as a basal taxon to root the tree.

All *Oecetis* sequences formed yet another, smaller dataset. The only other New Zealand species of *Oecetis; O. iti,* was not included, however is known only from few sites in the central South Island and the upper North Island and is unlikely to be important in the ancestry of *O. chathamensis* (contrast to *O. unicolor* which is widespread). No close relatives to serve as approptiate outgroups were obtainable for this analysis (which would likely be closely related species from Australia). Due to only two taxa being used, the *Oecetis* dataset was left unrooted, and explored using only a simple neighbour-joining analysis in MEGA (10,000 bootstrap replicates, default assumptions: maximum composite likelihood, homogenous pattern among lineages, uniform rate among sites).

For the remaining datasets, phylogenetic analyses were performed using the software packages MrBayes 3.1 (Heulsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) and PAUP* 4.0 (Swofford 1999).The program MODELTEST 3.7 (Posada & Crandall 1998) was implemented through PAUP* to identify the most likely suitable evolutionary model using the Akiake information (AIC), selecting a GTR+I+G modelfor both COI datasets (Chathamiidae and Hydrobiosidae) and selecting a GTR+I model for 16s.

Maximum parsimony (MP, Farris 1970) and Maximum Likelihood (ML, Felsenstein 1981) analyses were estimated using PAUP* on the COI dataset for the Chathamiidae. Maximum parsimony was performed using a heuristic search model, and bootstrapped using 10,000 replicates. Maximum likelihood was run using the model selected by MODELTEST and run under a heuristic search criterion using 10,000 replicates for bootstrap support.

Bayesian analyses were implemented in MrBayes 3.1, again using the closest model possible as suggested by MODELTEST, and run for all datasets. For each dataset one cold and three heated Markov chains were run for a total of either 10,000,000 generations sampled every 1,000 to obtain a total of 1,000 trees (for both Chathamiidae sets) or 20,000,000 generations for a sample of 2,000 trees (for the Hydrobiosidae). The first 25% of trees were discarded as a burn-in phase, with the remaining trees used to estimate the posterior probabilities.

3.2.4 Estimating divergences and molecular clock

In order to infer the ages of the species a molecular clock was attempted on the dataset. The prospect by which an independent clock can be used in this study is limited however. Helicophidae from the purbeck beds of England (154.8-137.2 Ma, Sukatsheva & Jarzembowski 2001), and two fossil taxa with affinites to the Calocidae, Helicophidae and Conoesucidae have been identified from Baltic Amber (85-74 Ma, Botosaneanu & Wichard 1983), although one has since been ascribed to the Northern family Sericostomatidae (Wietchat & Wichard 1998), neither of which therefore suitable as reliable estimates for a recent common ancestor. Excepting perhaps the probable Pleistocene age of the Chatham Islands, no fossil or geological dates can be used here with any confidence.

A divergence rate was estimated using two strict molecular clocks used from Brower 1994 (2.3% per Ma) and Papadopoulou *et al*. 2010 (3.54 / 2.69% for COI / COI+16s). Pairwise distances used for calibration were estimated in MEGA, using corrected (between mean) group distances, with each species analysed with its assumed nearest sister taxon as inferred by estimated phylogeny and/or genetic distance. The species *Philanisus fasciatus* and *Chathamia integripennis* are assumed to form sister taxa based on molecular distance. Also as the phylogeny of *Hydrobiosis* remained largely unresolved (see results, fig 3.8), distances were estimated between *H. lindsayi* and the mean divergence from the closest remaining 'Umbripennis' *Hydrobiosis* species (*H. copis*, *H. budgei*, *H. umbripennis*, *H. parumbripennis*, *H. johnsi*, *H. styracine* and *H. falcis*), Standard error was calculated with in MEGA with 10,000 replicates with use of the Kimura-2 parameter model.

3.3 Results

3.3.1 Sample collection

Most target species were successfully collected from Chatham Island. Only larvae and pupae of *Chathamia brevipennis* were found, all from near Kaingaroa township in the North-West of the Island amongst coralline algae, in spite of repeated light trapping for adults in near Port Hutt,

Waitangi and Owenga. No *Hudsonema* were collected from the island although a possible abandoned larval case was found in Awatotara creek, near the southern end of Chatham Island, indicating a breeding population, although this may have been of *Oecetis chathamensis.* Adults of *O. chathamensis* by contrast were common and easily collected in number throughout the island including from Te Whanga lagoon, lakes and streams. *Hydrobiosis lindsayi* were readily collectable as larvae, pupae and adults from or near any clean running stream. From the remainder of New Zealand samples of adult *Oecetis unicolor*, *P. plebeius*, *P. fasciatus* and *C. integripennis* were readily obtained, although no *P. mataua* were collected.

Also in addition, large numbers of the microcaddisfly genera *Paraoxyethira* and *Oxyethira* were collected from both New Zealand and Chatham Islands. However as the species on the Chathams are the same from New Zealand and other distant islands; an in-depth phylogeographic analysis would be needed for each species, which would be logistically difficult as Hydroptilid caddisflies are among the most common and ubiquitous insects in New Zealand. Whilst in the duration of this study it was eventually decided not to analyse these species.

3.3.2 Sequences and sequence statistics

The *Philanisus mataua* sequence had a number of sites shown in chromatograms with twin peaking, taken to be the presence of two mitochondrial sequences within the same individual. This species was thus analysed separately (see methods). The COI dataset for the Chathamiidae (omitting *P. mataua* and outgroups) constituted 618 base pairs, with 70 variable sites and 66 parsimony informative sites. The Hydrobiosidae dataset constituted 620 base pairs, which within *Hydrobiosis* and *Edpercivalia* alone 200 of which were variable and 158 parsimony informative. The *Oecetis* dataset was 633 base pairs, with 30 variable sites and 21 parsimony informative sites. The 16S dataset (only Chathamiidae) was 418 base pairs with 44 variable; however only 3 were parsimony informative – likely due to the extreme singular divergence of *C. brevipennis* for this gene (see Fig. 3.5). Nucleotide compositions were generally consistent across all lineages; 32.6-40.8% T, 13.8-

19.4% C, 29.4-33.5% A, 11.8-17.4% G for COI, and 40.40-40.6% T, 7.1-7.2% C, 39.7-40.1% A, 12.2-12.6 G% for 16s. However the Chathamiidae were generally more A-T rich, and the Hydrobiosidae more G-C rich for COI.

Pairwise divergences from within groups and between groups means are shown in Tables 3.4-3.6 (also see Table 3.7 for *Oecetis*). Distance analysis alone strongly indicates a high divergence for *C. brevipennis*, and confirming separate species status for *Oecetis chathamenis* and *Hydrobiosis lindsayi*. High interspecific divergences (>1%) are indicated for *P. plebeius* and *O. unicolor*, and little or no variation for *C. brevipennis*, *P. fasciatus* or *H. lindsayi*. Additionally no variation was found within 16s for *Philanisus plebeius* (not shown in figures).

3.3.3 Phylogeny of the Chathamiidae

Maximum likelihood, Maximum parsimony and Bayesian trees of COI & 16S data all Chathamiidae species, excepting *P. mataua*, are shown in figs 3.3-3.6. Monophyly of either genus was not supported. *C. brevipennis* was established as a relatively distant sister taxon to *P. plebeius*, *P. fasciatus* and *C. integripennis* which form a closely related monophyletic group (the 'Philanisid clade') strongly supported by both 16s and COI data. Within the 'Philanisid' group relationships were unclear, although Bayesian analyses indicated a *C. integripennis-P. fasciatus* sister relationship for COI and a *C.integripennis-P.plebeius* sister relationship for 16s.

C. integripennis was the most widely sampled taxon (not including *P. plebeius* from Chapter Two) and displayed the most genetic variation (0.6%). The 'Sydney Chathamiid', a larva previously assumed to be *P. plebeius* based on location is indicated unusually to be a *C. integripennis* occurring in Australia, closely related to one haplotype in particular from New Zealand ("05" – Hogg *et al*. 2009, 1 base pair difference).

3.3.4 Phylogenetic placement of *Philanisus mataua*

The short sequence obtained from the single sample of *P. matau*a was highly ambiguous, and chromatograms consistently confirmed the presence of two separate mitochondrial sequences (the region was amplified and sequenced twice, and in both cases chromatograms showed the same results). Within the region of COI sequenced for *P. mataua* (418 base pairs), there were 20 variable sites within the Philanisid clade, 7 of which were heterogeneous for *P. mataua*. In addition no site changes were unique to this sequence; all variable sites fell within variation for either *P. plebeius* or *C. integripennis*. Uncorrected pairwise distances (not including ambiguous regions) were identical between the *P. mataua* sequence and either the 'Sydney Chathamiid' (see above) or a New Zealand *C. integrippenis* sequence ("05") imported from Hogg *et al*. (2009), both indistinguishable at the COI region concerned (the two ultimately differ elsewhere however). 3 singleton sites (4 if *P. fasicatus* is excluded) unique to these *C. integripennis* sequences converged with heterogeneous base pair regions within the *P. mataua* sequence, indicating one sequence to match the *C. integripennis* haplotype.

If the phasing is assumed to be the same as with the C. *integripennis* sequence, then was possible to form a hypothetical counter-sequence (*P. mataua* 'A' and 'B'; with 'A' identical to *C. integripennis*). The B sequence was found to fall within variation exhibited within *P. plebeius*, although divergent and unrelated to any known haplotype**.** Uncorrected divergence distances between *P. mataua* 'B' and *P. plebeius* ranged from 0.79-1.49%, (only 0.79-0.99% to *P. plebeius* clade A). Neighbour-joining trees for the unmodified and modified *P. mataua* sequence/s and all available *Philanisus* data (including *C. integripennis*) are shown in Figs 3.9-3.10.

3.3.5 Phylogeny of Hydrobiosis and Oecetis

Oecetis chathamensis was found to be distinct from *O. unicolor* supporting separate species status (see Fig 3.7) However genetic diversity was considerably higher within *O. unicolor* (1.5% vs 0.5), approaching levels found between species. The dataset of the Hydrobiosidae generally showed varying levels of Bayesian support. *Edpercivalia* was weakly suggested to be nested within

Hydrobiosis sister to the species *H. charadraea*, however at the very least is indicated to form the sister taxon followed by the genus *Costachorema*. The inner phylogeny of *Hydrobiosis* itself was less clear although monophyly of the 'Umbripennis' group was well supported, with *H. gollanis* basal to the other species (including *H. lindsayi*), in turn shown to be closely related.

3.3.6 Divergences and Age assumptions

Mean corrected pairwise divergences for each taxon of interest with hypothesised divergence ages are shown in Table 3.6. *Chathamia brevipennis* is indicated to represent the oldest lineage analysed at 4.91-2.46 Ma in age, followed by at *Hydrobiosis lindsayi* at 4-2.1 Ma. The remainder of the Chathamiidae are indicated to have diverged more recently during the Pleistocene 1.39-0.56 Ma, the most recent split being with *Chathamia integripennis* and *Philanisus fasciatus* 0.92-0.34 Ma. *Oecetis chathamensis* and *O. unicolor* are suggested to have diverged 1.78-0.79 Ma. The molecular models had a high margin of error (using two clocks), however *C. brevipennis* was indicated to show largely congruent ages using each clock and gene (roughly ~4 Ma), with the exception of the 3.59% clock from Papadopoulou *et al*. (2010) for COI.

3.4 Discussion

3.4.1 Phylogeny and taxonomy of the Chathamiidae

As clearly shown by both COI and 16S data, both *Chathamia* and *Philanisus* are polyphletic taxa with the species *C. integripennis* nested within *Philanisus* (related to *P. fasciatus* in particular) together comprising a a 'Philanisid' clade. The only other species of *Chathamia*, *C. brevipennis*, by contrast formed a remote sister taxon to this grouping. This relationship is also supported by biogeographic inference as the northerly-distributed *C. integripennis* makes a poor candidate to be close to the ancestry of the Chatham Island *C. brevipennis*. As *C. brevipennis* is the type species of *Chathamia*, this can be easily resolved by transferring *C. integripennis* to *Philanisus* (creating the new

species name *Philanisus integripennis*), rendering both genera monophyletic and leaving *Chathamia* monotypic and endemic to the Chatham Islands.

The high genetic distance between *C. brevipennis* and the remainder of the family also supports the retention of the genus *Chathamia* as distinct from *Philanisus*, although distances suggestive of subfamily status were not supported in agreement with Riek (1976*)*. Any morphological features identified by Riek between *Chathamia integripennis* and *C. brevipennis* are considered to be analogous convergences, or alternatively plesiomorphic features (however this would indicate the species to have been the basal 'Philanisid' in regard to the probable singular evolution of '*Philanisus'* features, which was never supported – the basal Philanisid was likely either *P. plebeius* or *P. fasciatus*). The extant representatives of the Chathamiidae are indicated to have begun radiation in the Pliocene to early Pleistocene (with *C. brevipennis*), with the Philanisid group diverging in the late Pleistocene, roughly 1 Ma.

Another significant result was the identification of a larval *C. integripennis* to be found in Sydney New South Wales. Possible error was accounted for as the specimen was re-extracted and sequenced again, confirming the result. This is the first time this species has been found outside of Northern New Zealand, and joins *P. plebeius* as the only New Zealand caddisfly species to be also found in Australia. The sample also being larval clearly demonstrates a breeding population (as opposed to a rare adult vagrant). However the sample was identified very close to one NZ haplotype (1 base pair difference) indicating a very recent origin or possibly a human introduction (however the NZ sequence was not from this study and the collection locality remains unknown). The singular pair difference is also congruent with that found between the New Zealand and Australian populations of *Philanisus plebeius*, (see Chapter Two) although sampling in this species was much more thorough.

Philanisus mataua raised the most issue with phylogenetic placement, with sequenced data showing two mitochondrial sequences. This was considered not to reflect degeneration due to the age of the sample (a 17 year old museum specimen) as the heterogenous sites occurred only in areas variable in *Philanisus* (including *C. integripennis*), and either represents contamination or

heteroplasmy. The two *P.mataua* sequences were considerably different (7/20 variable 'Philanisid' sites differed between them). Naturally occurring and divergent heteroplasmic sequences are generally the result non-maternal mitochondrial transmission, or 'paternal leakage' (Lansman *et al.* 1983). In insects, paternal leakage of mitochondrial DNA has been documented in species of bee (Meusel & Moritz 1993, Magnacca & Brown 2010 *a, b*), *Drosophila* (Satta *et al.* 1988, Kondo *et al.* 1990, 1992, Matsuura *et al*. 1991, Sherengul *et al.* 2006), mosquitoes (Paduan & Ribolla 2008), moths (Arunkumar *et al.* 2006) and cicadas (Fonataine *et al*. 2007).

If the two sequences represent heteroplasmy then *P. mataua* appears to represent a hybrid between *P. plebeius* and *C. integripennis* (see Figs 3.9 & 3.10). This is biogeographically plausible; *P. mataua* is rare and occurs sympatrically with both *P. plebeius* and *C. integripennis* over most of its distribution (in the upper North Island see fig 3.1). Natural intraspecific hybridisation is not well documented in Trichoptera although is evidenced to occur in a number of studies (Blahnik 1995, Leese 2004, Pauls 2004, Wells 2006, Pauls *et al*. 2009, 2010). Heteroplasmy is also more frequent in hybrids; it has been suggested that intra-specific hybridisation leads to frequent heteroplasmy as oocyte enzymes are less likely to recognise and counter unrelated mitochondria (Kondo *et al*. 1990, Kaneda *et al*. 1995, Kvist *et al*. 2003, Ballard et al. 2004). Heteroplasmy does not seem to be commonly inherited (Gyllensten 1991) indicating this sample would likely be a direct hybrid.

However even among intraspecific hybrids heteroplasmy is generally uncommon (Kondo *et al.* 1990, Gyllensten 1991); thus the likelihood of one sample to be heteroplasmic is unlikely. It is also is unclear what extent the morphology of *P. mataua* is intermediate between *C. integripennis* and *P. plebeius*; several morphological features are distinct to the species (Ward 1994, 1995). Additionally *C. integripennis* is not recorded from the Three Kings Islands; an apparent stronghold of *P. mataua* (5/12 of all collection records, and 8 of about 20 known specimens). It is perhaps more probable the sample is simply contaminated by *C. integripennis* DNA, made only more likely as most of the sample was used for DNA extraction. Cannibalism is known to occur in normally herbivorous caddisfly larvae (Mecom 1972, Wissinger *et al.* 1996, 2004) and is known in *P. plebeius* (Leader 1976), although it seems unlikely consumed material as a larva would transmit through to adulthood.

Contamination is probably the most parsimonious explanation although new *P. mataua* sequences are necessary to confirm either hypothesis.

Overall the taxonomy of the family needs some revising. It is proposed that *Chathamia integripennis* be transferred to the genus *Philanisu*s. This would leave *Chathamia* a monotypic Chatham Island taxon, and *Philanisus* comprising all of the four species found in mainland New Zealand, the Kermadecs and Australia. Additionally new molecular data should be collected for *P. mataua* in order to test for its phylogenetic status. It cannot be ignored that possible hybridisation and molecular introgression may have considerable implications inferring the phylogeny of the Chathamiidae, especially as only mitochondrial markers were used in this study and that heteroplasmy is indicated (Posada & Crandall 2002, Sackton et al. 2003, Piganeau *et al.* 2004).

3.4.2 Phylogenetic placement of *Hydrobiosis lindsayi* and *Oecetis chathamensis*

The placement of *Edpercivalia* as a subtaxon of *Hydrobiosis* was not well supported in this study. Morphological data supports the close relationship of *Edpercivalia* and *Hydrobiosis*, although *Edpercivalia* shares a number of presumably pleisomorphic features with *Costachorema* and separate genus status seems likely (Ward *et al*. 2004). Separate species status of *H. lindsayi* was supported, as is the placement of *H. lindsayi* within the Umbripennis group of *Hydrobiosis* (Schmid 1989, Smith 1998). Molecular divergence suggests the divergence of *H. lindsayi* from the remaining *Hydrobiosis* 4-2.1Ma, not fully congruent with a Pleistocene date for the origin of the Chatham Islands.

Oecetis chathamensis was also supported as distinct from *O. unicolor*, supporting morphological differences such as ‘turquoise’ coloured larvae unique to the taxon (Champion & Clayton 2004), both diverging 1.78-0.79 Ma. The younger age of *O. chathamensis* (Leptoceridae) contrast to *H. lindsayi* (Hydrobiosidae) is likely expected. Although the Hydrobiosidae is the most diverse caddisfly family in New Zealand (roughly half of described species), Hydrobiosids are apparently poor long-distance dispersers with all native genera entirely endemic and globally have a largely southern Gondwanan biogeography (Schmid 1989, de Moor & Ivanov 2008), although two

have dispersed from New Zealand and are endemic to the Auckland Islands (Wise 1976, Schmid 1989, Micheaux &Leschen 2004). Leptocerids are by contrast among the most widespread of Trichoptera and are among the few families to commonly inhabit oceanic islands (e.g. Malicky 1992, Smithers 2000, Wells 2004). In addition *O. chathamensis* and *O. unicolor* closely resembles the species *O. umbra* from Tasmania, and the whole New Zealand lineage is likely a recent Australian dispersal (Neboiss 1979, Wells 2004).

3.4.3 *Philanisus fasciatus* and the Kermadec Islands.

P. fasicatus was shown to form a distinct taxon, although closely related to the species *P. plebeius*, *P. mataua* and *C. integripennis* (the 'Philanisid' clade). Nevertheless, the species is indicated by COI data to have diverged from *C. integripennis* between 0.34-0.92 Ma, well into the early to mid Pleistocene. The date compares with the only other molecular study of a Kermadec insect; that found for Kermadec cicadas (0.39-0.71 Ma, Arensburger *et al*. 2004). The lineage that constitutes the Kermadec pohutukawa (*Metrosideros kermadecensis*) is believed to have dispersed out of New Zealand 0.5-1 Ma, although includes a number of Pacific species as far north as Hawaii (the 'excelsa' lineage) and some gene flow between *M. kermadecensis* and the New Zealand *M. excelsa* has been indicated (Wright *et al*. 2000, 2001, Gardner *et al.* 2004). No other molecular studies have been undertaken including any of the endemic Kermadec fauna or flora with the exception of two species of limpet (Wood & Gardner 2007), however this only discussed local connectivity and no molecular clock was used.

From the current genetic evidence however it can be proposed that there has been continuous land in the Kermadec region suitable for terrestrial inhabitation >0.3 Ma and possibly considerably longer, also supported by the general existence of other species and subspecies endemic to the Islands. Using geological records to estimate an age of the islands is difficult, as being the tops of active volcanoes they are likely to have had an ephemeral history having risen and fallen numerous times in the past. The two largest islands (Raoul and Macauley Islands) are both closely linked to massive,

near-surfacing submarine calderas both representing recent eruptive collapse events. Macauley Island was likely over 10 times its current size before the SBT eruption 6 ka (Brothers & Martin 1970, Lloyd et al. 1996, Smith *et al.* 2003 *a*), and even the comparatively large Raoul was probably more massive until the collapse of Denham caldera 2.2 ka (Worthington *et al*. 1999). Such events would have had a catastrophic effect on the biodiversity; for example the eruption 2.2 Ka on Denham caldera is estimated to be comparable in volume to the 1883 Krakatau eruption (Worthington *et al*. 1999), and the SBT eruption is possibly one of the largest eruptions in the entire Holocene (Latter *et al*. 1992, Lloyd *et al*. 1996). If recent Holocene ages of Raoul and Macauley islands is assumed it is probable that there must have been sufficient continuous land elsewhere in the region long enough for some unique biota to form.

As is known with Macauley (Brothers & Martin 1970, Lloyd et al. 1996, Smith *et al.* 2003 *a*), some or even all of the smaller islands (and their volcano bodies) were likely once much larger prior to caldera collapse and erosion. All islands would have also been considerably more massive during lower sea levels in the Pleistocene as recently as the last glacial maximum (~20 ka). In addition the Kermadec chain also constitutes at least 26 major volcanic centres comprising numbers of submarine volcanoes and seamounts, some (such as the Giggenbach volcano) are high enough to have formed islands over 50 m high during this period (Wright 1994, Ballace *et al*. 1999, Smith *et al.* 2003 *b*, Wright *et al*. 2006). Collapse and re-eruption is common in the Kermadec-Tonga arc (Ballance *et al.* 1999, Wright *et al.* 2008), and thus there have likely been a number of islands, now long submerged. As a result of these factors the emergent areas of the Kermadec region have likely been under a period of considerable flux.

Therefore in spite of a volatile history it seems plausible there has been continuous land suitable to support terrestrial animal and plant life in the Kermadec region since the early-mid Pleistocene 0.3-0.5 Ma and perhaps earlier. However the Kermadec biota can therefore probably be assumed to have undergone a series of historical ‘island-hopping’ episodes and repeatedly subjected to regular local extinctions or volcanic ‘sterilisations’ with the current restriction of most species to Raoul and Macauley Islands untypical. Species such as *P. fasciatus* being largely intertidal, and *M.*

kermadecensis able to colonise fresh lava flows (Clarkson 1990), may have been better suited for prolonged survival in this environment. This may also explain why overall diversity in the islands is low, although many more molecular studies are still needed.

3.4.4 *Chathamia brevipennis, Oecetis chathamensis, Hydrobiosis lindsayi* and the Chatham Islands.

C. brevipennis was indicated conclusively by both COI and 16s to be highly divergent from the remainder of the Chathamiidae and that *Chathamia* should be rendered a monotypic genus endemic to the island group (*Chathamia integripennis* being unrelated and nested within *Philanisus*). This may be significant as although endemicity in the Chatham Islands is high, levels only very rarely reach above the species or sub-species level (Emberson 1995, 1998 Heenan *et al.* 2010). For age estimates, *Chathamia brevipennis* diverged from the remaining Chathamiidae 2.46-4.91 Ma ago, supporting a Pliocene origin of the species. The genetic divergence (~10% for COI and 16s) and the age (>2.4 Ma) are among the highest found for any Chatham Island species. Additionally *Hydrobiosis lindsayi* was also indicated a possibly old taxon 4-2.1 Ma in age, although the lower estimates are congruent with geological records. *Oecetis chathamensis* was indicated conclusively to be a recent Pleistocene dispersal roughly 1 Ma in age, typical of most Chatham species.

However these dates should perhaps be considered carefully, Brower's substitution rate in particular has been known to considerably overestimate probable divergence times in Lepidoptera (Gratton *et al*. 2008). However such rates invariably differ between lineages, and other subsequent studies on insects using independent clock models have inferred a wide variety of divergence rates (Papadopoulo *et al*. 2010). In addition strict models assume a constant rate of divergence; however rates are known to be non-linear, rapidly decreasing with age as nucleotide saturation and selection pressures begin to play an increasing role (Brown et al. 1979, 1982, Arbogast et al. 2002).

Relaxed molecular clocks allow rates to differ and have been used in caddisflies finding substitution rates as high as 5-6% per Ma for recently diverged taxa (less than 1Ma), with rates

rapidly decreasing with apparent age (Espeland & Johanson 2010 *a*, *b*). Divergences over 10% in these studies are suggested to reflect ages well in excess of 5 Ma, for example divergences under 12% have been proposed as roughly 20 Ma in age in the genus *Agmina* (Espeland & Johanson *b*). Additionally, 16s shows an unusually high level of divergence between *C. brevipennis* and the remaining Chathamiidae, roughly the same found in COI despite the slower evolution rate of 16s (~3x slower in Papadopoulou *et al*. 2010). This may suggest further mutation of COI has slowed due to negative selection; further supporting a Pliocene age for *C. brevipennis* (a minimum age of 3.12 Ma is suggested when both genes are combined).

Thus an ultimate minimum age of 2.46 Ma is probably conservative and potentially much higher. If the divergence and the speciation of *C. brevipennis* is directly related to the allopatric event of dispersal to the Chatham islands, then the divergence must directly relate to continuous land in the region since the assumed time (additionally the lineage need not reflect the whole age of the island group). Alternatively *C. brevipennis* can be hypothesised as having originated from a now-extinct lineage from New Zealand and thus not directly related to the remaining Chathamiidae. This argument will always remain a possibility; as the in spite of their presumed antiquity (thus reaching family status) the Chathamiidae have a remarkably low diversity and are likely to have passed through a recent evolutionary bottleneck. An island group such as the Chathams may have provided a refuge for an older lineage of Chathamiids, only now recently extinct in New Zealand.

Two species flightless stag beetle from the Chatham Islands (*Geodorcus* spp.) were aged at ~6 Ma (Trewick 2000), although it was decided in the particular study the closest mainland relative was not included. Only three *Geodorcus* species (just one from the mainland) were analysed out of a described 17 (Holloway 1996, 2007) making this inference likley. All other divergences (see introduction section for full review) for Chatham Island invertebrates, including spiders, damselflies, cicadas, stick insects, cockroaches, isopods and amphipods show much more recent ~1-2 Ma arrivals within New Zealand. Similarly the endemic Chatham mudfish and the majority of the flora show a recent origin. No bird taxa are found to be any more recent than the mid-early Pleistocene; the entirety of the endemic Chatham avifauna is restricted to species or subspecies level. However three extinct

monotypic bird genera; *Pachyanas*, *Cabalus* and *Diaphorapteryx* (Chatham Island duck, and Chatham and Hawkin's rails) were endemic to the islands and may have older origins (Holdaway *et al*. 2001).

High distances have however been found for at least four plant taxa (6.18-10.97 Ma, Heenan *et al*. 2008) and for the Chatham skink (5.86-7.29 Ma, *Oligosoma nigiriplantare nigiriplantare*, Liggins *et al*. 2008), although the closest mainland relatives to the Chatham skink may have not been sampled. For a hypothetical explanation regarding the old age of some plant taxa, Heenan *et al*. discussed the confirmed Miocene-Pliocene emergence of the Mangere volcano (Campbell & Hutching 2007) and also cited the evidence of fossiliferous and palynological records of Opoitian (5.28-3.6 Ma) and Waipipian-Mangapanian (3.6-2.4 Ma) age, apparently carrying though to present (Mildenhall 1994). Also discussed was one Pliocene bone indistinguishable from the modern Chatham Pigeon (*Hemiphaga chathamensis*, Eagle *et al.* 2005), although this is now believed a recent Holocene intrusion (Worthy *et al*. 2009).

If the Mangere-volcano hypothesis is considered, there has been continuous land in the wider Chatham region for up to 6 Ma, as much as 4 Ma longer than has been suggested by recent geological studies. It is possible that some of the Rangitihi Volcanics (the most recent of all the Chatham volcanoes) were emergent earlier, or that there was emergent land elsewhere in the Chatham Rise allowing for a tenacious evolutionary connection between the 'Mangere' and 'Chatham' biotas (Campbell *et al*. 1988, Heenan *et al.* 2010). Intertidal Chathamiids may have fared better than most terrestrial invertebrates and the skink *O. nigiriplantare* can survive on small rock stacks (McCann 1955), so both animals may have persisted through a geographical bottleneck. This explanation is purely conjectural and may be based off overestimated ages, however does explain why some of the Chatham biota appear to have Pliocene or even Miocene origins whilst the vast majority is evidently much younger. With this indicated more molecular studies should be undertaken of select taxa linking the Chatham Islands to New Zealand, and some taxa such as *Geodorcus* should be re-investigated. In addition the geological possibility of a Plio-Pleistocene link 2-4 Ma between Mangere volcano and the modern Chathams should perhaps be more seriously considered.

3.5 Figures

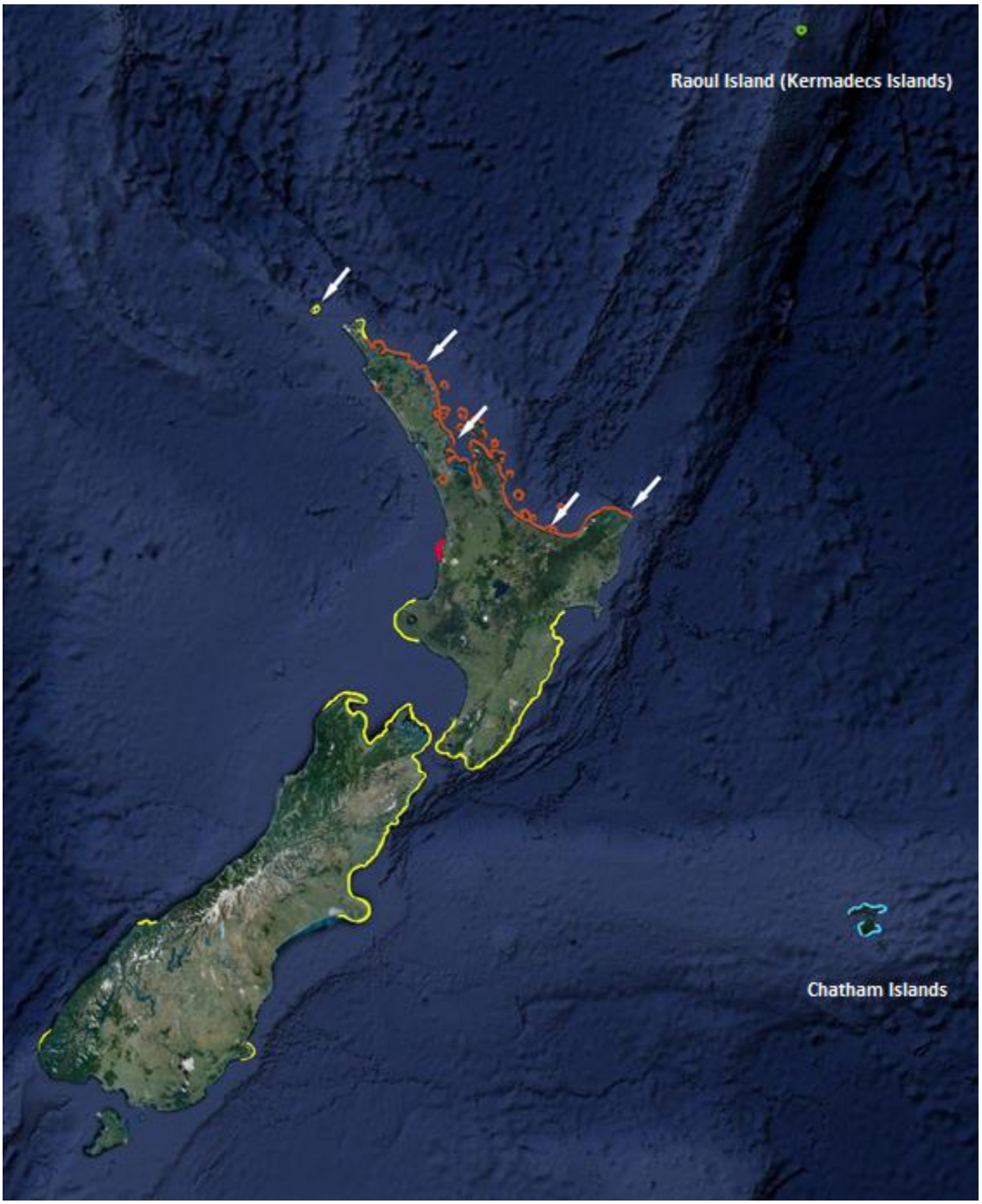


FIG 3.1) Estimated distribution of the Chathamiidae in New Zealand as inferred from collection records at the New Zealand trichoptera database (http://http://nzcaddis.massey.ac.nz/). As follows Yellow = *Philanisus plebeius*, Red = *Chathamia integripennis*, Orange = *Philanisus plebeius* / *Chathamia integripennis*, Blue = *Chathamia brevipennis*, Green = *Philanisus fasciatus*. White arrows designate the localities where *Philanisus mataua* has been collected. Not shown is the Australian distribution of *Philanisus plebeius* (Roughly 40km of coastline from Sydney Southwards), and also one dubious record from Stewart Island. Distribution disjunctions, especially in the South Island and the eastern North Island may reflect sampling bias.

TABLE 3.1) Full list of taxa, specimens and sequences included (including Chathamiidae + outgroups, Hydrobiosidae and Oecetis). Where possible collection details are shown. Source if not this study shows reference and genbank accession numbers. References: **1**; Johanson (2007), **2**; Johanson & Keijsner (2008), **3**; Hogg *et al*. (2009), **4**; Espeland *et al*. 2008, **5**;Johanson *et al*. (2009), **6**; Johanson & Espeland (2010), **7**; Shan *et al*. (unpublished).

*Distribution of whole species, specific sample collection site unknown. ** Unidentified prior to sequencing.

Species	Gene	Specimen No.	Ontogeny	No.	Source	Collector	Locality	Coordinates	Date
Philanisus plebeius "A"	COI & 16s	L201	Adult	1	This study	Ian Henderson	Marbourough Sounds	41°14’25 S, 174°16’14 E	30/12/2008
Philanisus plebeius "B"	COI & 16s	A1002	Adult	1	This study	Alex Boast	Tauranga	37°42’29 S, 174°53’15 E	16/02/2009
Philanisus plebeius "C"	COI & 16s	B303	Adult	1	This study	Alex Boast	Mangakuri Beach	39°57’59 S, 176°55’14 E	27/02/2009
Philanisus mataua	COI	PM2	Adult	1	This study	John Ward	Whangapaparoa	36°35’35 S, 174°49’40 E	24/01/1993
Philanisus fasciatus	COI & 16s	PF101	Adult	1	This study	Karen Baird	Raoul Island	29°14’56 S, 177°55’14 E	20/10/2009
Philanisus fasciatus	COI	PF102-3, 301-3	Adult	2	This study	Karen Baird	Raoul Island	29°14’56 S, 177°55’14 E	20/10/2009
Chathamia integripennis	COI	CI1	Adult	1	This study	Alex Boast	Waiwera	36°32’56 S, 174°42’32 E	8/03/2009
Chathamia integripennis	COI	CI201-2,301-2	Adults	4	This study	Alex Boast	Mt. Maunganui	37°37’30 S, 174°10’29 E	17/02/2009
Chathamia integripennis	COI	"05"	-	1	**3** GU263323	-	New Zealand*	-	-
Chathamiia integripennis	COI	DP2	-	1	**5** FJ263238	-	New Zealand*	-	-
Chathamia integripennis **	COI	K901	Larva	1	This study	Alice Wells	Sydney	33°49’25 S, 151°16’37 E	22/08/2008
Chathamia brevipennis	COI & 16s	CB1	Larva	5	This study	Alex Boast	Kaingaroa, Chat. Is.	43°43’49 S, 176°16’07 E	14/02/2010
Chathamia brevipennis	COI	CB2-5	Larvae/Pupae	4	This study	Alex Boast	Kaingaroa, Chat. Is.	43°43’49 S, 176°16’07 E	14/02/2010
Olinga feredayi	COI & 16s	BM5	-	1	**2** EF395045-4980	-	New Zealand*	-	-
Zelolessica cheira	COI & 16s	BP4	-	1	**2**EF395047-4982	-	New Zealand*	-	-
Hydrobiosis lindsayi	COI	HB101-201	Larva/Pupa	2	This study	Alex Boast	Awatotara Ck., Chat. Is.	44°03’34 S, 176°37’12 E	15/02/2010
Hydrobiosis lindsayi	COI	HB301	Adult	1	This study	Alex Boast	Makara R. Chat. Is.	43°59’19 S, 176°27’13 E	16/02/2010
Hydrobiosis budgei	COI	"59"	-	1	**3** GU263339	-	New Zealand*	-	-
Hydrobiosis chardraea	COI	"47"	-	1	**3** GU263340	-	New Zealand*	-	-
Hydrobiosis copis	COI	"23"	-	1	**3** GU263344	-	New Zealand*	-	-
Hydrobiosis falcis	COI	"44"	-	1	**3** GU263345	-	New Zealand*	-	-
Hydrobiosis gollanis	COI	"09"	-	1	**3** GU263346	-	New Zealand*	-	-
Hydrobiosis harpidiosa	COI	"42"	-	1	**3** GU263347	-	New Zealand*	-	-
Hydrobiosis johnsi	COI	"39"	-	1	**3** GU263348	-	New Zealand*	-	-

TABLE 3.1) Continued.

Species	Gene	Specimen No.	Ontogeny	No.	Source	Collector	Locality	Coordinates	Date
Hydrobiosis parumbripennis	COI	"22"	-	1	**3** GU263349	-	New Zealand*	-	-
Hydrobiosis soror	COI	"14"	-	1	**3** GU263352	-	New Zealand*	-	-
Hydrobiosis spatulata	COI	"08"	-	1	**3** GU263353	-	New Zealand*	-	-
Hydrobiosis styracine	COI	"21"	-	1	**3** GU263354	-	New Zealand*	-	-
Hydrobiosis umbripennis	COI	"60"	-	1	**3** GU263355	-	New Zealand*	-	-
Edpercivalia cassicola	COI	"43"	-	1	**3** GU263334	-	New Zealand*	-	-
Edpercivalia thomasoni	COI	"19"	-	1	**3** GU263335	-	New Zealand*	-	-
Atrachorema mangu	COI	"30"	-	1	**3** GU263321	-	New Zealand*	-	-
Costachorema callistum	COI	"45"	-	1	**3** GU263326	-	New Zealand*	-	-
Costachorema hecton	COI	"26"	-	1	**3** GU263327	-	New Zealand*	-	-
Costachorema xanthopterum	COI	"28"	-	1	**3** GU263330	-	New Zealand*	-	-
Neurochorema armstrongi	COI	"52"	-	1	**3** GU263358	-	New Zealand*	-	-
Neurochorema confusum	COI	"57"	-	1	**3** GU263361	-	New Zealand*	-	-
Psilochorema leptoharpax	COI	AO1	-	1	**4** AM902790	-	New Zealand*	-	-
Psilochorema mimicum	COI	"18"	-	1	**3** GU263391	-	New Zealand*	-	-
Xanthochorema bifurcatum	COI	W2	-	1	**1** DQ485522	-	New Caledonia*	-	-
Moruya charadra	COI	DC6	-	1	**6** FN179076	-	Australia*	-	-
Tiphobiosis cowiei	COI	"66"	-	1	**3** GU263407	-	New Zealand*	-	-
Tiphobiosis kleinpastei	COI	"72"	-	1	**3** GU263409	-	New Zealand*	-	-
Tiphobiosis veniflex	COI	"50"	-	1	**3** GU263410	-	New Zealand*	-	-
Synchorema zygoneura	COI	BY5	-	1	**4** AM902799	-	New Zealand*	-	-
Apsilochorema hwangi	COI	?	-	1	**7** AY490798	-	China*	-	-
Oecetis chathamensis	COI	OC201	Adult	1	This study	Alex Boast	Henga L., Chat. Is.	43°51’60 S, 176°33.12 E	12/02/2010
Oecetis chathamensis	COI	OC601	Adult	1	This study	Alex Boast	Te Whanga L., Chat. Is	43°49’59 S, 176°30.19 E	14/02/2010
Oecetis chathamensis	COI	OC701	Adult	1	This study	Alex Boast	Makara R., Chat. Is.	43°59’19 S, 176°27’13 E	16/02/2010
Oecetis unicolor	COI	OC8-9	Adult	2	This study	Alex Boast	St Arnaud	41°48’12 S, 172°50.44 E	27/02/2010
Oecetis unicolor	COI	OC11	Adult	1	This study	Alex Boast	MangakuriBeach	39°57’59 S, 176°55’14 E	5/12/2009

TABLE 3.2) List of primers used for amplification and sequencing.

Gene	Primer Name	Primer sequences (5' - 3')	Reference
COI	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al*. 1994
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*. 1994
COI	ChatP12	GAAATACCAGCTAAATGTAAAG	This study
16s	16sARL	CGCCTGTTTATCAAAAACAT	Palumbi (1996)
16s	16sBRH	CCGGTCTGAACTCAGATCACGT	Palumbi (1996)

TABLE 3.3) Interspecific divergence means (d) for COI for all species sequenced for this study (except *P. mataua*), with standard error (S.E.). Calculated in MEGA v 4 with 10,000 replicates using the Kimura-2 parameter model.

Species	d	S.E.
Philanisus plebeius	0.013	0.004
Philanisus fasciatus	0.001	0.001
Chathamia integripennis	0.006	0.002
Chathamia brevipennis	0	0
Hydrobiosis lindsayi	0	0
Oecetis chathamensis	0.005	0.002
Oecetis unicolor	0.015	0.004

TABLE 3.4) Intraspecific pairwise divergence means for COI (bottom-left) between species of the Chathamiidae and outgroups with standard error (top-right). Calculated in MEGA v 4 with 10,000 replicates using the Kimura-2 parameter model.

Species	1)	2)	3)	4)	5)	6)
1) *Philanisus plebeius*		0.006	0.006	0.013	0.018	0.017
2) *Philanisus fasciatus*	0.027		0.005	0.014	0.017	0.018
3) *Chathamia integripennis*	0.026	0.016		0.013	0.017	0.018
4) *Chathamia brevipennis*	0.102	0.100	0.099		0.019	0.018
5) *Olinga feredayi*	0.179	0.170	0.167	0.198		0.019
6) *Zelolessica cheira*	0.167	0.171	0.175	0.176	0.196	

TABLE 3.5) Intraspecific pairwise divergence means for 16S (bottom-left) between species of the Chathamiidae and outgroups with standard error (top-right). Calculated in MEGA v 4 with 10,000 replicates using the Kimura-2 parameter model.

Species	1)	2)	3)	4)	5)	6)
1) *Philanisus plebeius*		0.005	0.003	0.015	0.021	0.020
2) *Philanisus fasciatus*	0.010		0.005	0.015	0.021	0.020
3) *Chathamia integripennis*	0.005	0.010		0.015	0.021	0.020
4) *Chathamia brevipennis*	0.081	0.079	0.081		0.020	0.021
5) *Olinga feredayi*	0.163	0.160	0.163	0.154		0.020
6) *Zelolessica cheira*	0.148	0.142	0.148	0.166	0.154	

TABLE 3.6) Intraspecific pairwise divergences between all *Hydrobiosis* and *Epdercivalia* sequences (down-right), with standard error (up-left). This study is concerned primarily with *Hydrobiosis lindsayi* only so other Hydrobiosidae were omitted from this analysis. Calculated in MEGA v 4 with 10,000 replicates using the Kimura-2 parameter model.

	1)	**2)**	**3)**	**4)**	**5)**	**6)**	**7)**	**8)**	**9)**	**10)**	**11)**	**12)**	**13)**	**14)**	**15)**
1) *Hydrobiosis umbripennis*		0.014	0.016	0.011	0.011	0.011	0.011	0.017	0.008	0.01	0.015	0.018	0.011	0.018	0.018
2) *Hydrobiosis soror*	0.121		0.016	0.016	0.016	0.015	0.016	0.016	0.015	0.016	0.015	0.016	0.016	0.017	0.017
3) *Hydrobiosis spatulata*	0.140	0.136		0.016	0.017	0.017	0.017	0.016	0.015	0.016	0.017	0.017	0.016	0.018	0.018
4) *Hydrobiosis styracine*	0.077	0.152	0.156		0.012	0.011	0.006	0.018	0.012	0.012	0.016	0.019	0.012	0.018	0.02
5) *Hydrobiosis budgei*	0.081	0.152	0.170	0.099		0.011	0.012	0.016	0.012	0.014	0.016	0.018	0.012	0.019	0.019
6) *Hyrobiosis copis*	0.081	0.137	0.150	0.082	0.072		0.011	0.017	0.011	0.012	0.015	0.018	0.012	0.017	0.019
7) *Hydrobiosis falcis*	0.083	0.155	0.162	0.023	0.092	0.081		0.018	0.012	0.011	0.015	0.018	0.012	0.018	0.020
8) *Hydrobiosis harpidiosa*	0.156	0.133	0.152	0.176	0.163	0.165	0.181		0.017	0.017	0.017	0.018	0.017	0.020	0.018
9) *Hydrobiosis parumbripennis*	0.040	0.127	0.136	0.089	0.086	0.077	0.091	0.167		0.011	0.016	0.017	0.012	0.018	0.018
10) *Hydrobiosis johnsi*	0.066	0.141	0.146	0.081	0.111	0.087	0.079	0.163	0.072		0.016	0.017	0.013	0.019	0.019
11) *Hydrobiosis gollanis*	0.128	0.131	0.153	0.131	0.133	0.130	0.125	0.159	0.142	0.148		0.019	0.015	0.019	0.019
12) *Hydrobiosis charadraea*	0.161	0.134	0.147	0.179	0.173	0.173	0.169	0.165	0.154	0.16	0.177		0.019	0.018	0.018
13) *Hydrobiosis lindsayi*	0.072	0.141	0.154	0.09	0.086	0.085	0.087	0.161	0.081	0.094	0.126	0.184		0.018	0.019
14) *Edpercivalia cassicola*	0.168	0.152	0.168	0.176	0.186	0.161	0.172	0.189	0.174	0.184	0.178	0.152	0.168		0.017
15) *Edpercivalia thomasoni*	0.174	0.154	0.162	0.197	0.189	0.19	0.95	0.172	0.172	0.192	0.178	0.166	0.188	0.158	

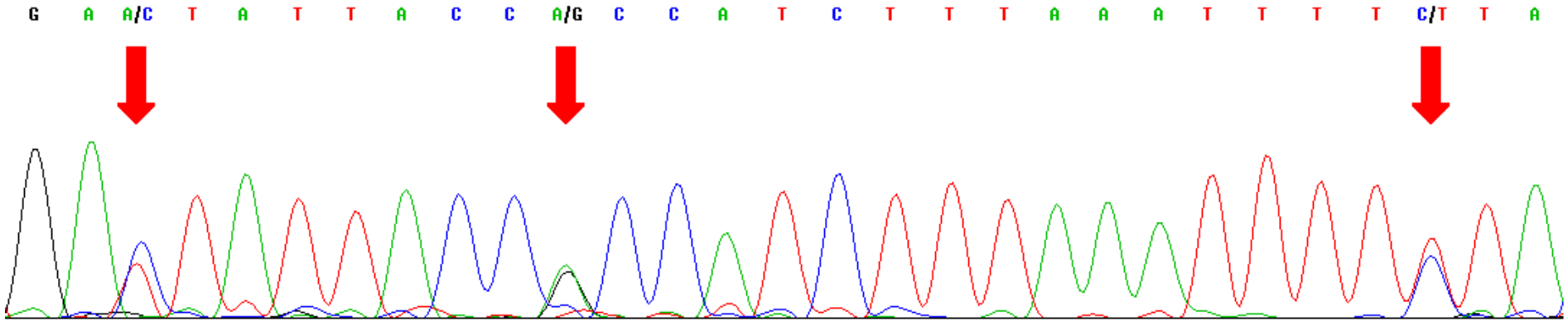


FIGURE 3.2) Sample region of the *Philanisus mataua* COI sequence chromatogram as viewed in Chromas V. 145, showing clear twin peaking (3/7 such sites), indicating contamination or heteroplasmy. All such regions coincided with variable sites indicative either of *Philanisus plebieus* or *Chathamia integripennis*.

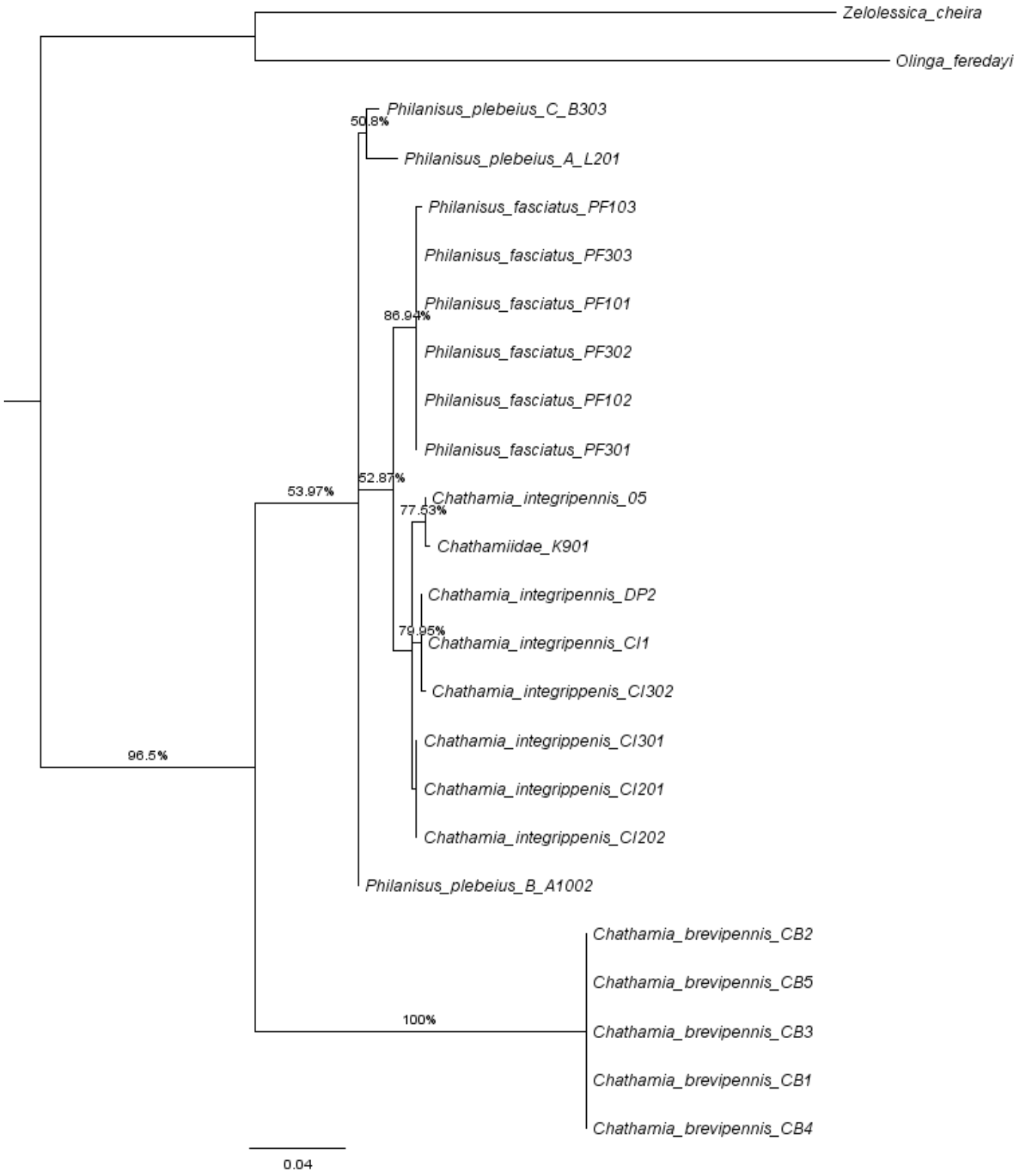


FIG 3.3) Bootstrap consensus Maximum likelihood (ML) tree of the Chathamiidae (10,000,000 replicates, Heuristic search logarithm) as inferred in PAUP* through COI data. Bootstrap values (%) are shown.

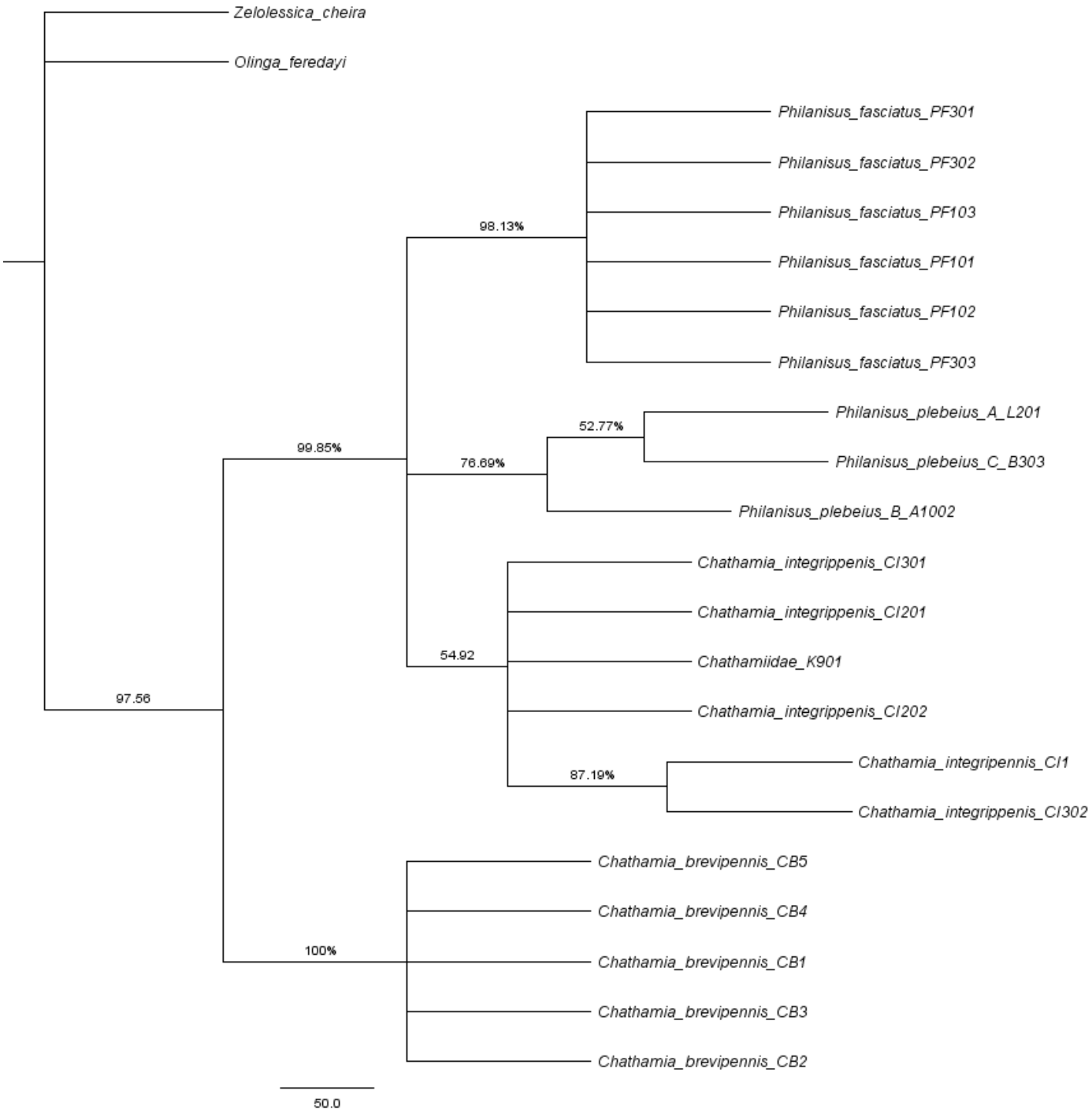


FIG 3.4) Bootstrap consensus Maximum Parsimony (MP) tree of the Chathamiidae (10,000,000 replicates, Heuristic search logarithm) as inferred in PAUP* through COI data. Bootstrap values (%) are shown.

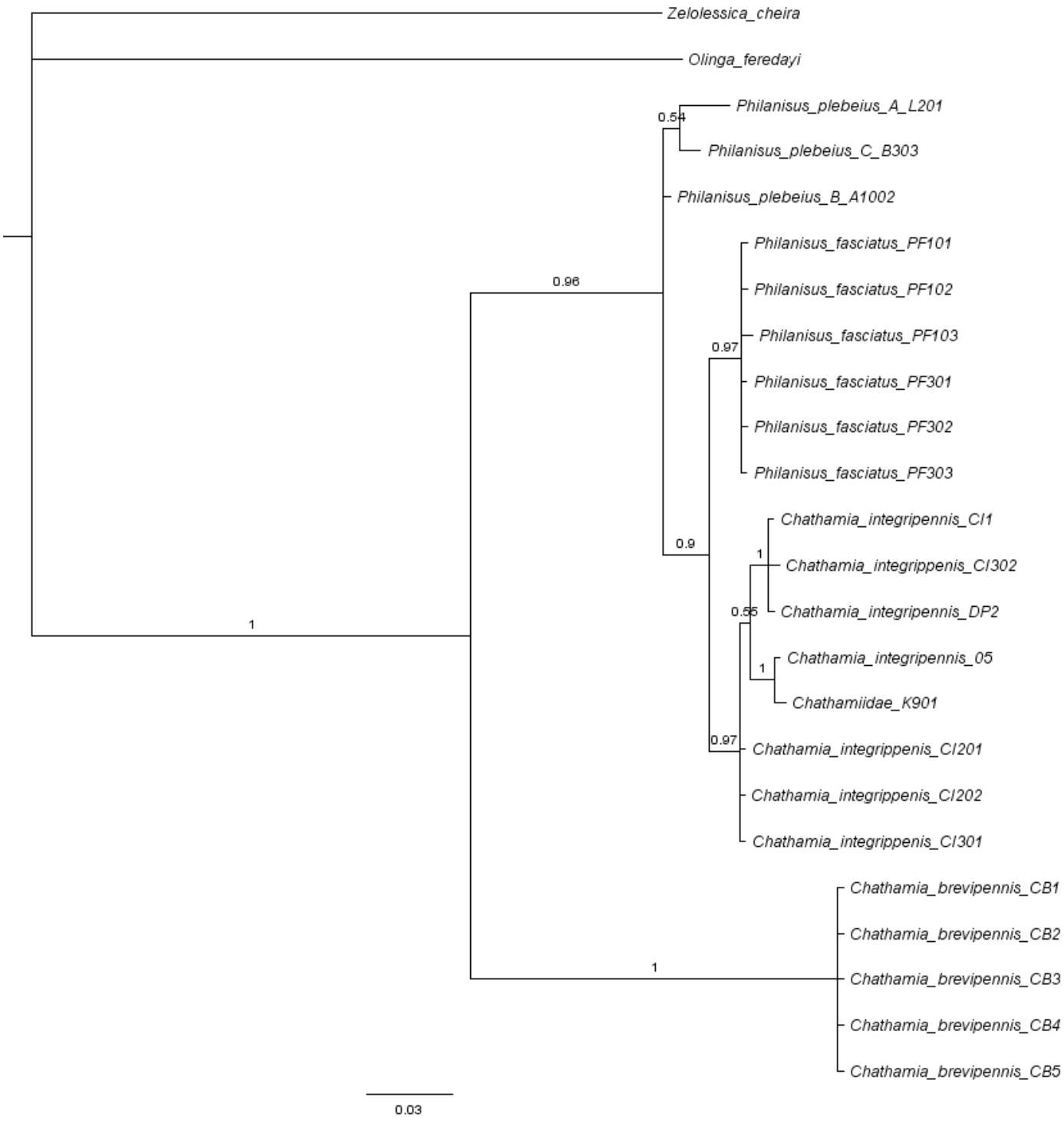


FIG 3.5) Bayesian analysis tree of the Chathamiidae as inferred by COI data and analysed through MrBayes. Posterior Bayesian probability indices are shown.

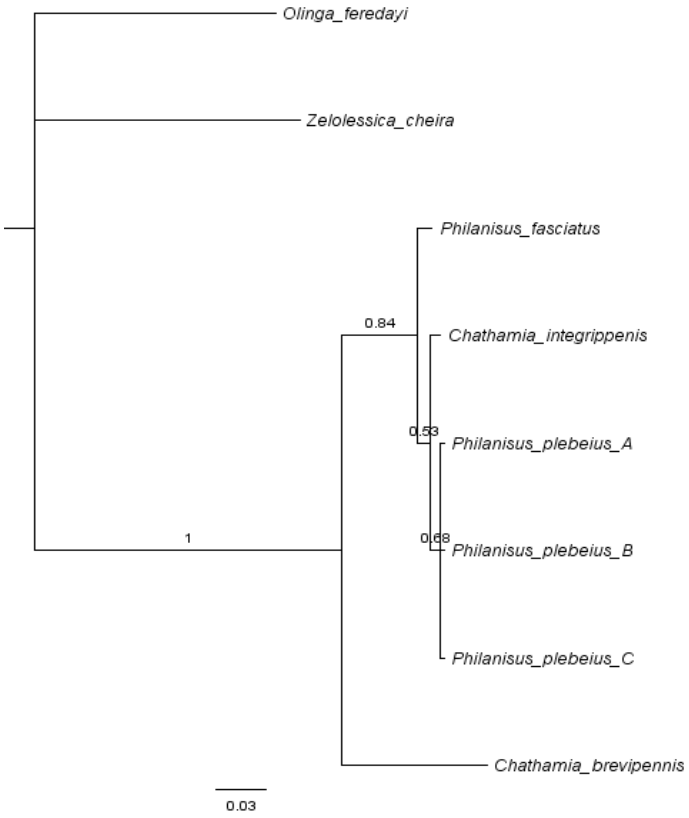


FIG 3.6) Bayesian analysis tree of the Chathamiidae as inferred by 16s data and analysed through MrBayes. Posterior Bayesian probability indices are shown.

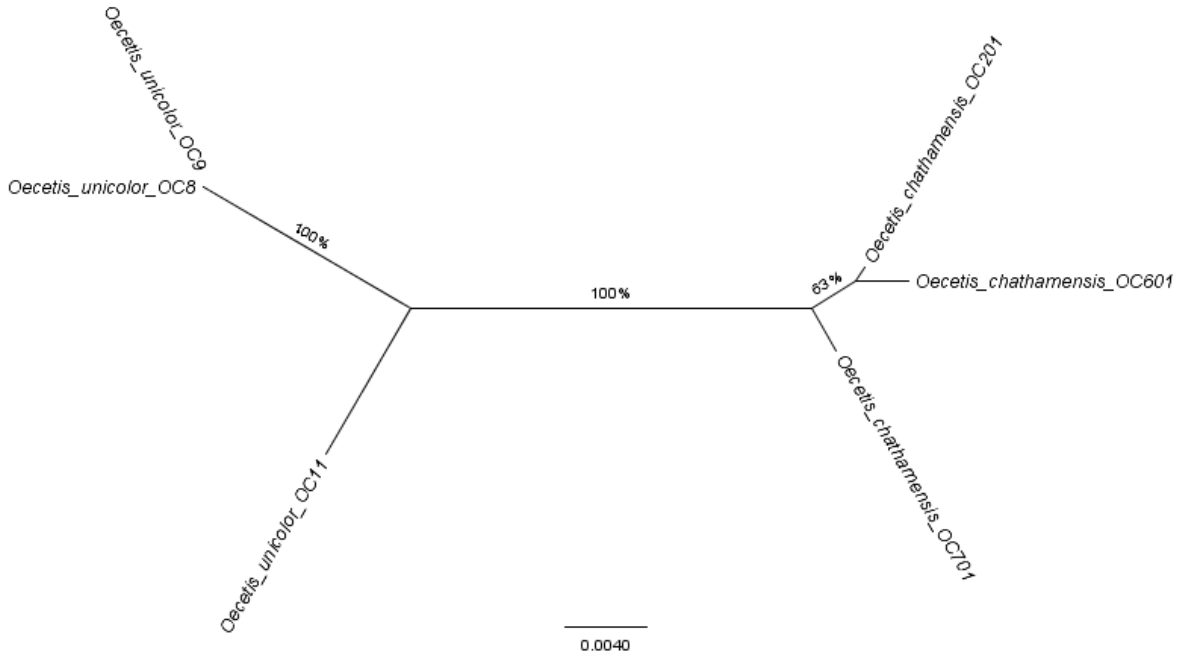


FIG 3.7) Unrooted neighbor joining tree of Oecetis chathamensis and O. unicolor as calculated in MEGA v 4. Separate species status is here supported.Bootstrap values shown from 10,000 replicates using maximum composite likelihood model(Tamura-Nei).

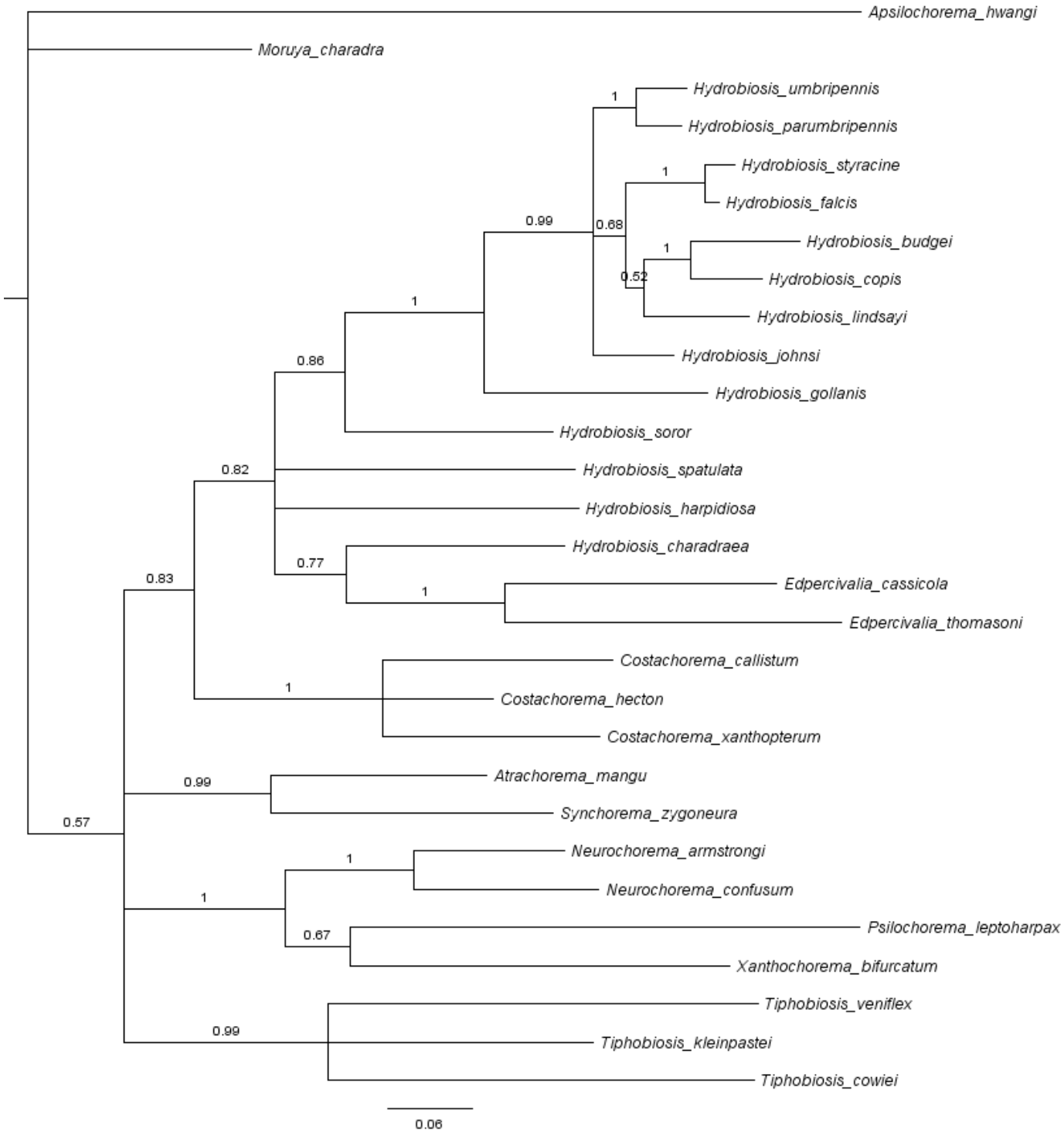
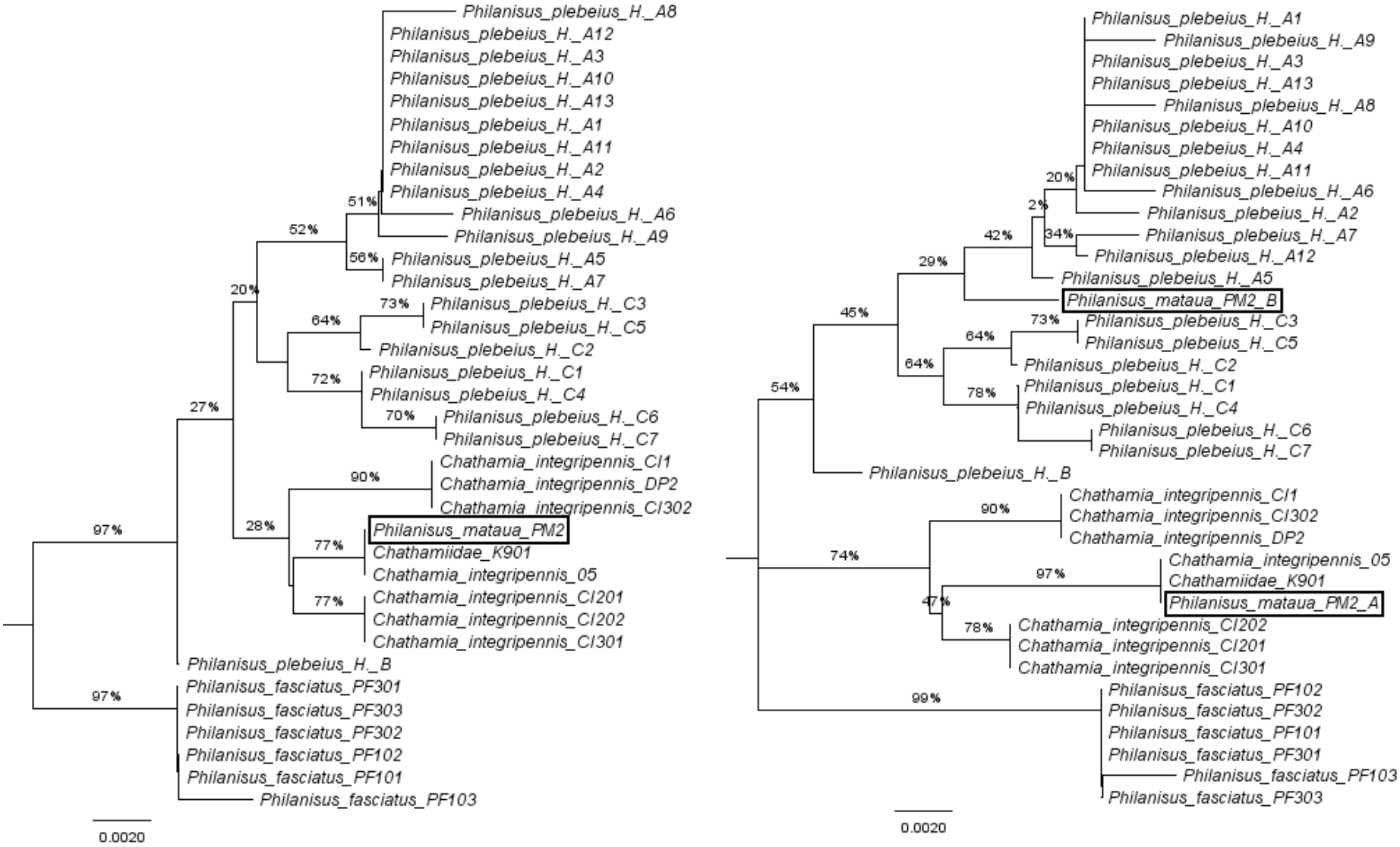


FIG 3.8) Bayesian analysis tree of the Hydrobiosidae primarily to infer the phylogenetic placement of the species *Hydrobiosis lindsayi*, as inferred by COI data and analysed through MrBayes. Posterior Bayesian probability indices are shown.



FIGS 3.9 & 3.10) Neighbour joining bootstrap consensus trees of COI inferred through MEGA v 4 software, exploring the Phylogenetic position of *Philanisus mataua* in the *Philanisus* complex, indicated as to have two mitochondrial sequences. Figure 3.9 (left) explores the uncorrected sequence with ambiguous regions included, wheras Figure 3.10 (right) explores the two most likely sequences ('A' & 'B') and their independent phylogenetic placements. Bootstrap values based on 10,000 replicates, maxiumum composite likelihood model (Tamura - Nei).

TABLE 3.7) Estimated age divergences in Ma as inferred through varying rough molecular clocks. Corrected (mean) pairwise divergence of each species to closest sister group (see methods for more detail) ± Standard error; analysed for separately if possible for both COI and COI + 16s combined, calculated in MEGA v 4 with 10,000 replicates using the Kimura-2 parameter model. Clock 1 uses a 2.3% mitochondrial DNA divergence rate of 2.3% per Ma from Brower et al. (1994). Clock 2 uses the more recent divergences from Papadopoulou *et al.* (2010), with separate divergence estimates for COI (3.54% per Ma) and COI+16s combined (2.69% per Ma).

			Age estimate (Ma)	
Species	**Gene**	**Corrected Divergence**	**Clock 1 (2.3%)**	**Clock 2 (3.54/2.69%)**
Chathamia brevipennis	COI	0.100 ± 0.013	4.35 ± 0.56	2.83 ± 0.37
	COI + 16s	0.093 ± 0.009	4.04 ± 0.39	3.46 ± 0.34
Philanisus plebeius	COI	0.027 ± 0.005	1.17 ± 0.22	0.76 ± 0.14
	COI + 16s	0.019 ± 0.004	0.83 ± 0.17	0.71 ± 0.15
Philanisus fasciatus	COI	0.016 ± 0.005	0.70 ± 0.22	0.45 ± 0.14
	COI + 16s	0.012 ± 0.003	0.52 ± 0.13	0.45 ± 0.11
Chathamia integripennis	COI	0.016 ± 0.005	0.70 ± 0.22	0.45 ± 0.14
	COI + 16s	0.012 ± 0.003	0.52 ± 0.13	0.45 ± 0.11
Hydrobiosis lindsayi	COI	0.083 ± 0.009	3.61 ± 0.39	2.35 ± 0.25
Oecetis chathamensis	COI	0.034 ± 0.006	1.48 ± 0.30	0.96 ± 0.17

3.6 References:

Arbogast BS, Edwards SV, Wakely J, Beerli P (2002). Estimating Divergence Times from Molecular Data on Phylogenetic and Population Genetic Timescales. *Annual Review of Ecology and Systematics* **33:** 707-740

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Chapter Four: Are New Zealand's marine caddisflies a 'Ghost of Gondwana'? Phylogenetic placement of the Chathamiidae.

4.1 Introduction

New Zealand biogeographically has characteristics of both a continental landmass and also an oceanic island (Cooper & Millener 1993, Wallis & Trewick 2009). Having broken from Gondwana over 80 million years ago the ancient subcontinent Zealandia today remains largely submerged with the major exception of modern New Zealand. New Zealand itself is often referred to as a literal 'Moa's ark' (Bellamy *et al*. 1990), host to ancient plants and animals argued to be of a Gondwanan or vicariant origin (e.g Fleming 1962, 1967, Stevens *et al*. 1988). However New Zealand is also characterised as being 'naturally depauperate but secondarily rich' (Daugherty *et al*. 1993) containing an unbalanced biota predominantly more indicative of long distance dispersal (Pole 1994, McGlone 2005, McDowall 2008, Goldberg *et al*. 2008). Although levels of endemicity are among the highest in the world; the number of endemic groups of family status or higher is much lower than would be expected of a continental landmass (Pole 1994, 2000, Macphail 1997, Gibbs 2006). It is now increasingly argued that a marine submergence during the Oligocene (roughly 23 Ma) known to have reduced New Zealand to at most a few islands (Fleming 1962, Cooper & Cooper 1995), may have been total (Trewick *et al.* 2007, Landis et al. 2008). This raises the biogeographic concept of 'New Zealand' as a fully modern entity, biologically separate from 'Zealandia' (Campbell & Hutching 2007, Trewick *et al*. 2007, Landis *et al*. 2008). Numerous taxa do however appear to be congruent with Gondwanan origins, for example Ratite birds, Leiopelmatid frogs, Tuatara, and and various plants and invertebrates; all now central to the debate of inferring the ancient history of New Zealand.

The five species and two genera of case-making caddisfly that comprise the Chathamiidae are among the more unusual aspects of New Zealand's fauna, being some of very few insects in the world that breeds in the marine environment. Additionally, they are one of only three insect families often considered endemic to New Zealand (Gibbs 1979, 2006, Gleeson *et al*. 2000, Wiegman *et al*. 2002,

Kutty *et al*. 2010). At least two Chathamiid species are found in a small region of New South Wales, Australia, although these are both likely due to singular dispersal events from New Zealand less 20,000 years ago (Refer to Chapters Two and Three). On an evolutionary timescale, the Chathamiidae can be considered a group unique to New Zealand. The freshwater fauna of New Zealand includes many likely candidates for a vicariant Gondwanan origin, as most species are poor dispersers and many are unable to survive prolonged exposure to seawater inhibiting long distance dispersal (Gibbs 2006). Chathamiid caddisflies have the unusual distinction of being marine, although are evolved from freshwater species, thus the biogeographic inference of these groups still has relevance.

A possible ancient vicariant origin of many of freshwater groups is supported by their global distributions; many found only on the Gondwanan fragments of South America, Australia and New Zealand, which with Antarctica comprised the late Gondwanan remnant of 'Australis' (Gibbs 2006); New Zealand being the first to rift roughly 80-85 Ma (Luyendyk 1995, Sutherland 1999). A complete 'Australis' distribution is represented by four caddisfly families (de Moor & Ivanov) four Mayfly families (Barber-James *et al*. 2008), four stonefly families (Fochetti & de Figueroa 2008), the dobsonfly genus *Archichauliodes* (Cover & Resh 2008), the scorpionfly family Nannochoristidae (Ferrington 2008), the dragonfly family Austropetaliidae (Kalkman *et al*. 2008), four genera of craneflies (de Jong *et al*. 2008), the dipteran subfamily Ceratomerinae (Plant 1991, Wagner *et al*. 2008), two families of syncarid 'shrimps' (Camacho & Valdecasas 2008), crayfish of the Parastacidae (Crandall & Buhay 2008) and the freshwater mussel family Hyriidae (Bogan 2008), all with representatives in New Zealand. As of yet very few of these groups have been subjected to molecular analysis, vicariant origins in New Zealand being supported for two mayfly families, and Hyriid mussels, and unsupported for one mayfly family (Ogden & Whiting 2005, Fenwick 2006).

The caddisfly families Hydrobiosidae, Kokiriidae, Philorheithridae, Helicophidae; the subfamily Triplectidinae (Leptoceridae), and also two families not found in New Zealand; the Atriplectididae and the Tasimiidae, have distributions demonstrating an Australis pattern (Ross 1967, de Moor & Ivanov 2008). The phylogeny of the Helicophidae was analysed by Johanson & Keijsner (2008), who found a tree topology inconsistent with Gondwanan vicariance, with New Zealand's

species polyphyletic; sister to species either from South America or Australia. The phylogeny of the Hydrobiosidae, represented by over at least 87 species in 10 genera endemic to New Zealand, has been subject of morphology-based taxonomy (Neboiss 1977, Ward *et al*. 2004), which similarly indicated a complex history difficult to assign to vicariance or dispersal alone.

Other families with possible Gondwanan roots in New Zealand include those restricted to Australasia, although some oceanic dispersal in these groups seems probable. In the trichoptera this comprises four families, the Calocidae, Conoesucidae, Oeconesidae and the Chathamiidae. Both the Calocidae and the Conoesucidae have been subject to phylogenetic analysis (Johanson *et al*. 2009, Johanson & Malm 2010). Basal New Zealand relationships have been indicated in both cases possibly supporting vicariance, although at least one trans-Tasman dispersal event is demonstrated in the Conoesucidae. Five of six genera of the Oeconesidae excepting the monotypic *Tascuna* from Tasmania, are endemic to New Zealand. Two other taxa are also of special note. The endemic genus *Alloecentrella* has been of taxonomic interest, being transferred between the Bereaidae, Helicophidae and the Calocidae (Ward 1999), although the original placement in the Helicophidae is now supported (Henderson & Ward 2007, Johanson & Keijsner 2008, Johanson & Malm 2010). One other endemic genus, the monotypic *Rakiura* is known from a disjunct distribution in North West Nelson and Stewart Island (Michaelis 1973). *Rakiura vernale* has importance as is the most basal member of the Helicopsychidae (the 'spiral-cased' caddisflies), the remainder of which is comprised entirely of over 192 described species of *Helicopsyche* with highest diversity in Australia, South-East Asia and South America, although the genus has a global distribution (de Moor & Ivanov 2008).

Of all New Zealand's aquatic invertebrate families, only the Chathamiidae come so close to full endemic status, although the exact evolutionary relationships of the group itself remain unclear. Riek (1976) first suggested a relationship with the large and widespread family Leptoceridae (superfamily Leptoceroidea). More modern revisions of the taxonomy of the Trichoptera now place the Chathamiidae within the superfamily Sericostomatoidea, the sister grouping to the Leptoceroidea which together comprise the Brevitentoria (Weaver 1984, 1992, Frannia & Wiggins 1997). Morphological studies have supported various topologies although a relationship between the

Southern Sericostomatoidean families of the Calocidae, Chathamiidae, Conoesucidae and the Helicophidae is commonly suggested (Scott & de Moor 1993, Henderson & Ward 2007). The comprehensive phyogenies of Kjer *et al*. (2000, 2001) using 28S ribosomal DNA as a conservative marker have also supported this relationship. However recent phylogenies using 16S have instead indicated a possible relationship of the Chathamiidae with the Helicopsychidae and Leptoceridae in spite of the placement of the latter in a different superfamily (Johanson *et al*. 2009, Johanson & Malm 2010). The status of the Leptoceroidea as a monophyletic group, although supported by Kjer et al. (2000, 2001), has also been discredited in some recent phylogenies (Frannia & Wiggins 1997, Morse 1997, Johanson *et al*. 2009, Johanson & Malm 2010).

In spite of its unclear relations, separate family status and monophyly of the Chathamiidae has been consistently supported. The Chathamiidae as revised in this Thesis comprise four species of *Philanisus* (one of which is currently placed in *Chathamia*, see Chapter 3), and the monotypic *Chathamia* endemic to the Chatham Islands. One molecular clock based on fossil calibrations suggests an early Cretaceous origin for the family roughly 140 Ma in age (Grimaldi & Engel 2005); predating New Zealand's continental rifting by some 60 million years. This is almost certainly an overestimate, being partly based on a dubious early cretaceous record of a Helicophid from the purbeck beds of England (Ivanov & Jarzembowski 2001). A Mesozoic age of origin alone does not fully support a vicariant origin however; as any taxon may have dispersed recently only become recently extinct elsewhere (Waters & Craw 2006). Additionally the family is comprised of only five closely related species suggested to have diverged in the Pliocene roughly 4-3 Ma, an over 40-fold difference in age if an early Cretaceous origin is accepted (refer to Chapter Three of thesis). The family appears fully capable of oceanic dispersal having crossed the Tasman at least twice, and two species are found in of New Zealand's outermost island groups; the Kermadec and Chatham Islands (Riek 1976). Thus the Chathamiidae are congruent both with an ancient Gondwanan lineage, and also as a more typical recent dispersal taxon.

Although in some respects the phylogenetic placement of the Chathamiidae is well studied there is need for improved resolution. To date all phylogenetic studies have focused only on a single

conserved marker (either 28S or 16S), supported by a number of faster evolving sequences (including COI among others). By combining data from a number of studies, and with the addition of new taxa this study aims to test the effectiveness of phylogenetic reconstruction in the Trichoptera using conserved ribosomal sequences only. Additionally, by including the basal *C. brevipennis*, this is the first time that the full evolutionary diversity of the Chathamiidae has been included in such a phylogeny. This will aim to help understand the phylogenetic placement of the Chathamiidae, and with the addition of fossil data this can be expanded to incorporate a relaxed molecular clock to test the hypothesis of a vicariant age for the family (> 80 Ma). As this analysis will explore a number of taxa, this study will also address other questions of interest, including the age and phylogenetic relationships of taxa such as *Alloecentrella* and *Rakiura*; and the relationships between the Leptoceroidea and Sericostomatoidea superfamilies.

4.2 Materials and Methods

4.2.1 Taxon sampling

This study constructed a phylogeny of the Chathamiidae and related caddisfly families using two regions (D1 and D3) of the nuclear ribosomal gene 28S, and also the mitochondrial ribosomal gene 16S. For this analysis a number of new sequences were developed from both marine and freshwater species collected from a number of sites around New Zealand, which included all New Zealand case-making genera with the exception of *Triplectidina* (Leptoceridae); *Kokiria* (Kokiriidae), *Periwinkla* (Conoesucidae); and *Pseudoeconesus* and *Tarapsyche* (Oeconesidae). Species were collected by a number of methods, including using a UV light trap or net sweeping for adults, or use of a surber net or hand searching in substrate for larvae. All specimens used in the final analysis are listed in Table 4.1

This study also incorporated sequences from a number of previous studies (Kjer et al. 2001, 2002, Johanson & Keijsner 2008, Johanson *et al*. 2009, Johanson & Malm 2010), which included both

16S and 28S sequences for the New Zealand species *Philanisus plebeius* (Chathamiidae); and *Pycnocentrodes aureolus* and *Olinga feredayi* (Conoesucidae). Both sequences were present for many overseas genera, (although congeneric species had to be combined in some cases) of *Sericostoma* spp. (Sericostomatidae), *Austreithrus* spp. (Philorheithridae), *Limnocentropus insolitus* (Limnocentropodidae), *Molanna* spp. (Molannidae); and *Caenota plicata* and *Caloca saneva* (Calocidae).

DNA was extracted using a standard-phenol chloroform method, and regions of the mitochondrial genes COI and 16S, and also two regions (D1 and D3) of the nuclear gene 28S were amplified using a total of nine different primers (see Table 4.2). The COI region was amplified first and used as a DNA 'barcode' to confirm species status for some specimens, compared with data available on GenBank. The parameters for amplification have been presented in Chapter Two. DNA sequencing generally used one primer only; HCO2198 for COI, 28SD1f for 28SD1, 28SD3f for 28SD3, and 16BRH for 16S although some were sequenced from both sides if retrieved data needed improved resolution. Some species were never successfully amplified in all regions and were eventually omitted from further analysis including *Zelolessica* (Helicophidae), *Hudsonema* and *Oecetis* (Leptoceridae), *Confluens* (Oeconesidae) and *Pycnocentrella* (Calocidae). Representatives from each family still remained in the analysis however, and these omissions were not considered significant.

All sequences used are listed in Table 4.3. For the Chathamiidae the species *Philanisus plebeius* and *Chathamia brevipennis* were used to represent both genera and the Chathamiidae as a whole. Together this dataset comprised six of the families of the Sericostomatoidea. However this excluded the Bereaidae, Anomalopsychidae, Antipodoeciidae, Barbarochthonidae, Hydrosalpingidae and the Petrothrincidae as none of these families are found in New Zealand and data for both genetic regions was not available. Some phylogenetic studies have placed almost all these families near the Chathamiidae at some point, including the Southern African Hydrosaplingidae (Scott & de Moor 1993), the Southern African and Madagascan Petrothrincidae (Henderson & Ward 2007), the Northern hemisphere Bereaidae (Henderson & Ward 2007), and the South American

Anomalopsychidae (Kjer *et al*. 2001); although none of these relationships are well supported. The Antipodoeciidae, represented only by the species *Antipodoecia turneri* from Southern Australia, have not been included in any molecular study to date although morphology does not suggest a close relationship to the Chathamiidae (Ross 1967, de Moor & Scott 1993, Henderson & Ward 2007, Holzenthal *et al*. 2007). The analysis also included three families of the Leptoceroidea (the Molannidae, Leptoceridae and the Limnocentropodidae), and finally one family of the Plenitentoria (the Oeconesidae) for use as an outgroup taxon.

4.2.2 Alignment, phylogenetic analysis and molecular clock.

Sequences were aligned in CLUSTALX algorithm in MEGA V 4 (Kumar *et al.* 2007, 2008), using default parameters and with finer scale editing by eye. All sequences had large number of insertions, thus some regions could not be aligned with certainty and were removed from analysis. Each of the three regions were analysed separately and concatenated using the model selection software jMODELTEST (Posada 2008), selecting a GTR + G (General time reversal model + Gamma) in all cases. The dataset was run in the phylogenetic software MrBayes 3.1 (Heulsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) using a total of 10,000,000 generations sampled every 10,000 to gain a total of 1,000 trees. The first 25% of the trees were discarded as a burn-in phase and the remainder used to estimate the posterior probabilities.

To test for a clock-like evolution of the gene sequences and possible gene saturation, the dataset was analysed using the phylogenetic software DAMBE (Data Analysis in Molecular Biology and Evolution, Xia 2001, Xia & Xie 2001). The likelihood ratio test used a base tree generated by neighbour-joining with use of a GTR model and the Oeconesidae as an outgroup. Taxa were sampled for use in a Relative Rates test, which included two sister species and their nearest outgroup as inferred by the Bayesian analysis, and was repeated using random sequences throughout the tree. To further test for saturation, a false test of substitution saturation was also run (Xia et al. 2003, Xia & Lemey 2009).

The software BEAST v1.6.1. (Drummond & Rambaut 2007) was used to implement a molecular clock on the data. Parameters were set using a GTR + G model and a Yule speciation model. A molecular clock was run using a relaxed uncorrelated lognormal estimate, with prior age estimates given normal distributions ± standard deviation (see below for calibrations used). The Bayesian analysis was run using 10,000,000 generations with a sample frequency of 10,000, with the first 25% discarded as a burn-in phase. The log file was then also reviewed in Tracer v. 1.5.

For calibration points a fossil identified as being from the Helicophidae from the Purbeck beds of England (140 Ma) was used (Shukatsheva & Jarzembowski 2001). However the association with the Helicophidae is dubious as the family is otherwise known only from the Southern Hemisphere, and other Northern hemisphere fossils of the family have since been ascribed to the extant northern hemisphere family Sericostomatidae (Wietchat & Wichard 1998). This point was therefore used as a node calibration for the origin of the Seriocostomatoidea, assumed as representing the oldest known record of a caddisfly from this group. For a second calibration point a Cretaceous fossil of a larval Leptoceridae from the Baissa deposit of Siberia was used, dated at roughly 135 Ma (Grimaldi & Engel 2005, Ivanov 2006). This age was used as the estimate for most recent common ancestor of the Leptoceridae and its nearest sister taxon as inferred from the prior bayesian phylogeny. The geological age of the Leptocerid fossil source strata is less certain than that of the 'Helicophid' (Rasnitystn & Zherikhin 2002) therefore these groups were given normal distribution prior estimates with standard deviations of 20 and 5 million years respectively. Also due to the taxonomic uncertainty of the Helicophid fossil, the analysis was also run using the Leptocerid fossil only. A smaller deviation of only 10 million years was used in this instance, here being the only calibration point available. Although the possible 'correct' sister taxon to the Leptoceridae was not included (such as the Calamoceratidae), this would presumably only allow for an underestimation of the age of the last common ancestor.

4.3 Results

4.3.1 Phylogenetic Analysis

The final Bayesian tree is shown in Fig 1.1. Here monophyly of the Chathamiidae was supported, and was strongly indicated to be sister group to a monophyletic Conoesucidae (99.999% Bayesian posterior support). This in turn was shown likely to be related to the Helicophidae and the Calocidae together comprising a clade. However this group was also suggested to contain the Molannidae, a Leptoceroidean family. Placement of *Alloecentrella* in the Helicophidae and *Rakiura* in the Helicopsychidae were supported. The Sericostomatidae was suggested as basal to other Sericostomatoidean families, and the Leptoceridae and the Philorheithridae were suggested sister taxa (the common ancestor of the latter group were used for fossil calibration). Basal position of the Oeconesidae, shown to comprise a closely related monophyletic group, was also supported.

4.3.2 Molecular Clock & Saturation Tests

The likelihood ratio test found a non-significant result ($P = 0.0613$, > 0.01), thus all taxa are assumed an equidistance from the root of the tree, congruent with a constant evolutionary rate. All relative rates test consistently returned non-significant P-values also supporting this result. The false test of substitution saturation returned a significant P-value (< 0.01) and showed an ISS value of 0.1016 and an ISSC of 0.7285. The ISS < ISSC relationship with the significant P-value indicated little saturation, and indicated a genetic sequence useful for phylogenetic purposes.

Of the two Bayesian trees inferred in BEAST, the tree using both calibration dates (see methods) showed younger (and more conservative) ages than that using only the Leptoceridae which were both older and also more variable. Therefore the tree using both calibrations was used, shown in Fig. 4.2. As inferred in Tracer, the Chathamiidae diverged from the Conoesucidae roughly 93.5 Ma (95% HPD range of 73-119.5 Ma), and *Chathamia* and *Philanisus* are inferred to have diverged 30 Ma (95% HPD range of 13-53 Ma). Other ages include a divergence of the Conoesucidae at 65 Ma

(95% HPD range of 41-83 Ma), a divergence of *Helicopsyche* and *Rakiura* at 80 Ma (95% HPD range of 56-108 Ma), a divergence of the Helicophidae and Calocidae at 80 Ma (95% HPD range of 57-105 Ma), and a divergence of *Philorheithrus* and *Austreithrus* at 64 Ma (95% HPD range of 34-91 Ma).

4.4 Discussion

The phylogeny found in this study generally showed robust Bayesian support and is consistent with previous genetic and morphological based phylogenies. The only feature that stood out as unusual was the placement of the Molannidae well within the Sericostomatoidea. This is almost certainly an artefact of long-branch attraction or issues with DNA alignment, as the Molannidae are known to be closely related to the Leptoceridae, strongly demonstrated both by morphological and genetic evidence (eg. Scott & de Moor 1993, Kjer *et al*. 2001, 2002, Johanson & Malm 2010). Placement of *Alloecentrella* and *Rakiura* within the Helicophidae and Helicopsychidae respectively was supported. Most relevant to this study in particular was the strongly supported relationship (Bayesian support of almost 100%) between the Conoesucidae and the Chathamiidae. This group in turn (if the Molannidae are then excluded) was sister to clade containing the Helicophide and the Calocidae. Using the characters listed by Henderson & Ward (2007), the Conoesucidae and Chathamiidae share some larval morphological features not found in the Helicophidae and Calocidae. These include the presence of branched abdominal gills and a quadrangular ventral apotome (sclerotized plates under the head); opposed to simple or absent gills, and a triangular apotome in the Helicophidae and Calocidae. Other common features, including those of the adults are generally shared between all four families.

A probable close relationship of these families has been indicated in the past; however this particular topology is new to this study. For example a basal Helicophidae and a Conoesucidae-Calocidae relationship (Johanson & Keijsner 2008, Johanson *et al*. 2009, Johanson & Malm 2010), a basal Conoesucidae and a Chathamiidae-Helicophidae relationship (Henderson & Ward 2007), and a

Chathamiidae-Calocidae relationship (Scott & de Moor 1993) have been demonstrated. None have demonstrated monophyly *per se* however; the clade containing these four families has also been inferred to include the Sericostomatidae, Petrothrincidae and Anomalopsychidae among others, although a close relationship to any other specific family has not been repeatedly supported. However as several families were not included in this phylogeny, the implications of a more thorough phylogeny to the topology found here must be considered. Other points in the tree include the basal position of the Sericostomatidae, followed by the Helicopsychidae, and also a sister grouping of the Philorheithridae and the Leptoceridae.

The molecular clock found an age of the Chathamiidae consistent with a vicariant origin, with the age of the last common ancestor of the Chathamiidae and the Coneoseucidae dated at roughly 94 Ma (mean age, total distance of 73-113 Ma). The molecular clock also estimated a wide range of ages for the divergence of *Philanisus* and *Chathamia*, between 53-13 Ma (mean 30 Ma). This has implications considering the endemicity status of *Chathamia* to the Chatham Islands which are likely no older than Pleistocene in age, and conflicts with the assumed Pliocene age of the taxon (~3Ma) estimated in Chapter Three. It may be that *Chathamia* represents an old taxon now extinct in mainland New Zealand, the clock is wholly innacurate, or that the molecular clock used here is inappropriate for estimating more recent divergences.

Although the age of the Chathamiidae may seem to agree with New Zealand's rifting from Australia, is important to note that the inferred closest taxon to the Chathamiidae, the Coneosucidae, is well represented in New Zealand. A prior phylogeny of the Conoesucidae indicated at least three clades, one wholly New Zealand, one wholly Australian and one found in both regions (Johansen *et al.* 2009). This study included taxa representative of all these groupings, and although a Cretaceous divergence of extant taxa was indicated, an age of these diversions congruent with vicariance was not well supported (83-43 Ma, mean 65 Ma). New Zealand or Australian origins for the Conoesucidae both remain possibilities, although Johansen *et al*. (2009) assumed a New Zealand origin to be more parsimonious as the arrangement of the clades weakly suggested New Zealand's species to form an ancestral paraphyly, also supported here. If the Conoesucidae and the Chathamiidae comprise a clade

of New Zealand origin, this may have implications as the inferred age of this group diverging from the Calocidae and Helicophidae is indicated 133-89 Ma in age (mean 114 Ma), predating New Zealand's continental age somewhat (although the lower estimates are compatible).

It is possible that the fossil calibrations in this study were overestimates. The fossil used to date the node of the Sericostomatoidea was based on some incomplete fossilised wings from near the Jurassic-Cretaceous boundary, and may represent an unrelated taxon. However, a late Jurassic origin of the Sericostomatoidea may have to be expected if the group's biogeography is inferred to reflect continental drift. All families, perhaps excepting the cosmopolitan Helicopsychidae are restricted either to Africa and Madagascar (Barbarochthonidae, Petrothrincidae, Hydrosalpingidae); Eurasia(Beraeidae, Sericostomatidae); or the 'Australis' regions of Australia, South America, New Zealand and New Caledonia (Anomalopsychidae, Antipodoeciidae, Calocidae, Chathamiidae, Conoesucidae, Helicophidae). Pangea and Gondwana (then including Africa) did not fully separate until the late Jurassic, followed by Africa then separating in the Mid-Cretaceous opening the South Atlantic (Golonka & Bocharova 2000, Jokat *et al*. 2003). Although biogeography may suggest continental vicariance, monophyly of each geographic grouping is currently equivocal, although weakly indicated in some cases (e.g. Kjer *et al.* 2002).

An alternative possibility is that Gondwanan India may have transported the Sericostomatidae and Beraeidae into Eurasia, by which the Sericostomatoidea may have reached its present distribution more recently. However this seems unlikely as a fossil attributed to the Sericostomatidae is described from Baltic amber roughly late Creatceous in age (Botosaneanu & Wichard 1983, Wietchat & Wichard 1998) and India did not reach Eurasia until the late Cenozoic (Ali *et al*. 2008). Additionally the role of extinction cannot be discredited, as many contemporary 'Gondwanan' taxa are well represented by Mesozoic fossils in Laurasia, such as the conifer genus *Araucaria* (Kunzmann 2007). Mesozoic fossils with affinities to the Calocidae, Helicophidae and Coneoesucidae are known from Europe, although their placement within any of these families is dubious (Botosaneanu & Wichard 1983). The historical biogeography of the Sericostomatoidea is an exciting prospect for further research, however is not within the scope of this particular study.

Other ages include the divergence of *Austreithrus* and *Philorheithrus* as roughly Paleocene in age (~60 Ma). The Philorheithridae have a current distribution indicative of Gondwanan continental drift, restricted to Australia, New Zealand, South America and also possibly Madagascar (Weaver *et al*. 2008). However the age here suggests that New Zealand's lineage is unlikely to reflect vicariance, although this prospect cannot be rejected (a maximum age of 92 Ma was suggested). However, only two genera of the total nine were used. Various morphological features shared between *Philorheithrus* and other members of the family are absent in *Austreithrus*, thus the two genera are unlikely to be closely related (Henderson & Ward 2006), in which case an even more recent age of *Philorheithrus* would be demonstrated.

By contrast, a vicariant age of New Zealand's endemic *Rakiura* was supported (56-108 Ma in age, with mean age of 80 Ma). *Rakiura* is almost certainly the most basal member of the Helicopsycidae or the 'snail-cased' caddisflies. The only other genus, *Helicopsyche,* is found worldwide and comprises roughly 250 known species including seven in New Zealand (Johanson 2001, Holzenthal *et al*. 2007). *Helicopsyche* contains a total of six subgenera, one restricted to Australasia (*Saotrichia*) one to South America (*Cochilopsyche*), one to Madagascar and the Seychelles (*Petrotricia*), one to the Americas (*Feropsyche*), and two (*Galopsyche* and *Helicopsyche*) to the Palearctic and Oriental regions (Johanson 1998). *Helicopsyche* attributable to *Feropsyche* are known from Dominican amber (Johanson & Wichard 1996, Weaver 2007), indicating by roughly 20-25 Ma all subgenera were well established (Poinar & Poinar 1999). It is plausible the common ancestor of *Rakiura* and *Helicopsyche* was found on Gondwanan 'Australis', with Helicopsyche evolving on South America and Australia and later dispersing elsewhere, including to New Zealand. However this argument may be weakened as the Helicopsychidae is possibly more closely related to the Northern Sericostomatidae and Beraeidae than the other supposedly 'Gondwanan' families (Scott & de Moor 1993, Kjer *et a*l. 2002), although not evidenced in this study. Again, considerable further study is needed.

This study set out to explore the phylogenetic affiliations of the Chathamiidae and to test if its evolutionary age was congruent with a Gondwanan origin in New Zealand. Here a Chathamiidae

Conoesucidae sister relationship was well supported. Molecular clock estimates did find an age close to the geological rifting in New Zealand, however the possibility of the taxon to have gone extinct elsewhere, as with many of New Zealand's other ancient plants and animals, will always remain a distinct possibility (Waters & Craw 2006). Overall the Chathamiidae and indeed many closely related caddisflies represent a fascinating, largely untapped group for extensive historical biogeographical study, in New Zealand and worldwide. It is concluded here that the evidence supports the Chathamiidae representing a taxon of a vicariant Gondwanan origin, and significantly predates the Oligocene submergence of New Zealand.

4.5 Figures

TABLE 4.1) List of all specimens used in this study for use of new sequences.

Species	Family	Code	Collector	Ontogeny	Location	Date	Coordinates
Oeconesus maori	Oeconesusidae	OEC2	Alex Boast	Adult	Pukerua Bay	26/03/2009	41°02'15 S, 174°53'12 E
Zelandopsyche ingens	Oeconesusidae	ZE101	Ian Henderson	Larva	-	15/01/2008	-
Zepsyche acinaces	Oeconesusidae	ZP101	Ian Henderson	Adult	Tangarakau Gorge	-	-
Triplectides dolichos	Leptoceridae	LE202	Ian Henderson	Adult	Mauatotara Falls	9/02/2008	-
Philorheithrus sp.	Philorheithridae	PS101	Alex Boast	Larva	St. Arnaud Ranges	-	41°51'15 S, 172°52'60 E
Chathamia brevipennis	Chathamiidae	CB3	Alex Boast	Larva	Chatham Island	14/02/2010	43°43’49 S, 176°16’07 E
Alloecentrella magnicornis	Helicophidae	AL2	Ian Henderson	Adult	Tangarakau Gorge	29/01/2010	-
Bereaoptera roria	Conoesucidae	BE201	Alex Boast	Larva	Ohakune	2/09/2009	39°24;09 S, 175°24'41 E
Pycnocentria evecta	Conoesucidae	PC6	Alex Boast	Larva	Ohakune	1/09/2009	39°25'19 S, 175°24'47 E
Helicopsyche albecens	Helicopsychidae	HE201	Alex Boast	Larva	Ohakune	2/09/2009	39°24;09 S, 175°24'41 E
Rakiura vernale	Helicopsychidae	RV101	Alex Boast	Larva	Westhaven	25/02/2010	40°36'43 S, 172°34'37 E

TABLE 4.2) List of all primers used for amplification and sequencing.

Gene/Region	Primer Name	Primer sequences (5' - 3')	Reference
COI	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al*. 1994
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*. 1994
16s	16sARL	CGCCTGTTTATCAAAAACAT	Palumbi 1996
16s	16sBRH	CCGGTCTGAACTCAGATCACGT	Palumbi 1996
28S D1	28SD1f	GGAGGAAAAGAAACTAACAAGGATT	Kjer *et al.* 2002
28S D1	28SD1r	CAACTTTCCCTTACGGTACT	Kjer *et al.* 2002
28S D3	28SD3f	ACCCGTCTTGAAACACGGAC	Kjer *et al.* 2002
28S D3	28SD3r1	ATTCCCCTGACTTCGACCTGA	Kjer *et al.* 2002
28S D3	28SD3r2	CTATCCTGAGGGAAACTTCGGA	Kjer *et al.* 2002

TABLE 4.3) List of all sequences used in this study. References: **1** Kjer et al. (2001), **2** Hayashi *et al.* (2008), **3** Johanson *et al.* (2009), Johanson & Keijsner (2008), **5** Johanson & Malm (2010)

			Source		
Genus/Species	**Family**	**Biogeography**	**16S**	**28SD1**	**28SD3**
Oeconesus maori	Oeconesidae	New Zealand	This study	**1** AF436174	**1** AF436295
Zelandopsyche ingens	Oeconesidae	New Zealand	This study	This study	This study
Zepsyche acinaces	Oeconesidae	New Zealand	This study	This study	This study
Triplectides dolichos	Leptoceridae	New Zealand	This study	This study	This study
Limnocentropus insolitus	Limnocentropodidae	Japan	**2** AB365798	**1** AF436175	**1** AF436296
Molanna angustata/*uniophila*	Molannidae	Europe	**3** FJ263197	**1** AF436201	**1** AF436321
Austrheithrus glyma/*ronewa*	Philorheithridae	Australia	**3** FJ263221	**1** AF436207	**1** AF436327
Philorheithrus sp.	Philorheithridae	New Zealand	This study	This study	This study
Alloecentrella magnicornis	Helicophidae	New Zealnad	**4** EF394983	This study	This study
Caenota plicata	Calocidae	Australia	**4** EF395003	**1** AF436191	**1** AF436311
Caloca saneva	Calocidae	Australia	**5** FN257670	**1** AF436195	**1** AF436315
Chathamia brevipennis	Chathamiidae	Chatham Island	This study	This study	This study
Philanisus plebeius	Chathamiidae	Australasia	**3** FJ263205	**1** AF436196	**1** AF436316
Bereaoptera roria	Conoesucidae	New Zealand	**3** FJ263202	This study	This study
Costora delora	Conoesucidae	Australia	**4** EF395004	**1** AF436192	**1** AF436312
Olinga feredayi	Conoesucidae	New Zealand	**4** EF394980	**1** AF436194	**1** AF436314
Pycnocentria evecta	Conoesucidae	New Zealand	**3** FJ263199	This study	This study
Pycnocentrodes aureolus	Conoesucidae	New Zealand	**3** FJ263198	**1** AF436193	**1** AF436313
Alloecella grisea	Helicophidae	Australia	**4** EF395006	**1** AF436181	**1** AF436302
Helicopsyche albecens	Helicopsychidae	New Zealand	**4** EF394986	This study	This study
Rakiura vernale	Helicopsychidae	New Zealand	**4** EF394976	This study	This study
Sericostoma clypeatum / sp.	Sericostomatidae	Europe	**3** FJ263207	**1** AF436185	**1** AF436306

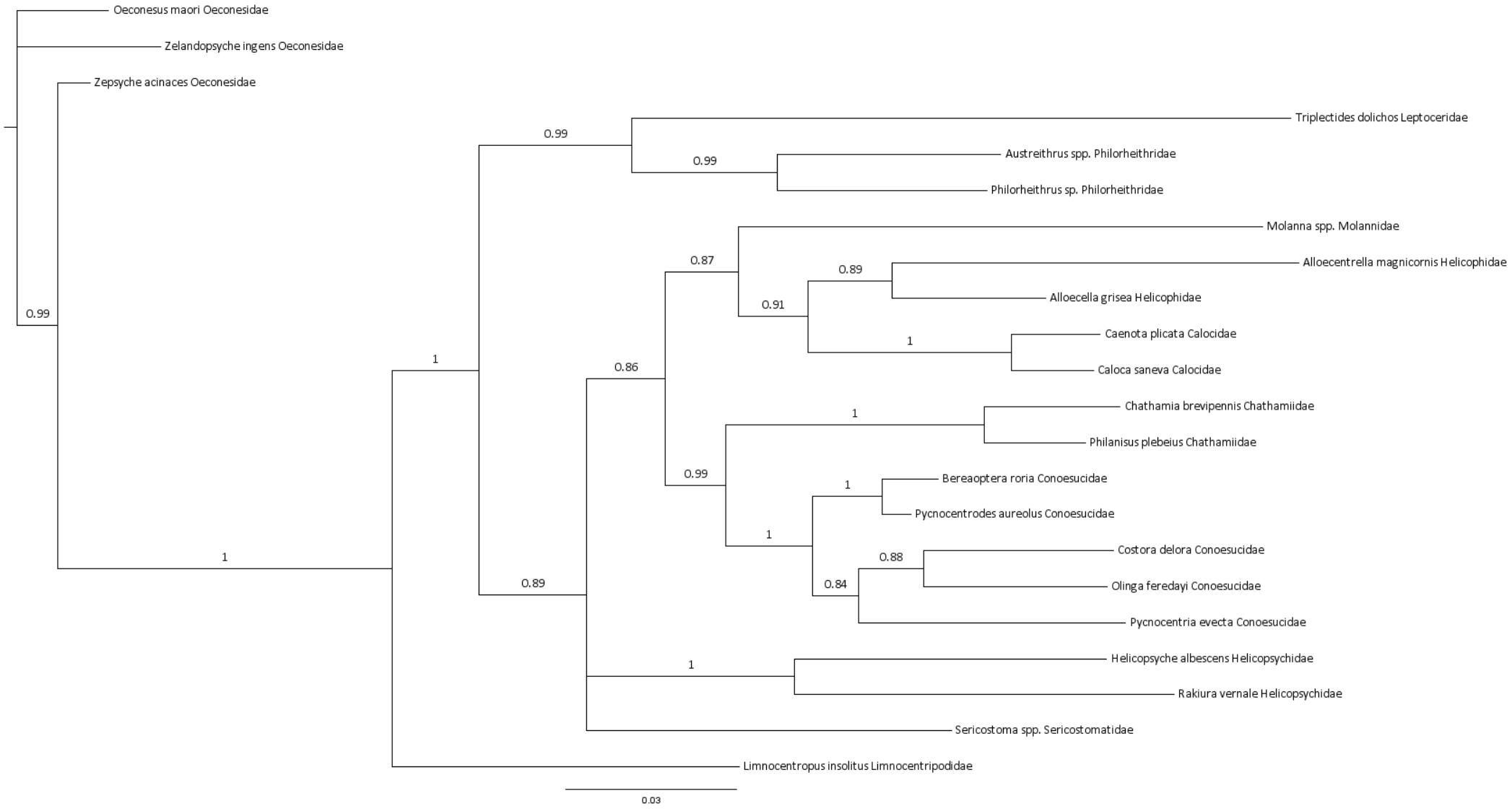

FIG 4.1) Bayesian analysis tree as inferred through MrBayes. Posterior Bayesian probability indices are shown.

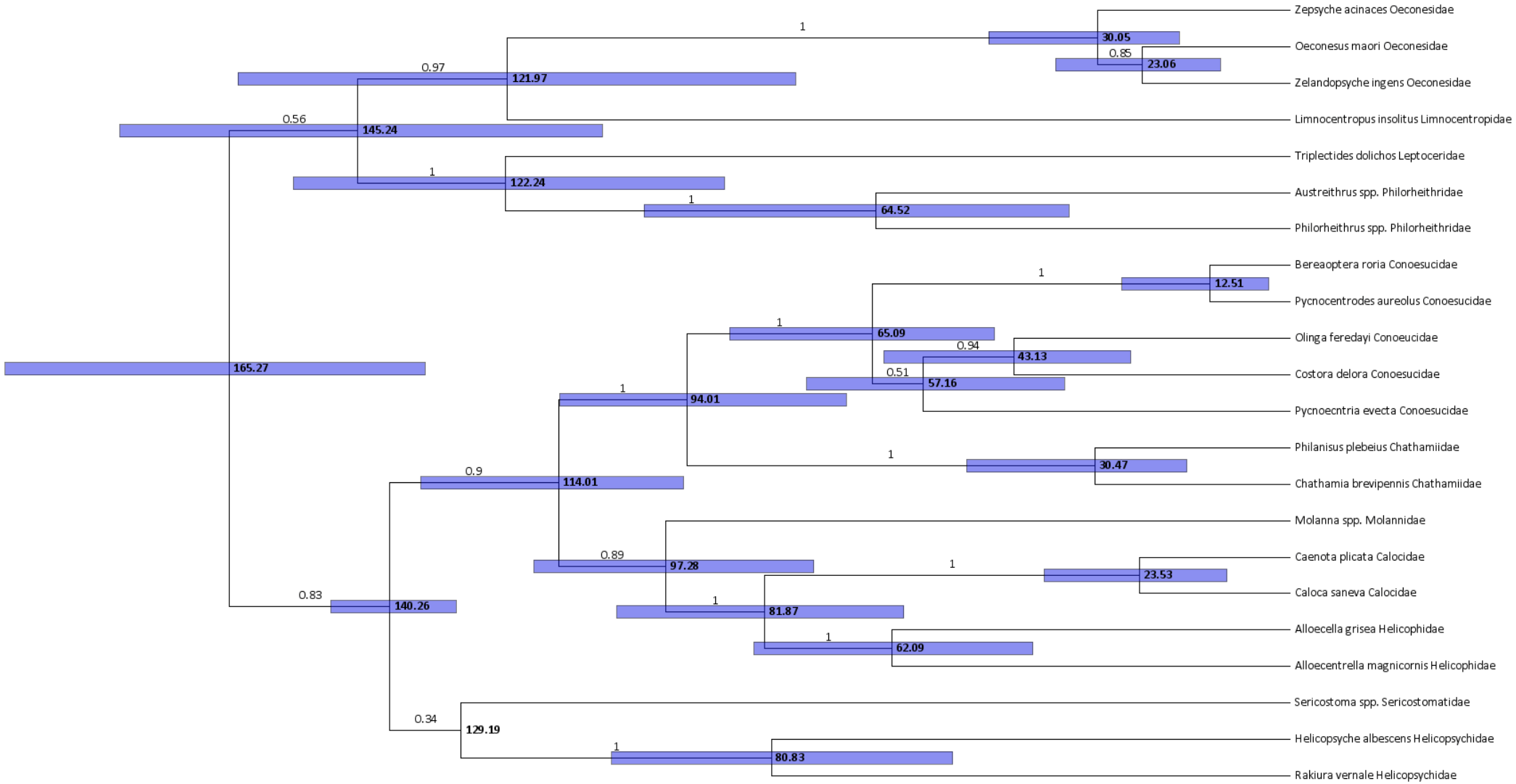

FIG 4.2) Bayesian analysis as run through BEAST with molecular clock estimates shown. Posterior probabilites shown on left of nodes, and estimated age in bold (in Ma). Bars show 95% HPD.

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Chapter Five: General Discussion and Conclusions.

5.1 Major results of this study.

In this thesis, the evolutionary history of New Zealand's marine caddisflies, the Chathamiidae, was examined through the use of a thorough genetic analysis. Firstly an analysis was performed on the common and widespread species *Philanisus plebeius* from samples throughout New Zealand and also New South Wales Australia, to investigate the phylogeographic structure of this species in New Zealand and also to determine the origin of the population in Australia. Secondly, the inner phylogeny of the Chathamiidae was examined with a special emphasis on the island species *Philanisus fasciatus* from the Kermadec Islands, and *Chathamia brevipennis* from the Chatham Islands. In addition, two other species of caddisfly; *Oecetis chathamensis* and *Hydrobiosis lindsayi* from Chatham Island were also used as a comparison for *C. brevipennis*. Finally a family level phylogeny of the Chathamiidae and related families was undertaken, using both sequences new to this study and also drawing on data from a number of previous studies. This thesis found a number of new and interesting results in each of these chapters.

The first chapter discussing results (Chapter Two) focused entirely on *Philanisus plebeius*. It was found that the New Zealand population of *P. plebeius* comprised almost all of the genetic diversity found within the species; a total of 21 haplotypes in 3 identifiable haplogroups. By contrast all Australian sequences shared just one haplotype not found in New Zealand, although this differed from the dominant New Zealand haplotype by only a single base pair, and nested within one of the haplogroups identified. Based on the available genetic information, a human introduction to Australia is not supported although a recent dispersal from New Zealand since the LGM (~20 ka ago) is largely conclusive. However a human introduction would be very strongly supported should the Australian haplotype be discovered to be resident in a New Zealand population.

The trans-Tasman nature of the Chathamiidae was also found to be more complex than previously thought, as whilst studying *P. plebeius* samples from Australia a single larval sample from Sydney was found to be that of *Chathamia integripennis*. This again differed from a New Zealand haplotype of the species by a single base pair. The species *C. integripennis* was previously thought entirely endemic to New Zealand, and to the upper North Island in particular, so this result was wholly unexpected. It was decided that this particular result fell outside of the scope of Chapter Two and was instead discussed in the following chapter.

In New Zealand itself there was a distinct cryptic population structure found in two closely associated sites in the central eastern North Island, just south of Hawke's bay (comprising the entirety of one of the haplogroups described above), evolutionarily distinct from the remainder of the species. The northern boundary of this cryptic group was not identified, but there was found to be a discrete genetic switch of the genetic groups between Mangakuri and Whangaehu beaches, a geographic distance of just 52 km. A number of possible hypotheses for this break were proposed, including oceanic currents, volcanism and Pleistocene climate change, however none were considered conclusive. The reasoning for this structure is still considered largely unknown, although similar patterns have been found in some marine studies (Nakano & Spencer 2007, Nickel 2009). Overall this was the major result of this particular Chapter, and together these observations raise the possibility for this population to represent a previously unidentified cryptic species.

The remainder of *Philanisus plebeius* was shown to contain little genetic diversity, almost all fell within the other major haplogroup, and in turn most of these differing by only one base pair from the most common haplotype which was found throughout New Zealand. It seems probable that this haplogroup represents a radiation following the last glacial maximum, and only this grouping has successfully re-dispersed throughout the remainder of New Zealand. It seems likely that during the glacial cycles, environmental limitations restricted the species largely to the North of New Zealand although more evidence from this region is needed to support this fully. This radiation event also included the single haplotype found in Australia. There was a single sample that was found to be genetically intermediate between the two major groupings (comprising a third and final haplogroup),

and remains somewhat enigmatic. The distribution of this haplogroup remains largely unknown, although is likely restricted to Northern New Zealand (the sequence came from one sample collected in Tauranga estuary).

The next main chapter (Chapter Three) analysed the phylogeny of the Chathamiidae and also investigated two other unrelated caddisfly species from the Chatham Islands. It was found that the Chathamiid species *Chathamia integripennis* from the upper North Island (and also evidently Australia, see above), was nested within the genus *Philanisus*. The type (and only remaining) species of *Chathamia, C. brevipennis,* instead formed a distant sister taxon to all the remaining Chathamiidae. It was suggested that *C. integripennis* be transferred to *Philanisus*, an inference that would leave *Chathamia* a monotypic genus endemic to the Chatham Islands.

Age estimates using strict substitution rates from Brower (1994) and Papadopoulou *et al.* (2010) suggested an age of roughly 3-500,000 years for the lineage represented by *P. fasciatus* evidencing that land has been present in the Kermadec region since the mid Pleistocene. A Pliocene age of roughly three million years for the species *C. brevipennis* was also found. However the age for *C. brevipennis* by relaxed molecular clock using fossil calibrations in the final main chapter (Chapter Four) suggested a minimum age of 12 million years (with a mean of 30 million years). It is unknown which clock is more accurate. However the possibility for *C. brevipennis* to represent a taxon of Pliocene age or older seems likely, possibly significant as the modern Chathams are generally considered no older than Pleistocene in age (Campbell & Hutching 2007). This may indicate that the *C. brevipennis* lineage has since gone extinct in New Zealand, or that there has been continuous land in the region longer than current geological estimates suggest. There are a number of studies that do suggest Pliocene ages for at least some Chatham Island taxa (e.g. Liggins *et al*. 2008, Heenan *et al*. 2010), congruent with this finding. This study also demonstrated an early Pleistocene age for the species *Hydrobiosis lindsayi*, and a mid-late Pleistocene age for the species *Oecetis chathamensis*.

In this study only a small fragment of the gene COI was ever sequenced from the species *Philanisus mataua*. This sample was a museum specimen roughly 17 years in age (collected in 1993),

and had been stored in 70% ethanol at room temperature since this time. Amplification of this sequence involved numerous attempts over several weeks, and was only successful using one new primer developed in this study (paired with the universal primer LCO1490). Another primer was developed to be paired with the universal primer HCO1498 to comprise the remainder of the sequence however was never successful. Eventually the region was successfully amplified and sequenced twice, and the data received demonstrated both times two DNA sequences within each sample (thus either contamination or a heteroplasmic sample). This comprised a total of seven such heterogenous sites, all corresponding exactly with regions either indicative with haplotypes of *Philanisus plebeius* or *Chathamia integripennis*. It is considered that *P. mataua* may therefore be a hybrid between these two species, or represents a close relative of *P. plebeius* and the data was contaminated by *C. integripennis* DNA (the reconstructed *P. plebeius* sample was divergent from any known *P. plebieus* haplotype, although still fell within the diversity exhibited by this species). New sequences of *P. mataua* are of some interest here, to see which (if either) hypothesis is correct.

The final chapter (Chapter Four) addressed the phylogenetic position of the Chathamiidae among related families and also used a relaxed molecular clock. This particular phylogeny was the first in caddisflies to combine the two ribosomal DNA sequences 28S and 16S in a single analysis. However the number of taxa was limited. Time constraints and amplification issues resulted in a number of taxa being omitted, in spite of having been successfully collected. Additionally families not found in New Zealand were poorly represented, as these were limited to what had been sequenced in past studies. Only by chance some genera therefore had sequences of both regions available (as opposed to species, as this phylogeny was forced to combine different congeneric species into single sequences). However the tree topology found was robust, and strongly suggested the Chathamiidae to form a sister taxon to the Australasian family Conoesucidae, in turn related to the other mostly Australasian families of the Calocidae and the Helicophidae. A molecular clock was used based on fossil calibrations, and is considered to be conservative and the dates found are thus unlikely to represent overestimates. In this case a late Cretaceous origin of the Chathamiidae was found,

consistent with a Gondwanan origin in New Zealand. The potential of 28S and 16S used together to construct a robust phylogeny using a larger number of taxa is well supported.

5.2 Limitations of this study and future research

The limitations of this study were in most part, logistical. The phylogeography of *P. plebeius* did not include many sites in the upper North Island excepting from around Auckland and a few samples from Tauranga. No sites from the west coast of the South Island were used, and a number of geographically isolated regions (especially New Plymouth) were limited to very few samples. Additionally the use of only mitochondrial genes can lead to a biased perspective of gene flow in populations due to lineage sorting (Moore 1995). As a result future study of the phylogeographic structure of *P. plebeius* using nuclear genes would be of particular interest. Future research should include a large number of sites in the upper North Island, hypothesised in this study to provide the glacial refuge of the species and thus likely to represent most of the evolutionary diversity. Samples from the western South Island would also be of some interest although are predicted here to show very low genetic diversity, and are unlikely to possess any unique hapotypes. Wider sampling to also test for the possible gaps in the species' distribution (such as the North Eastern North Island and Stewart Island) may need to be undertaken, and also the use of a nuclear gene (such as EF-1a) should perhaps be considered. *P. plebeius* was well demonstrated, in spite of the limitations here, to have a high degree of interesting genetic features, and should be of interest for a much more thorough analysis. More sampling in New South Wales should also be of interest, especially to confirm or identify the distribution of *C. integripennis* there.

It is considered here that the phylogenetic relationship between the species of the Chathamiidae was robustly tested, excepting the species *Philanisus mataua*, from which new complete sequences are critically needed. Again no nuclear genes were used however, ignoring the effects of possible prior introgression events misleading the results, such as for example the observed

placement of *C. integripennis* within *Philanisus* (Posada & Crandall 2002). The family level phylogeny although finding arguably the most robust phylogenetic placement of the Chathamiidae to date, was nonetheless limited by its use of only half of the families of the Sericostomatoidea, and the use of only a few species per family. A larger scale phylogeny of case making caddisflies, or at the very least the Sericostomatoidea, incorporating several conservative genes and all families and also using a molecular clock would provide a fascinating biogeographic study regarding the presumed radiation of this group in the Mesozoic. It is also argued in this study that many more studies on the biotas of New Zealand's outermost islands are urgently needed, especially the Kermadec Islands, and also the sub-Antarctic Islands which have been subject of no phylogeographic studies to date. It is also suggested here that some of the species endemic to the Chatham Islands be selected to further provide evidence for, or against, a pre-Pleistocene age of the Chatham biota.

5.3 References

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