Population genetics and morphometrics of the black tunnelweb spider *Porrhothele* (Mygalomorphae, Porrhothelidae)

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Shaun Thompson

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(Photo credit to Steve Trewick)

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Abstract

The Mygalomorphae are a taxonomically challenging group due to their morphologically conserved nature. Similarly, the phylogenetic relationships of Mygalomorphae within New Zealand are poorly resolved. The *Porrhothele* (Mygalomorphae:Porrhothelidae) have poorly defined species that may not accurately represent the true diversity of the genus. This thesis utilizes genetics and morphometrics to 1) provide a hypothesis for how New Zealand's Mygalomorphae relate to one another 2) clarify whether *Porrhothele antipodiana* is composed of multiple species 3) determine if traditionally used morphological traits can effectively separate *Porrhothele* mtDNA clades from one or another or even from *Hexathele*, a morphologically and ecologically similar group. Mygalomorphae were collected throughout New Zealand and had the CO1 mtDNA gene sequenced. Combined with online data, a phylogenetic tree representing all five of New Zealand's Mygalomorphae genera was generated. The multiple genera tree hypothesizes that *Migas* is the closest relative to *Porrhothele* within New Zealand. CO1 mtDNA data from *Porrhothele* was used to generate phylogenetic trees for the genus. Morphological traits were measured and used in a principal components analysis to determine whether they could separate genera and mtDNA clades. An unsupervised cluster analysis was also used to determine whether mtDNA clades and genera could be separated. The *Porrhothele* phylogenetic trees provide some evidence that *P. antipodiana* may represent more than one species, but it still appears that *P. antipodiana* is a widespread species. Additionally, the *Porrhothele* phylogenetic trees provide some evidence for the presence of three undescribed species. The CO1 mtDNA clades within *Porrhothele* could not be separated from one another using the selected morphological traits in the PCA but were able to separate *Porrhothele* from *Hexathele*. However, the cluster analysis was unable to separate mtDNA clades and genera. Metatarsus length was found to be the most effective trait at separating *Porrhothele* from *Hexathele* but cannot completely separate them. It was also found that the number of spermatheca lobes in females provide support for the new mtDNA lineages being undescribed *Porrhothele* species, but caution is needed as some *Porrhothele* species and individuals are variable for this character.

Table of Contents

List of Tables

List of Figures

The known biogeography of New Zealand's Mygalomorphae

Introduction

New Zealand biogeographical studies often attempt to determine the origin of New Zealand species (Fleming 1962; Wallis & Jorge 2018). While broad, this question can ultimately be answered by a Gondwanan vicariance hypothesis (the species existed here when Zealandia split away from Gondwana and have persisted) or by a dispersal hypothesis (the species travelled to New Zealand and colonized after the split) (Trewick et al., 2011). Evidence for both vicariant origins and dispersal origins have been revealed from molecular phylogenetic studies. For example, evidence for a Gondwanan origin in Leiopelmatidae frogs has been found whereas there is strong evidence that the ancestors of moa arrived relatively recently (Feng et al., 2017; Phillips et al., 2010). The origins of many taxa in New Zealand are of interest, but some particularly so; for instance, the Mygalomorphae, which are a large group of spiders with morphological traits that have been inferred as ancestral within the Araneae order (Bond et al. 2012). For example, their chelicerae (fangs) are orientated to face downwards (Hedin et al. 2018) which is considered an ancestral state. The origins of this group of spiders in New Zealand are interesting to study because they are thought to have poor dispersal capabilities. Mygalomorphae were probably present in Gondwana, and fossil records indicate that they were present in New Zealand at least 23 million years ago (Selden et al., 2006; Selden & Kaulfuss, 2019). Mygalomorphae have two pairs of book lungs which is considered to be the ancestral state, and this is used to place them sister to the Araneomorphae spiders (with diagonally pointing fangs) (Coddington & Levi, 1991). More book lungs may increase the rate of desiccation and therefore restrict these spiders to more damp habitats as they are likely sensitive to changes in the environment which may restrict their ability to disperse over long distances (Perez-Miles & Perafan, 2017). New Zealand's Mygalomorphae fauna has representatives of five families, four of which (Hexathelidae, Nemesiidae, Idiopidae and Migidae) have overseas members. The only endemic family of Mygalomorphae in New Zealand is the Porrhothelidae, which contains only one genus, *Porrhothele* (Hedin et al., 2018) which contains five species. The New Zealand lineage is sister to a group of Australian species including the venomous Sydney funnel-web spider (*Atrax robusttus*; Hedin et al. 2018). *Porrhothele antipodiana* is widespread throughout

New Zealand, whereas the other four described species of *Porrhothele* have restricted distributions (Forster & Wilton, 1968). Morphological variation within *P. antipodiana* could be explained in two ways: either it is a single morphologically variable species, or it is a cryptic species complex and represents many similar undescribed species (Forster & Wilton, 1968).

Here, the available literature on the biogeography of Mygalomorphae is reviewed, with emphasis on implications for the origins of various genera of Mygalomorphae in New Zealand. This review focuses on *Porrhothele* as the study species for the origins of Mygalomorphae in New Zealand. Also discussed in this review is the literature regarding species complexes in the Mygalomorphae, with implications for the possibility of a species complex in *Porrhothele*.

New Zealand geological history

New Zealand's geological history gives rise to questions about the origins of New Zealand's many endemic species. In the mid cretaceous period 105 MYA, the supercontinent known as "Gondwanaland" began to shift from subducting to rifting in the region of Zealandia, that would later become modern-day New Zealand (Luyendyk, 1995; Tulloch et al., 2009). Roughly 82-85 MYA, this rifting caused Zealandia to split away from Gondwanaland and it has remained as its own separate landmass ever since (Cooper & Millener, 1993; Thomsom et al., 1991). A consequence of this is that some Gondwanan flora and fauna that were present on Zealandia when it split away could have persisted there until present day, meaning that these endemic species are of vicariant origin (Trewick et al., 2011). However, an issue with this is that roughly 30 MYA during the Oligocene epoch, there was a bottleneck event referred to as the "Oligocene drowning", where the continent of Zealandia sunk into the ocean and with very little exposed landmass remaining (Cooper & Cooper, 1995; Stockler et al., 2002). There is debate in the current literature about whether Zealandia was fully submerged during this event, which presumably would have obliterated all non-marine Gondwanan species present on the landmass (Landis et al., 2008; Waters & Craw, 2006; Strogen et al., 2014). If Zealandia was completely submerged, the implication of this is that all species in New Zealand are the result of dispersal over the ocean, a process referred to as "trans-oceanic dispersal" (Fox, 1973; Pole, 1994).

Methods of trans-oceanic dispersal:

There are many means of natural trans-oceanic dispersal in arthropods. Some animals may be able to disperse via "rafts", either as adults or as resistant eggs or pupae that can tolerate the seawater (Smith, 2002; Fraser et al., 2010; Nikula et al., 2013). Others may disperse via flight or aerial drifting, relying on wind currents to float them great distances (Fox, 1973; Klass-Douwe, n.d.; Asahina, 1970). A more peculiar example of this sort of dispersal is when one animal hitchhikes on another animal that can fly long distances. Migratory birds fly to New Zealand from the Northern Hemisphere every year, revealing that for some organisms, New Zealand is not isolated. A more common method of dispersal is by travelling within ocean currents in the water (Queiroz, 2005; Coulson et al., 2002; Scheltema, 1968). However, this requires the animal to be able to tolerate submergence for long periods without rafts (Coulson et al., 2002; Pfingstl, 2013; Renault, 2011). These three means of dispersal apply broadly to the animal kingdom, particularly to the spiders.

These three means of trans-oceanic dispersal have been implicated in the biogeography of spiders, albeit to different extents, and the available research indicates that spiders can use a small variety of trans-oceanic dispersal methods. Like most other arthropods, spiders are assumed to have rafting capabilities, which may explain the distributions of many species (Harrison et al., 2017; Smith, 2016). Previous studies have not directly observed spiders rafting or their ability to tolerate long periods on the ocean, so this gap in research may have to be addressed at some point. However, spiders are arguably best suited for wind dispersal due to their ability to glide in the air using "ballooning", which has been implicated in the distribution of some species of spider (Gillespie, 2002; Kuntner & Agnarsson, 2011; Soto et al., 2017). With just a few exceptions, the Mygalomorphae are not capable of ballooning (Decae, 1987; Coyle, 1983). Except for a small number of genera (particularly the *Amaurobioides*), the ability of spiders to disperse using ocean currents has been less-well studied (Ramirez, 1995; Baehr et al., 2017).

Widespread Mygalomorph background:

Although there is no truly cosmopolitan family of Mygalomorphae, there are several families that are exceptionally widespread, some of which are represented in New Zealand. The

Theraphosidae family, better known as the tarantulas, are perhaps the best-known family of Mygalomorphae. Many species of Theraphosidae can be found throughout Africa and in a few locations in Europe but are most commonly found in the North and South American continents (World Spider Catalog, 2019a). However, an exception to this is the sub-family Selenocosmiinae (aptly referred to as Asia-Pacific tarantulas) which can also be found in Australia and in South-East Asia (World Spider Catalog, 2019a). The Pycnothelidae family, referred to as the funnel-web tarantulas also seem to be widespread. Pycnotheliids have been described in North and South America, Australia, South-Eastern Asia, Africa and in Europe (World Spider Catalog, 2019b). Pycnothelidae is also represented in New Zealand by the *Stanwellia* genus (World Spider Catalog, 2019b). Similar to these two families, the Idiopidae family (Trapdoor spiders) is also widespread. Idiopidae is known to be distributed in North and South America, Africa, Australia, South-Eastern Asia and in parts of Europe (World Spider Catalog, 2019c). This family is represented in New Zealand by the *Cantuaria* genus (World Spider Catalog, 2019c).

Phylogeography in New Zealand

Phylogeography is the study of how historical processes have shaped the geographic distribution of taxa (Avise, 2000). The fundamental principle of phylogeography is that the distribution of all living organisms can ultimately be explained by historical processes such as dispersal, vicariance, extinction and speciation. To make inferences about the historical processes that have shaped a species distribution, we must examine the spatial distribution of genetic variation and the amount of dissimilarity between genetic variants (Trewick et al, 2011). This information thereby hints at the nature of the event that shaped these populations. For instance, if a population has weak genetic differentiation and this variation has a heterogeneous distribution, this may indicate a relatively recent spatial partitioning event (Trewick et al, 2011). This phylogeographic pattern has been observed in the *Acanthophlebia* mayflies for instance, which have been found to have heterogeneous populations, but relatively low diversity (Smith et al, 2005). The implication of this is that the spatial partitioning of *Acanthophlebia* may be a result of a recent event (< 1MYA), which has been inferred to be the relatively recent Taupo volcanic eruption (Smith et al, 2005).

The phylogeography of New Zealand's wide range of plant and animal life has received significant attention in the past couple of decades. The results from a wide variety of

phylogeographic studies in New Zealand has revealed several types of historical events that have structured New Zealand's biota.

Glaciation has been a driving factor creating many phylogeographic patterns in New Zealand's fauna and flora. During the Pleistocene, glaciers were widespread throughout New Zealand (Rother et al, 2014). Large glaciers could form gaps in a species distribution. If these gaps are maintained, gene flow between populations may become reduced or prevented entirely. This may enable different haplotypes to accumulate in the different populations and ultimately drive speciation. Additionally, once the glaciers receded, populations that colonize areas previously covered by the glaciers may have less haplotype diversity since they have had less time to accumulate new haplotypes. Historic glaciation shaping phylogeography in New Zealand can be seen in the New Zealand mudsnail *Potamopyrgus antipodarum*, where *P. antipodarum* distributed throughout the South Island appears to have a North-South genetic division, which has been suggested to be due to Pleistocene glaciation (Neiman & Lively, 2004). Similar patterns of glaciation shaping the phylogeography of New Zealand's biota can be seen in a variety of taxa (Trewick, 2001; Buckley et al, 2009; Marshall et al, 2009).

Mountain building has also partially shaped the phylogeographic patterns of New Zealand's biota. During the Pliocene epoch, extensive mountain building began in the South Island that resulted in the formation of the Southern Alps (Whitehouse & Pearce, 1992). As the mountains rose, populations that occupied alpine habitats would have become adapted to better survive on their geographic peaks. As the populations became adapted to their high-altitude peaks, gene flow would have been reduced because climatic changes may have narrowed the zones these populations could occupy, causing a more patchy distribution. This process could also have been enhanced by the presence of glaciation throughout the Southern Alps. The isolation of many populations could then facilitate adaptive radiation and result in the formation of many distinct lineages. This process has been implicated in *Celatoblatta* cockroaches, which occupy the South Island and are estimated to have diversified 3.7-4.2 mya, the same time as uplift began (Chinn & Gemmell, 2004; Trewick, 2001). Similar pattern of diversification by uplift in the Southern Alps has been implicated in a variety of taxa (Buckley & Simon, 2007; Trewick et al, 2001; Wagstaff & Garnock-Jones, 1997).

To a lesser extent, volcanism has also been responsible for the phylogeographic patterns seen in some of New Zealand's biota. Large scale volcanic events could bury large areas in ash, wiping out a variety of life. This sort of event could wipe out local populations, which could result in the loss of haplotypes and a temporary separation of populations. When these areas are later colonized, haplotype diversity is likely to be low since new haplotypes have not had time to accumulate. For instance, haplotypes of the spleenwort *Asplenium hookerianum* distributed in the central North Island have been found to have an east-west pattern of separation, which has been implicated to be the result of relatively recent volcanic activity in the tongariro volcanic plateau (Shepherd et al, 2007). Similar patterns of low diversity in the central plateau have been observed in a variety of taxa (Holzapfel et al, 2002; Morgan-Richards et al, 2001; Baker et al, 2005).

Mygalomorphae Phylogeography

Mygalomorphae taxa seem to show a tendency to easily form genetically structured populations in response to environmental events and factors. This may be due to their low vagility and usually restricted habitat preferences (Perez-Miles & Perafan, 2017). In the Tallaganda region, the Sydney funnelweb spider (*Atrax*) and *Hadronyche* occupy restricted soil habitat and saproxylic habitat, respectively (Beavis & Rowell, 2006). As a result of this, when Pleistocene glacial cycles occurred 400,000 years ago, *Atrax* was able to persist in gully habitats, whereas *Hadronyche* populations became extinct due to a lack of wood habitats for them to occupy, so recolonized at a later date (Beavis & Rowell, 2006). As a result of this, *Atrax* has high levels of divergence within Tallaganda whereas *Hadronyche* has low levels, which appears to strongly reflect their biogeographic history (Beavis & Rowell, 2006). Similarly, other groups of Mygalomorphs show a tendency to form strongly structured populations due to factors such as Pleistocene climate change, mountain uplift and habitat fragmentation (Hamilton et al, 2011; Starrett & Hedin, 2007; Hedin et al, 2015).

Origins of some New Zealand Mygalomorphae:

New Zealand's five genera of Mygalomorphae (*Porrhothele*, *Hexathele*, *Migas*, *Cantuaria* and *Aparua*) were described in Forster & Wilton (1968) and have been mostly unchanged with the exception of the genus *Aparua* being recognized as a junior synonym of *Stanwellia* in Main, (1983). Of these genera, only *Cantuaria* and *Stanwellia* have research detailing their origins in New Zealand.

Recent evidence suggests that *Cantuaria* most likely dispersed to New Zealand after the breakup of Gondwana. In her doctoral research, Smith (2016) examined the biogeography of the *Cantuaria* genus. One of the main findings of Smith's research was that *Cantuaria* seemed to have diverged from its Tasmanian relative, *Misgolas*, about 18 mya (and subsequently dispersed to New Zealand). This is after both the breakup of Gondwana and the Oligocene drowning. Divergence dates were calibrated with substitution rates and tested by calibrating the molecular clock with geological dates. This resulted in mitochondrial substitution rates required for *Cantuaria* to be much lower than in any other Mygalomorphae, which seems unlikely.

In research by Wheeler et al., (2016), *Stanwellia* and the South American genus *Acanthogonatus* were found to both be sister groups to the rest of the Pycnothelidae, family, which is thought to be consistent with a Gondwanan vicariance origin for *Stanwellia*. However, the flaw to this argument is that the divergence between these two genera was not dated and thus it is unknown whether they diverged before or after Gondwana broke apart. In a study of the phylogenetic relationships within Pycnothelidae Harvey et al., (2018) used several species of *Stanwellia* from Australia and two species of *Stanwellia* from New Zealand. The first relevant finding was that these two species of *Stanwellia* formed a clade within the Australian *Stanwellia*, strongly indicating that the *Stanwellia* in New Zealand dispersed here from Australia. Harvey et al., (2018) also dated the radiation of *Stanwellia* and found that this occurred a maximum of 38 mya, which is long after Gondwana broke apart, further suggesting that New Zealand species of *Stanwellia* dispersed here from Australia. Furthermore, their estimate for time of the most recent common ancestor of the New Zealand and Australian *Stanwellia* species is mid to late Miocene, well after the Oligocene drowning.

It is currently not established whether *Porrhothele* is of vicariant origin or not, but it appears unlikely. As previously stated, it appears that Mygalomorphae are quite capable of transoceanic dispersal, so this would suggest it is certainly possible that ancestral *Porrhothele* could reach New Zealand (Harrison et al., 2017; Smith, 2016). There is also evidence that two of New Zealand's five Mygalomorphae dispersed to New Zealand and no evidence of the remaining three (including *Porrhothele*) being of vicariant origin (Harvey et al, 2018; Smith, 2016). While not conclusive, this tentatively suggests that *Porrhothele* is likely to be a more recent (in the last few hundred thousand years) arrival, rather than a Gondwanan relic.

***Porrhothele* biogeography:**

Porrhothele are interesting to use in biogeographical studies because their mygalomorph traits might lead to geographic structure of populations. Spiders can usually disperse widely because of ballooning behaviour in juvenile spiders but like most mygalomorphs, this behaviour has not yet been observed in *Porrhothele* (Coyle, 1983). Female *Porrhothele* do not travel once their tunnel web is set up; only the males are known to travel and only for short distances (Forster & Wilton, 1968). Further, *Porrhothele* are usually restricted to living in moist, damp areas (typically forests, but they have been found in sand dunes as well) under stones/logs and in tree trunks, which restricts the habitat they can occupy (Jackson & Pollard, 1990). These restrictions on dispersal suggest that gene flow between populations is likely limited and thus could foster genetic divergence between populations. One would expect to see evidence of isolation by distance which could make *Porrhothele* interesting for studying population structure and may also have resulted in the formation of cryptic species.

Literature on the biogeography of *Porrhothele* is very limited, with the only available study being a Master's thesis produced by Macdonald, (2013). As part of his thesis, Macdonald (2013) examined the phylogeography of *Porrhothele antipodiana* on Banks Peninsula. He found evidence of high levels of divergence and phylogeographic clustering of *Porrhothele* and suggested that this may have been caused by these spiders persisting in glacial refugia while the rest of the land was uninhabitable during Pleistocene glaciation (Macdonald, 2013). However,

this is just an interpretation of the pattern in the data. The author does not directly relate his data to patterns of glaciation on Banks Peninsula and does not generate lineage age estimations, so there is no convincing proof that this pattern can be attributed to Pleistocene glaciation.

Gaps in knowledge of Porrhothele:

In 2018, *Porrhothele* was moved to its own family, Porrhothelidae, based on molecular data that suggested it was distinctive enough for this change (Hedin et al., 2018). Because of this, *Porrhothele* is endemic at the family level, which may suggest a Gondwanan origin, but this is yet to be tested. However, if the study by Hedin et al. (2018) failed to sample close relatives in New Zealand or elsewhere, we would be misled into thinking the lineage is very distinct. This seems possible because close relatives may have gone extinct or be simply unknown, so therefore cannot be sampled. Another aspect of *Porrhothele* that is not well understood are the species themselves. Of the five species, four have very restricted distribution; for example, *P. quadrigyna* is only known in Northland (Forster & Wilton, 1968). In comparison, *P. antipodiana* has a relatively broad distribution, it is known to occur everywhere in the North Island except Northland and occurs over most of the South Island (Forster & Wilton, 1968). Throughout its range, *P. antipodiana* varies in size, colour and has minor differences in structures, especially the structure of the female reproductive system and the patterns of spines on the modified tibia of males (Forster & Wilton, 1968). This can especially be seen in the Queen Charlotte sounds area, where populations on different islands tend to have different sizes and small structural differences (Forster & Wilton, 1968). This species is known to occur in damp forest, sand dunes, rocky hillsides and urban areas (Laing, 1978). It is possible that *P. antipodiana* represents many undescribed species (an unrecognized cryptic species complex). If there were many species one would expect consistency in morphological traits (for example, all large individuals would also have a red/tan coloured dorsal carapace) and that these sets of traits would be concordant with genetic markers (for example, all large individuals would have a distinct mitochondrial lineage). The genotypic species definition provides a tool to recognise sympatric species (Mallet 1995). Distinct species, if ecologically divergent, might co-occur (for example, forests and sand dunes habitats may have different clusters). However, if species are allopatric, then relative levels of differentiation could be used to infer species.

Mygalomorphae taxonomic issues

Due to the life history and how morphologically conserved the Mygalomorphae are, taxonomy is more difficult than in the Araneomorphae. Because of their susceptibility to desiccation, Mygalomorphae are mainly fossorial and live in silk burrows (Perez-Miles & Perafan, 2017). This hinders taxonomy because specimens are difficult to collect, but it may also mean that most Mygalomorphae have similar selective pressures (Bond & Hedin, 2006). As a result of this, it is expected that morphological features in Mygalomorphae will evolve in similar ways and thus morphology will be conserved. This also predicts that homoplastic features are likely to arise, which may further complicate taxonomy (Bond & Hedin, 2006). Conserved morphology and the presence of homoplasy therefore makes it difficult to find reliable morphological taxonomic features (Goloboff, 1995). Because of this lack of reliability in morphological taxonomy, it is expected that there could be numerous species that may only be recognized through the use of molecular methods.

Cryptic species in Mygalomorphae

The biology of Mygalomorphae can, in some circumstances, predispose them to forming cryptic species complexes (CSC). It has been suggested that organisms that have non-visual mating systems may be more predisposed to forming CSCs than those dependent on visual cues (Bickford et al., 2007). This is because speciation caused by evolutionary changes in non-visual mating systems (e.g. changes in olfaction) usually will not result in any morphological changes, thus a morphologically indistinguishable species may arise (Bickford et al., 2007). For Mygalomorphae species, courtship behaviour frequently involves tactile-chemical communications using the female's web (Perez-Miles & Perafan, 2017). In addition, most species are nocturnal (as is evident from their poor vision) and mating occurs in the darkness of web-covered retreats (Land, 1985; Perez-Miles & Perafan, 2017). These traits suggest that vision is unlikely to be important for mate recognition in most Mygalomorphae. This may therefore mean that speciation will primarily involve non-visual cues, predisposing Mygalomorphae to be morphologically conserved (unless they are ecologically differentiated).

Recent molecular work in the last decade has revealed numerous examples of cryptic species complexes in Mygalomorphae. In one study, Satler et al. (2013) examined *Aliatypus thompsoni*,

a species of trapdoor spider in California. After sequencing six genes (five nuclear and one mitochondrial), the finding was that *A. thompsoni* represented three different species, two of which had previously been unknown (Satler et al, 2013). Similarly, Hamilton et al. (2014) used several species delimitation approaches with the aim of determining how many species are present in *Aphonopelma*, a genus of Mygalomorphae with few identifying morphological characteristics. The authors aimed to define species using several sets of criteria and their associated molecular methods. They used tree-based delimitation and defined species by clade monophyly and whether there was likely to be low gene flow between clades. DNA barcoding was also used to calculate genetic distances and Automatic Barcode Gap Discovery was used to establish a barcode gap to define species boundaries. One of the main findings was that mtDNA data is not enough on its own but that it is necessary to incorporate knowledge of morphology and natural history into the "species equation". Using this, 32 cryptic species were recognized in this spider complex (Hamilton et al, 2014). Cryptic species complexes have also been observed at much smaller scales. For example, Bond (2004) examined the *Apomastus* genus, which was thought to be monotypic and restricted to just a few mountains. After examination using a combination of morphological and mtDNA (cytochrome b) data, evidence suggested that this highly restricted lineage may be two species rather than one, something which was hinted at using morphological characteristics, but could not be justified using morphology alone (Bond, 2004). However, the author also suggested that an alternative explanation for the data is that one species had very high geographical structure (Bond, 2004).

Aims

This study has two aims: 1) to describe and comment on the phylogenetic relationships of New Zealand's native Mygalomorphae in relation to previous literature using mtDNA sequences. 2) to determine whether the widespread *Porrhothele antipodiana* species actually represents multiple undescribed cryptic species of *Porrhothele*. This will be accomplished using mtDNA sequences to get estimates of divergence between lineages while also testing if morphological features are consistent with the genetic evidence. It is predicted that the phylogenetic relationship produced will be shaped like Figure 1. This is based on previous phylogenies by Opatova et al, (2019) and Wheeler et al, (2016), that have been produced using the same Mygalomorphae families.

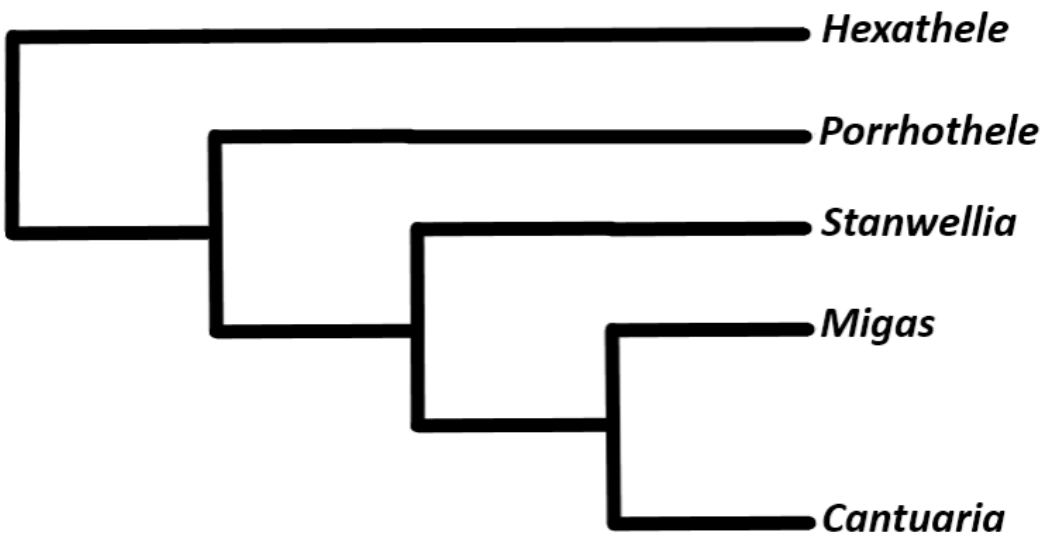

Figure 1. Predicted phylogenetic tree of the relationship of New Zealand's Mygalomorphae based on previous phylogenies produced by Opatova et al, (2019) and Wheeler et al, (2016)

Materials and Methods

Specimen collection

Mygalomorph spiders (*Porrhothele*, *Hexathele and Stanwellia*) were collected from 31 ecological districts throughout New Zealand, mostly from native forest and sand dune habitats (Table 3). Specimens from Cape Farewell were collected as part of a bioblitz organized by Farewell Whahariki HealthPost Nature Trust (November 2019) whilst specimens collected from Te Paki were collected as part of a bioblitz organized by Ngati Kūri (January 2020). All specimens collected from private property had the permission of the property owner to do so. Spiders were generally found by searching under logs and rocks, but a few were lured out of webs built into the side of rock faces and trees. A total of 58 specimens were donated by other collectors located throughout New Zealand (Table 3). Once collected, the spiders were euthanized by freezing and were then stored in 70% ethanol to preserve morphological features. For each spider, one leg IV was removed and stored in 99% ethanol to preserve the DNA. Spiders were identified to genus level using descriptions by Forster & Wilton (1968). *Porrhothele* and *Hexathele* were distinguished from one another by the number of spinnerets

(*Porrhothele* has four whereas *Hexathele* has six) and *Stanwellia* was separated from *Porrhothele* by the number of serration rows on the claws (*Stanwellia* has two rows whereas *Porrhothele* has one row). All specimens are currently held in the Phoenix Group collection at Massey University but will be deposited in the Museum of New Zealand Te Papa Tongarewa.

Morphometrics

A total of ten morphometric characters were measured in both *Porrhothele* and *Hexathele* specimens. Morphometric characters used are listed in Table 1 and are visually displayed in Figure 2. Male sexual morphological characters were not used in this study due to not being able to find enough material (male Mygalomorphae are difficult to find in sufficient numbers). Imaging and measuring of morphometric characters used an Olympus SZX7 stereomicroscope and an Olympus SC100 camera attachment with OLYMPUS Stream image analysis software under magnification. A principal components analysis of data from adult female *Porrhothele* and *Hexathele* was done using R studio with the ggplot2 and ggfortify packages (Wickham, 2016; Tang et al, 2016). The significance of each principal component was assessed by using the broken-stick test, average eigenvalue rule, scaled eigenvalue rule and by examining the shape of the scree plot for the presence of an "elbow" (Frontier, 1976; Jolliffe, 1972; Kaiser, 1960). The three most informative traits revealed by the principal component analysis as best able to separate *Porrhothele* from *Hexathele* were then used in a T-test to determine whether the mean of these traits differed significantly between *Porrhothele* and *Hexathele*.

Table 1. Ten morphometric characters used to investigate shape and size differences between adult female spiders in the genera *Porrhothele* and *Hexathele*

Trait	**Measurement**
Femur	The length of the femur
Patella	The length of the patella
Tibia	The length of the tibia
Metatarsus	The length of the metatarsus
Tarsus	The length of the tarsus
Head width	The width of the head at its widest point
Head length	The length of the head down the middle
Eye group width	The width of the complete eye group
Eye group length	The length of the complete eye group
Fovea position	The distance of the fovea from the posterior end of the head

Principal components of morphological variation were plotted to visualize the dataset. Two morphological traits which appeared to separate *Porrhothele* and *Hexathele* were then converted into a ratio and tested using the Wilcoxon-Mann-Whitney to see if this trait ratio could be used to separate the two genera.

In addition to this, the data was explored using Gaussian mixture models (a type of unsupervised learning algorithm that do not require *a priori* labels) in the R package mclust v5.4.5 (Scrucca et al, 2016) using the three most informative traits. Mclust creates models that group individuals together based on similarity. If the Gaussian mixture models formed clusters that separated *Porrhothele* from *Hexathele*, then this would indicate that they are morphologically distinct. Suitability of each model was assessed using Bayesian information criterion (BIC) and the model with the best fit was selected. Once the best model was selected, a plot was produced to visualize the clusters created for the dataset.

Figure 2. Morphometric characters used to investigate shape and size differences between adult female spiders in the genera *Porrhothele* and *Hexathele*

Reproductive morphology

The internal reproductive ducts and external genital structure (epigynum) of adult female specimens was dissected and removed from the body using size 1.20x38mm hypodermic medical needles. The removed epigynum was then left to soak in a 10% solution of KOH overnight to dissolve obstructive tissue to make the reproductive receptacles (also alternatively referred to as spermatheca) more visible. The shape and number of receptacle lobes were recorded and photographed under the microscope and then the epigynum was placed in a vial of 70% ethanol with its specimen of origin to preserve the structures.

Genetic data

Muscle tissue was extracted from the femur of one leg IV. The salting out procedure for DNA extraction followed that used in Trewick & Morgan-Richards (2005). For the molecular analysis, partial sequences of the mitochondrial cytochrome oxidase I (CO1) gene was used. The forward primer LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and the reverse primer HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') were used to amplify a fragment of CO1 with a length of ~700bp (Folmer et al, 1994). Polymerase chain reactions (PCR) for CO1 followed the protocol used by MacDonald (2013). The process began with denaturation at 94°C for 2 minutes, then with 40 cycles of 15 seconds at 94°C for denaturation, 30 seconds at 49°C for annealing (this temperature was sometimes raised and lowered depending on the quality of the samples), and 30 seconds at 72°C to elongate, with a final elongation of 7 minutes at 72°C. An attempt was made at also sequencing the ITS gene using the ITS_G923F (5'-CGTAACAAGGTTTCCGTAGGTGA-3') and ITS_G923R (5'AGAGAACTCGCGAATTCCACGG-3') primers, (Harrison et al, 2017). Although the ITS primers worked, the sequences produced had a very high error rate and thus were not used in the study. Once completed, the PCR products were visualized on a 1% agarose gel in TAE buffer. Successful amplification products of the expected size were sequenced using the Massey Genome Service's ABI 3730 genetic analyser (Applied Biosystems Inc., Carlsbad, CA). Sequences were then aligned using Geneious version 9.1.8 (Kearse et al, 2012). All sequences generated will be submitted to GenBank.

The *Porrhothele* dataset was also supplemented with CO1 sequences downloaded from GenBank. This data was produced by MacDonald (2013) using *Porrhothele* collected from Banks Peninsula. Other online data of mygalomorph genera that are represented in New Zealand and that were over 400 bp in length were also downloaded from Genbank (Table 2).

Table 2. DNA sequences of CO1 from Mygalomorphae spiders downloaded from NCBI GenBank.

GenBank Identifier	Genus/species	Length	Reference
KY017792	*Migas*	1076	Wheeler et al, 2016
KY615617	*Cantuaria wanganuiensis*	1133	Smith, 2016
KY615622	*Cantuaria*	1103	Smith, 2016
KY615631	*Cantuaria*	1133	Smith, 2016
KY615641	*Cantuaria*	1133	Smith, 2016
MG800167	*Stanwellia*	658	Harvey et al, 2018
MG800171	*Stanwellia*	658	Harvey et al, 2018
MG432408	*Porrhothele*	562	Macdonald, 2013
MG432409	*Porrhothele*	562	Macdonald, 2013
MG432410	*Porrhothele*	562	Macdonald, 2013
MG432411	*Porrhothele*	562	Macdonald, 2013
MG432412	*Porrhothele*	562	Macdonald, 2013
MG432413	*Porrhothele*	562	Macdonald, 2013
MG432414	*Porrhothele*	562	Macdonald, 2013
MG432415	*Porrhothele*	562	Macdonald, 2013
MG432416	*Porrhothele*	562	Macdonald, 2013
MG432417	*Porrhothele*	562	Macdonald, 2013
MG432418	*Porrhothele*	562	Macdonald, 2013
MG432419	*Porrhothele*	562	Macdonald, 2013
MG432420	*Porrhothele*	562	Macdonald, 2013
MG432421	*Porrhothele*	562	Macdonald, 2013
MG432422	*Porrhothele*	562	Macdonald, 2013
MG432423	*Porrhothele*	562	Macdonald, 2013
MG432424	*Porrhothele*	562	Macdonald, 2013
MG432425	*Porrhothele*	562	Macdonald, 2013
MG432426	*Porrhothele*	562	Macdonald, 2013
MG432427	*Porrhothele*	562	Macdonald, 2013
MG432428	*Porrhothele*	562	Macdonald, 2013
MG432429	*Porrhothele*	562	Macdonald, 2013
MG432430	*Porrhothele*	562	Macdonald, 2013
MG432431	*Porrhothele*	562	Macdonald, 2013
MG432432	*Porrhothele*	562	Macdonald, 2013
MG432433	*Porrhothele*	562	Macdonald, 2013
MG432434	*Porrhothele*	562	Macdonald, 2013
MG432435	*Porrhothele*	562	Macdonald, 2013
MG432436	*Porrhothele*	562	Macdonald, 2013
MG432437	*Porrhothele*	562	Macdonald, 2013
MG432438	*Porrhothele*	562	Macdonald, 2013
MG432439	*Porrhothele*	562	Macdonald, 2013

Phylogenetic analysis

For phylogenetic analysis to infer the evolutionary relationships of New Zealand's native Mygalomorphae spiders a dataset of mitochondrial DNA sequences was created. The alignment was 610 to 700 bp long and included 18 sequences representing all five of New Zealand's Mygalomorphae genera. The phylogenetic analysis used *Cantuaria* as the outgroup since this group is also a Mygalomorph and there are abundant sequences for this group. To test whether the five New Zealand mygalomorph genera are monophyletic I used a Bayesian estimation of the evolutionary relationships with the MrBayes plugin in Geneious Prime 2020 (Kearse et al, 2012) with the generalized time reversible substitution model default settings (Huelsenbeck & Ronquist, 2001). MrBayes uses Bayesian inference which attempts to find the highest likelihood phylogeny by calculating the posterior probability from different evolutionary models (Huelsenbeck & Ronquist, 2001). A Maximum Likelihood phylogeny was inferred to further test the monophyly using the PhyML plugin in Geneious with default settings and 1000 bootstrap replications (Guindon et al, 2010). PhyML uses Maximum Likelihood which calculates probability scores to determine the most probable relationships of a gene alignment. The probabilities are calculated with the mutation rate based on a given substitution model.

To examine the geographic structure of genetic diversity within *Porrhothele* I created a 610 bp alignment of 74 CO1 DNA sequences. A neighbour-joining phylogenetic tree (Saitou & Nei, 1987) of the *Porrhothele* genus was estimated using a homologous sequence of *Migas* sp. as an outgroup with 1000 bootstrap replications in Geneious using the default settings. (Kearse et al, 2012). A maximum likelihood tree was generated in Geneious with the PHYML plugin using default settings (Guindon et al, 2010). Additionally, a Bayesian tree was produced in Geneious using the MrBayes plugin with default settings (Huelsenbeck & Ronquist, 2001).

Table 3. Mygalomorph spiders collected for this study with locality, date, collector and code. "Used in phylogeny and morphometrics" indicates whether a specimen was used in both analyses (YY), just one (YN or NY) or none (NN). Ecological region and districts are based on the *The ecological regions and districts of New Zealand* (1987)

MY code	Genus	Location	Coordinates	Ecological Region/District	Sex	Collector	Collection	Date	Used in phylogeny & morphometrics?
29	*Porrhothele*	Rangiwhai Hut, Tussock Zone,	-39.895044, 176.043936	Ruahine/Ruahine	F, Adult	S. Thompson	Massey	13/10/2019	YY
1	*Porrhothele*	Waikawa Beach	-40.687896, 175.145217	Manawatu/Foxton	F, Adult	S. Thompson	Massey	13/12/2018	YY
3	*Porrhothele*	Waikawa Beach	-40.687896, 175.145217	Manawatu/Foxton	F, Adult	S. Thompson	Massey	13/12/2018	YY
30	*Porrhothele*	Waikanae Beach	-40.871197, 175.006681	Manawatu/Foxton	Juv	S. Thompson	Massey	6/09/2019	NN
31	*Porrhothele*	Kuku Beach	-40.668498, 175.154925	Manawatu/Foxton	F, Adult	S. Thompson	Massey	17/10/2019	YY
5	*Porrhothele*	Bledisloe Park	-40.382376, 175.618907	Manawatu/Manawatu Plains	F, Adult	S.Nielson	Massey	1-Oct	NY
6	*Porrhothele*	Bledisloe Park	-40.382376, 175.618907	Manawatu/Manawatu Plains	Juv	S. Thompson	Massey	9/02/2019	YN
7	*Porrhothele*	Prouse Bush, Levin	-40.633507, 175.280182	Manawatu/Manawatu Plains	F, Adult	S. Thompson	Massey	4/05/2019	NY
15	*Porrhothele*	Ashurst Domain	-40.303555, 175.758007	Manawatu/Manawatu Plains	F, Adult?	S. Thompson	Massey	9/06/2019	YN
16	*Porrhothele*	Bledisloe Park	-40.382376, 175.618907	Manawatu/Manawatu Plains	F, Adult?	S. Thompson	Massey	24/06/2019	YN

62	*Porrhothele*	Manawatu Gorge, Manawatu	-40.31, 175.77	Manawatu Gorge/Manawatu Gorge North	F, Adult	F.R. Schniteler	Te Papa	May-01	NY
4	*Porrhothele*	Murphys Rd, Pauatahanui	-41.118886, 174.930406	Sounds-Wellington/Wellington	F, Adult	S. Thompson	Massey	3/01/2019	YY
9	*Porrhothele*	Mt Kaukau, Wellington	-41.232829, 174.787034	Sounds-Wellington/Wellington	F, Adult?	S. Thompson	Massey	10/05/2019	NN
20	*Porrhothele*	Near Pauatahanui Reserve	-41.106407, 174.916712	Sounds-Wellington/Wellington	F, Adult	S. Thompson	Massey	26/07/2019	YY
42	*Porrhothele*	10 Hindipur Ter., Broadmeadows, Wellington	-41.235006, 174.793878	Sounds-Wellington/Wellington	F, Adult	G. Belchamber	Te Papa	11-Mar-96	NY
46	*Porrhothele*	Reefton	-42.11, 171.86	Sounds-Wellington/Wellington	M, Adult		Te Papa	Jul-93	NN
61	*Porrhothele*	1 Lincoln Street, Wellington	-41.309299, 174.764341	Sounds-Wellington/Wellington	M, Adult	A.J.D. Tennyson	Te Papa	4-Dec-05	NN
80	*Porrhothele*	Titahi Bay, Porirua, Wellington	-41.090921, 174.852144	Sounds-Wellington/Wellington	F, Adult?	S. Thompson	Massey	20/12/2019	NN
103	*Porrhothele*	Percy Reserve, Lower Hutt	-41.21, 174.87	Sounds Wellington/Wellington	Juv	B.N. McQuillan	Massey	26/04/2019	YN
64	*Porrhothele*	Wairapas, Te Awaiti	-41.470349, 175.524968	Eastern Wairapa/Eastern Wairapa	F, Adult	SAT/MMR	Massey	8/11/2019	YY
82	*Porrhothele*	Castlepoint, Wairarapa	-40.903604, 176.225697	Eastern Wairapa/Eastern Wairapa	F, Adult	S. Thompson	Massey	4/01/2020	YY
105	*Porrhothele*	Havelock North, 303 Durham Drive	-39.686290, 176.904810	Hawkes Bay/Heretaunga	F Adult?	SAT/MMR	Massey	15/02/2020	NN
106	*Porrhothele*	Havelock North, 303 Durham Drive	-39.686290, 176.904810	Hawkes Bay/Heretaunga	F Adult?	SAT/MMR	Massey	15/02/2020	YN
107	*Porrhothele*	Havelock North, 303 Durham Drive	-39.686290, 176.904810	Hawkes Bay/Heretaunga	F Adult?	SAT/MMR	Massey	15/02/2020	YN
108	*Porrhothele*	Havelock North, 303 Durham Drive	-39.686290, 176.904810	Hawkes Bay/Heretaunga	F Adult?	SAT/MMR	Massey	15/02/2020	YN
25	*Porrhothele*	Te Mata Peak (-396986449,1769002399)	-39.6986449, 176.9002399	Eastern Hawkes Bay/Eastern Hawkes Bay	F, Adult?	M. Lusk	Massey	3/10/2019	YN

26	*Porrhothele*	Te Mata Peak (-396986449,1769002399)	-39.6986449, 176.9002399	Eastern Hawkes Bay/Eastern Hawkes Bay	Juv	M. Lusk	Massey	3/11/2019	NN
27	*Porrhothele*	Te Mata Peak (-396986449,1769002399)	-39.6986449, 176.9002399	Eastern Hawkes Bay/Eastern Hawkes Bay	JUv	M. Lusk	Massey	3/12/2019	YN
65	*Porrhothele*	Cape Kidnappers	-39.66, 177.044	Eastern Hawkes Bay, Eastern Hawkes Bay	F, Adult	Mike Lusk	Massey	5/11/2019	YY
66	*Porrhothele*	Cape Kidnappers	-39.66, 177.04	Eastern Hawkes Bay, Eastern Hawkes Bay	Juv	Mike Lusk	Massey	5/11/2019	YN
67	*Porrhothele*	Cape Kidnappers	-39.66, 177.07	Eastern Hawkes Bay, Eastern Hawkes Bay	F, Adult?	Mike Lusk	Massey	5/11/2019	YY
68	*Porrhothele*	Cape Kidnappers	-39.65, 177.04	Eastern Hawkes Bay, Eastern Hawkes Bay	Juv	Mike Lusk	Massey	5/11/2019	YN
69	*Porrhothele*	Cape Kidnappers	-39.66, 177.07	Eastern Hawkes Bay, Eastern Hawkes Bay	Juv	Mike Lusk	Massey	5/11/2019	YN
70	*Porrhothele*	Cape Kidnappers	-39.66, 177.07	Eastern Hawkes Bay, Eastern Hawkes Bay	F, Adult?	Mike Lusk	Massey	5/11/2019	YY
71	*Porrhothele*	-39664464, 770447797	-39.664464, 177.0447797	Eastern Hawkes Bay, Eastern Hawkes Bay	F, Adult?	Mike Lusk	Massey	5/11/2019	YY
72	*Porrhothele*	Mohi Bush, Hawkes Bay	-39.85, 176.90	Eastern Hawkes Bay, Eastern Hawkes Bay	F, Adult	SAT	Massey	8/08/2014	YY
94	*Porrhothele*	Te Mata Peak	-39.69, 176.90	Eastern Hawkes Bay/Eastern Hawkes Bay	M, Adult	A.H. Simpson	Massey	19/04/2019	YN
95	*Porrhothele*	Te Mata Peak	-39.69, 176.90	Eastern Hawkes Bay/Eastern Hawkes Bay	F, Adult	A.H. Simpson	Massey	19/04/2019	YY
32	*Porrhothele*	Arthurs Pass, Grassland 3 West, Dan's Creek	-42.46, 172.40	Spenser/Lewis	M, Adult	K. Curtis	Massey	24/02/2019	NN
33	*Porrhothele*	Arthurs Pass, Shrubland 3 West, Dan's Creek	-42.466, 172.40	Spenser/Lewis	M, Adult	K. Curtis	Massey	1/04/2017	NN

34	*Porrhothele*	Grassland 2 Centre, Dan's Creek	-42.46, 172.40	Spenser/Lewis	M, Adult	K. Curtis	Massey	22/04/2014	NN
104	*Porrhothele*	Arthurs Pass, Nina Valley	-42.46, 172.32	Spenser/Lewis	F, Adult?	Kate Curtis	Massey	1/04/2018	YY
35	*Porrhothele*	"West Auckland"	-36.9, 174.5	Auckland/Waitakere	M, Adult	J. Welsh	Massey	10/10/2019	NN
36	*Porrhothele*	"West Auckland"	-36.9, 174.5	Auckland/Waitakere	M, Adult	J. Welsh	Massey	10/10/2019	NN
37	*Porrhothele*	Kennedy's Reserve, Canterbury	-43.616, 172.618	Banks/Port hills	M, Adult	C. Vink	Massey	21/10/2019	NN
49	*Porrhothele*	Omahu Reserve, Christchurch	-43.66, 172.61	Banks/Port hills	M, Adult	J.B. & G.M. Ward	Te Papa	22-Dec-05	NN
38	*Porrhothele quadrigyna? (male)*	Bream Head	-35.85, 174.57	Eastern Northland/Eastern Northland and Islands	M, Adult	?	Te Papa	Nov-12	NN
39	*Porrhothele quadrigyna*	Bream Head	-35.85, 174.57	Eastern Northland/Eastern Northland and Islands	M, Adult	?	Te Papa	Nov-12	NN
40	*Porrhothele quadrigyna*	Bream Head	-35.85, 174.57	Eastern Northland/Eastern Northland and Islands	M, Adult	?	Te Papa	Nov-12	NN
41	*Porrhothele quadrigyna*	Bream Head	-35.85, 174.57	Eastern Northland/Eastern Northland and Islands	M, Adult	?	Te Papa	Nov-12	NN
45	*Porrhothele*	Warawara Forest Park, Northland	-35.38, 173.31	Western Northland/Maungataniwha	M, Adult	D.S. Seldon	Te Papa	7-21/11/2009	NN
58	*Porrhothele*	Warawara Forest Park, Northland	-35.38, 173.31	Western Northland/Maungataniwha	M, Adult	D.S. Seldon	Te Papa	7-21/11/2009	NN
83	*Porrhothele*	Edge of Waipapa River, Puketi Forest, Kerikeri, Northland	-35.277, 173.680	Western Northland/Maungataniwha	F, Adult?	SAT/MMR	Massey	26/01/2020	YY
47	*Porrhothele*	Kelburn, Wellington	-41.28, 174.76	North-Westland/Reefton	m, Adult	F.R. Schniteler	Te Papa	30-Dec-01	NN
51	*Porrhothele*	Middle chain, Alderman Islands	-36.95, 176.08	Coromandel/Tairua	F, Adult	B.M. Fitzgerald	Te Papa	20-Feb-02	NY
52	*Porrhothele*	Te Roto, Chatham Islands	-43.82, -176.58	Chathams/Chathams	F, Adult	J.P. Thomas	Te Papa	15-Mar-95	NY

73	*Porrhothele*	Maipito Road, Chatham Islands	-43.95, -176.55	Chathams/Chathams	F, Adult?	SAT	Massey	25/01/2005	NY
18	*Porrhothele*	Dunedin (-45.865, 170.504)	-45.865, 170.504	Otago Coast/Dunedin	Juv	J.Tweed	Massey	?	NN
19	*Porrhothele*	Macraes Flat, Otago	-45.384, 170.423	Lamerlaw/Macraes	F, Adult	J. Tweed	Massey	?	YY
23	*Porrhothele*	Talbot Scenic Reserve, Canterbury	-44.084452, 171.238645	Pareora/Geraldine	?	S.A.Trewick	Massey	27/02/2009	NN
56	*Porrhothele*	NW Nelson, Gordons Pyramid	-41.20, 172.68	North-West Nelson/Arthur	Juv	A. Tennyson	Te Papa	16/04/2003	NN
96	*Porrhothele*	Cobb Valley, Takaka	-41.12, 172.60	North-West Nelson/Wangapeka	F, Adult	B.N. McQuillan	Massey	1/12/2019	YY
74	*Porrhothele*	Cave site, Fossil Point, Farewell Spit	-40.51, 172.84	North-West Nelson/ West Whanganui	Juv	SAT/MMR	Massey	30/11/2019	YN
75	*Porrhothele*	Cave site, Fossil Point, Farewell Spit	-40.51, 172.84	North-West Nelson/ West Whanganui	Juv	SAT/MMR	Massey	30/11/2019	YN
76	*Porrhothele*	Whararikí Beach, Cape Farewell	-40.50, 172.67	North-West Nelson/ West Whanganui	F, Adult?	B.N. McQuillan	Massey	29/11/2019	YY
77	*Porrhothele*	Rimu Gulley, Badlands, Cape Farewell	-40.51, 172.71	North-West Nelson/ West Whanganui	F, Adult?	B.N. McQuillan	Massey	1/12/2019	YY
78	*Porrhothele*	Cave site, Fossil Point, Farewell Spit	-40.51, 172.84	North-West Nelson/ West Whanganui	F, Adult	SAT/MMR	Massey	30/11/2019	YY
79	*Porrhothele*	Cave site, Fossil Point, Farewell Spit	-40.51, 172.84	North-West Nelson/ West Whanganui	F, Adult	SAT/MMR	Massey	30/11/2019	NY
81	*Porrhothele*	Atene Skywalk, Whanganui	-39.723245, 175.136854	Taranaki/Matemateaonga	Juv	S. Thompson	Massey	2/01/2020	YN
91	*Porrhothele*	Fern Walk, Pohangina Valley, Manawatu	-40.148315, 175.844960	Rangitikei/Rangitikei	Juv	S.Thompson	Massey	2/02/2020	YN
101	*Porrhothele*	Hakarimata Reserve	-37.66, 175.13	Tainui/Raglan	F, Adult	B.N. McQuillan	Massey	18/05/2019	NY
102	*Porrhothele*	Dansey Reserve	-38.08, 176.11	Northern Volcanic Plateau/Rotorua	Juv	B.N. McQuillan	Massey	4/10/2018	YN

54	*Porrhothele(?)*	Hinewai Reserve, Christchurch	-43.81, 173.03	Banks/Akaroa	F, Adult	P.J. Sirvid	Te Papa	27/08/1996	NY
2	*Hexathele*	Waikawa Beach	-40.687896, 175.145217	Manawatu/Foxton	Juv	S. Thompson	Massey	13/12/2018	YN
8	*Hexathele*	Totara Reserve, Pohangina	-40.121694, 175.853887	Rangitikei/Rangitikei	F, Adult	S. Thompson Z. Quested	Massey	5/05/2019	YY
92	*Hexathele*	Maharahara Peak Track, Pohangina Valley, Manawatu	-40.159, 175.893	Rangitikei/Rangitikei	F, Adult	Z.Quested	Massey	2/02/2020	NY
10	*Hexathele*	Linton Reserve	-40.415966, 175.596859	Manawatu/Manawatu Plains	Juv	S. Thompson	Massey	6/06/2019	NN
11	*Hexathele*	Linton Reserve	-40.415966, 175.596859	Manawatu/Manawatu Plains	Juv	S. Thompson	Massey	6/06/2019	NN
12	*Hexathele*	Linton Reserve	-40.415966, 175.596859	Manawatu/Manawatu Plains	Juv	S. Thompson	Massey	8/06/2019	NN
13	*Hexathele*	Linton Reserve	-40.415966, 175.596859	Manawatu/Manawatu Plains	F, Adult	S. Thompson	Massey	6/06/2019	NY
14	*Hexathele*	Linton Reserve	-40.415966, 175.596859	Manawatu/Manawatu Plains	Juv	S. Thompson	Massey	6/06/2019	NN
17	*Hexathele*	Sledge Track	-40.487450, 175.628410	Manawatu Gorge/Manawatu Gorge South	F, Adult	S. Thompson Z. Quested	Massey	27/06/2019	NY
21	*Hexathele*	NO.1 Line Track	-40.193668, 175.882450	Manawatu Gorge/Manawatu Gorge North	F, Adult	S. Thompson	Massey	18/08/2019	NY
22	*Hexathele*	Sledge Track	-40.471815, 175.615192	Manawatu Gorge/Manawatu Gorge South	F, Adult	S. Thompson	Massey	22/06/2019	NY
28	*Porrhothele*	Te Mata Peak (-396986,1769002)	-39.698644, 176.900239	Eastern Hawkes Bay/Eastern Hawkes Bay	Juv	M. Lusk	Massey	3/13/19	YY
44	*Hexathele*	Mt Victoria, Wellington	-41.29, 174.79	Sounds-Wellington/Wellington	F, Adult	M. Murray	Te Papa	16-Apr-02	NY
90	*Hexathele*	Belmont Regional Park, Wellington	-41.159969, 174.968988	Sounds-Wellington/Wellington	Juv	S.Thompson	Massey	1/02/2020	NN
48	*Hexathele*	Ihumoana, Auckland	-36.890, 174.439	Auckland/Waitakere	F, Adult	A. Tennyson	Te Papa	5-Jul-95	NY

55	*Hexathele*	Mokoia island, Rotorua	-38.0797, 176.287	Northern Volcanic Plateau/Rotorua	F, Adult	B.M. Fitzgerald	Te Papa	7-Feb-00	NY
93	*Hexathele*	Dansey Rd Scenic Reserve	-38.08, 176.12	Northern Volcanic Plateau/Rotorua	F, Adult	B.N. McQuillan	Massey	10/10/2018	NY
57	*Hexathele*	Mayor Island	-37.288, 176.254	Coromandel/Mayor	F, Adult	B.M. Fitzgerald	Te Papa	24-28 February 2003	NY
59	*Hexathele*	Mayor Island	-37.288, 176.254	Coromandel/Mayor	Juv	C.A. McGuinness	Te Papa	10-13 December 2001	NN
63	*Hexathele*	Wairapas, Te Awaiti	-41.470349, 175.524968	Eastern Wairapa/Eastern Wairapa	F, Adult	SAT/MMR	Massey	8/11/2019	YY
60	*Hexathele*	Hen Island	-35.89, 174.73	Eastern Northland/Eastern Northland and Islands	F, Adult	B.M. Fitzgerald	Te Papa	19-22 October 2001	NY
85	*Hexathele*	Kerikeri fairy pools, Northland	-35.218, 173.947	Eastern Northland/Eastern Northland and Islands	F, Adult	SAT/MMR	Massey	25/01/2020	YY
86	*Hexathele*	Kerikeri River Walk, Northland	-35.215, 173.960	Eastern Northland/Eastern Northland and Islands	F, Adult	SAT/MMR	Massey	26/01/2020	NY
97	*Hexathele*	Karioi Lodge, Raglan	-37.83, 174.80	Tainui/Kawhia	F, Adult	B.N. McQuillan	Massey	?	YY
98	*Hexathele*	Karioi Lodge, Raglan	-37.83, 174.80	Tainui/Kawhia	F, Adult	B.N. McQuillan	Massey	?	YY
99	*Hexathele*	Te Mata Peak	-39.69, 176.90	Eastern Hawkes Bay/Eastern Hawkes Bay	F, Adult	B.N. McQuillan	Massey	19/04/2019	NY
100	*Hexathele*	Te Mata Peak	-39.69, 176.90	Eastern Hawkes Bay/Eastern Hawkes Bay	M, Adult	B.N. McQuillan	Massey	19/04/2019	NN
24	*?*	Te Kaha, Bay of Plenty	-37.74, 177.67	Raukumara/Motu	?	S.A.Trewick	Massey	12-Apr-09	NN
43	*Stanwellia*	Korapuki, Mercury Island	-36.65, 175.84	Coromandel/Mercury Islands	Juv	C.I Green	Te Papa	6/12/1995	NN
50	*Stanwellia*	Mercury Island	-36.65, 175.84	Coromandel/Mercury Islands	F, Adult?	I.A.E. Atkinson	Te Papa	9-Dec-95	NN
53	*Cantuaria*	Aro Valley, Wellington	-41.29, 174.76	Sounds-Wellington/Wellington	M, Adult	R. Dwyer	Te Papa	Apr-02	NN

84	*Stanwellia*	Te Paki stream Road, Northland	-34.511, 172.789	Eastern Northland/Eastern Northland and Islands	F, Adult?	SAT/MMR	Massey	24/01/2020	NN
87	*Stanwellia*	Kerikeri River Walk, Northland	-35.215, 173.960	Eastern Northland/Eastern Northland and Islands	F, Adult?	SAT/MMR	Massey	26/01/2020	NN
88	*Stanwellia*	Lake Ngakeketa, Te Paki Stream Road, Northland	-34.520, 172.773	Eastern Northland/Eastern Northland and Islands	F, Adult?	SAT/MMR	Massey	24/01/2020	NN
89	*Stanwellia*	Kerikeri, Northland	-35.215, 173.960	Eastern Northland/Eastern Northland and Islands	F, Adult?	SAT/MMR	Massey	25/01/2020	NN

Results

A total of 108 Mygalomorph spiders were collected in New Zealand. Of these, 73 specimens were *Porrhothele*, 27 were *Hexathele*, 6 were *Stanwellia* and one was *Cantuaria*. Of the *Porrhothele* specimens, 36 were adult females (Table 3)

Two DNA sequence alignments were made, the first alignment used representatives of the five genera of New Zealand's Mygalomorphae. The alignment was 610 bp to 700bp and contained 19 sequences. The alignment contained a total of 246 variable sites. The GC-content for the alignment was 35.9% which appears to be within the typical range of GC-content for Mygalomorphs (Hamilton et al, 2014; Sanggaard et al, 2014). Once the reading frame was adjusted, none of the sequences had stop codons.

The second DNA alignment was of the *Porrhothele* genus. The amplified DNA fragment was about 700 bp long, but when trimmed each DNA sequence was between 610 and 650 bp long. The *Porrhothele* DNA alignment contained 73 sequences of *Porrhothele* with one sequence of *Migas* sp. as an outgroup and had 207 variable positions. The GC-content for the alignment was 37.6%, which appears to be within the typical range of GC-content for Mygalomorphs (Hamilton et al, 2014; Sanggaard et al, 2014). Once the reading frame was adjusted, none of the sequences had stop codons.

Phylogenetic analysis

Analysis of New Zealand's five genera of Mygalomorphae DNA sequence dataset supported the current classification of New Zealand Mygalomorphae spiders into five genera. Each genus was represented by between one and five individuals in my dataset. Phylogenetic hypotheses of the same topology were produced from the Bayesian estimation of phylogeny and maximum likelihood phylogeny of New Zealand's Mygalomorphae genera. The posterior probabilities and bootstrap values show moderate-strong support for the monophyly of *Stanwellia* and *Cantuaria*, and weak support for the monophyly of *Hexathele* and *Porrhothele* (Fig. 2). The only representative of *Migas* is sister to *Porrhothele*. The resulting phylogenetic tree had different

topology reported elsewhere . (Figure 1) and contained a polytomy between *Stanwellia*, *Hexathele* and the *Porrhothele*/*Migas* cluster due to a lack of phylogenetic signal.

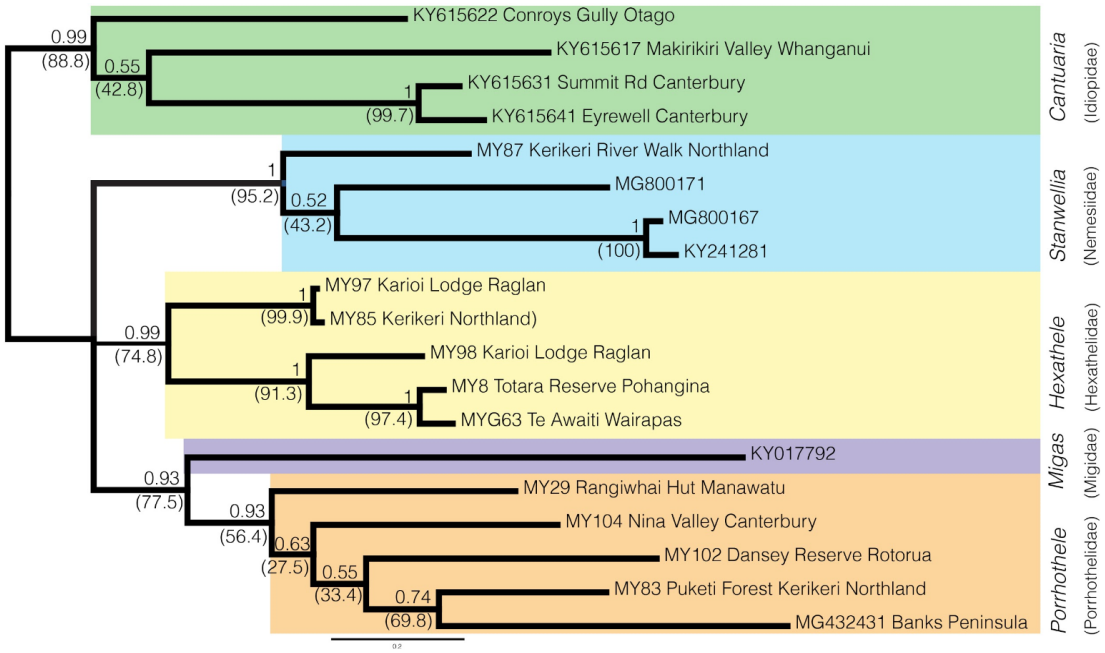

Figure 3. Evolutionary relations of New Zealand's five genera of Mygalomorphae spiders estimate with Bayesian phylogenetics from mtDNA sequences with posterior probability values. Maximum likelihood bootstrap proportion values are in brackets.

Population genetics

Fourty one *Porrhothele* individuals were sequenced for this study which generated 33 unique mtDNA haplotype sequences. I aligned these new sequences with publicly available data from *Porrhothele* from Banks Peninsula to create a dataset with 74 sequences. These CO1 sequences differed from one another by a maximum of 25.9%. Geographic structure is observed in the phylogenetic tree of *Porrhothele* using specimens from across New Zealand (Figure 4; neighbour-joining method. Fig. 5; maximum likelihood, Fig. 6; posterior probability). I identified one genetic cluster as representing the species *Porrhothele antipodiana* because almost all adult females within this sample had the three lobed receptacles that were identical in structure to the ones described in Forster & Wilton (1968). This genetic cluster identified as *Porrhothele antipodiana* includes individuals collected from throughout North Island, New Zealand, and Cape Farewell, Canterbury and Otago in South Island. All these locations are within the

previously described species range for *P. antipodiana* in Forster & Wilton, (1968). mtDNA sequences from several individuals of *Porrhothele* differ significantly from the *P. antipodiana* sequences, e.g. 0.191(19.1%), 0.195 (19.5%) and 0.2 (20%) and thus formed distinct branches (Figure 4). One sequence represents the only sampled specimen of the Northland species *Porrhothele quadrigyna* (MY83). Notably, despite being recognized as *P. antipodiana* by the previous author (MacDonald, 2013) the *Porrhothele* sequences from specimens collected from Banks Peninsula formed a genetic cluster separate by 0.221 (22.1%) from the widespread cluster I identified as *P. antipodiana*.

Table 4. Species delimitation statistics produced from the neighbour-joining phylogenetic tree

Clade	N	Closest clade	Intra distance	Inter distance-closest	Intra/inter	P ID (Strict)	P ID (Liberal)	Av (MRCA-tips)	P (Randomly Distinct)	Clade Support	Rosenberg's P(AB)
Porrhothele antipodiana	37	*Porrhothele* "Ruahine"	0.062	0.173	0.36	0.88 (0.83, 0.93)	0.97 (0.94, 0.99)	0.0466	0.56	99.90%	3.10E-23
Porrhothele "Banks Peninsula"	32	*Porrhothele quadrigyna*	0.055	0.193	0.29	0.90 (0.85, 0.95)	0.97 (0.94, 1.00)	0.0489	1	100.00%	1.89E-03
Porrhothele quadrigyna	1	*Porrhothele antipodiana*	NA	0.181	NA	NA	0.96 (0.83, 1.0)	NA	NA	NA	1.89E-03
Porrhothele "Ruahine"	1	*Porrhothele antipodiana*	NA	0.173	NA	NA	0.96 (0.83, 1.0)	NA	NA	NA	3.80E-04
Porrhothele "dwarf"	2	*Porrhothele antipodiana*	0.04	1.75E-01	0.23	0.47 (0.32, 0.63)	0.84 (0.69, 0.99)	0.02	6.20E-01	100.00%	1.00E-05

Table 5. Species delimitation statistics produced from the neighbour-joining phylogeny of clades within *Porrhothele antipodiana* and *Porrhothele* "Banks Peninsula"

Clade	N	Closest clade	Intra distance	Inter distance-closest	Intra/inter	P ID (Strict)	P ID (Liberal)	Av (MRCA-tips)	P (Randomly Distinct)	Clade Support	Rosenberg's P(AB)
P. antipodiana "East coast"	16	P. antipodiana "Wellington-Manawatu"	7.00E-03	0.065	0.11	0.95 (0.90, 1.0)	0.99 (0.96, 1.0)	0.0038	<0.05	100.00 %	4.70E-10
P. antipodiana "Wellington-Manawatu"	14	P. antipodiana "East coast"	0.046	0.065	0.7	0.72 (0.65, 0.79)	0.92 (0.87, 0.96)	0.0337	0.28	82.89%	4.70E-10
P. antipodiana "Nelson"	7	P. antipodiana "East coast"	0.04	0.091	0.44	0.72 (0.62, 0.83)	0.90 (0.84, 0.97)	0.0454	0.43	82.10%	5.30E-09
P. "Banks Peninsula" 1	12	P. "Banks Peninsula" 2	0.006	0.056	0.11	0.94 (0.88, 1.0)	0.98 (0.94, 1.0)	0.0069	<0.05	99.60%	3.10E-04
P. "Banks Peninsula" 2	3	P. "Banks Peninsula" 1	0.004	0.056	0.08	0.74 (0.57, 0.92)	0.96 (0.82, 1.0)	0.0034	<0.05	100.00 %	3.10E-04
P. "Banks Peninsula" 3	14	P. "Banks Peninsula" 1	0.01	0.077	0.12	0.94 (0.87, 1.0)	0.98 (0.94, 1.0)	0.0063	0.19	100.00 %	9.20E-10
P. "Banks Peninsula" 4	3	P. "Banks Peninsula" 3	0.002	0.086	0.02	0.78 (0.60, 0.95)	1.00 (0.85, 1.0)	0.14%	<0.05	100.00 %	1.30E-05

Figure 4. Neighbour-joining tree of *Porrhothele* mtDNA clades with consensus values. Pattern suggests a strong separation between *Porrhothele antipodiana* and *Porrhothele* "Banks Peninsula"

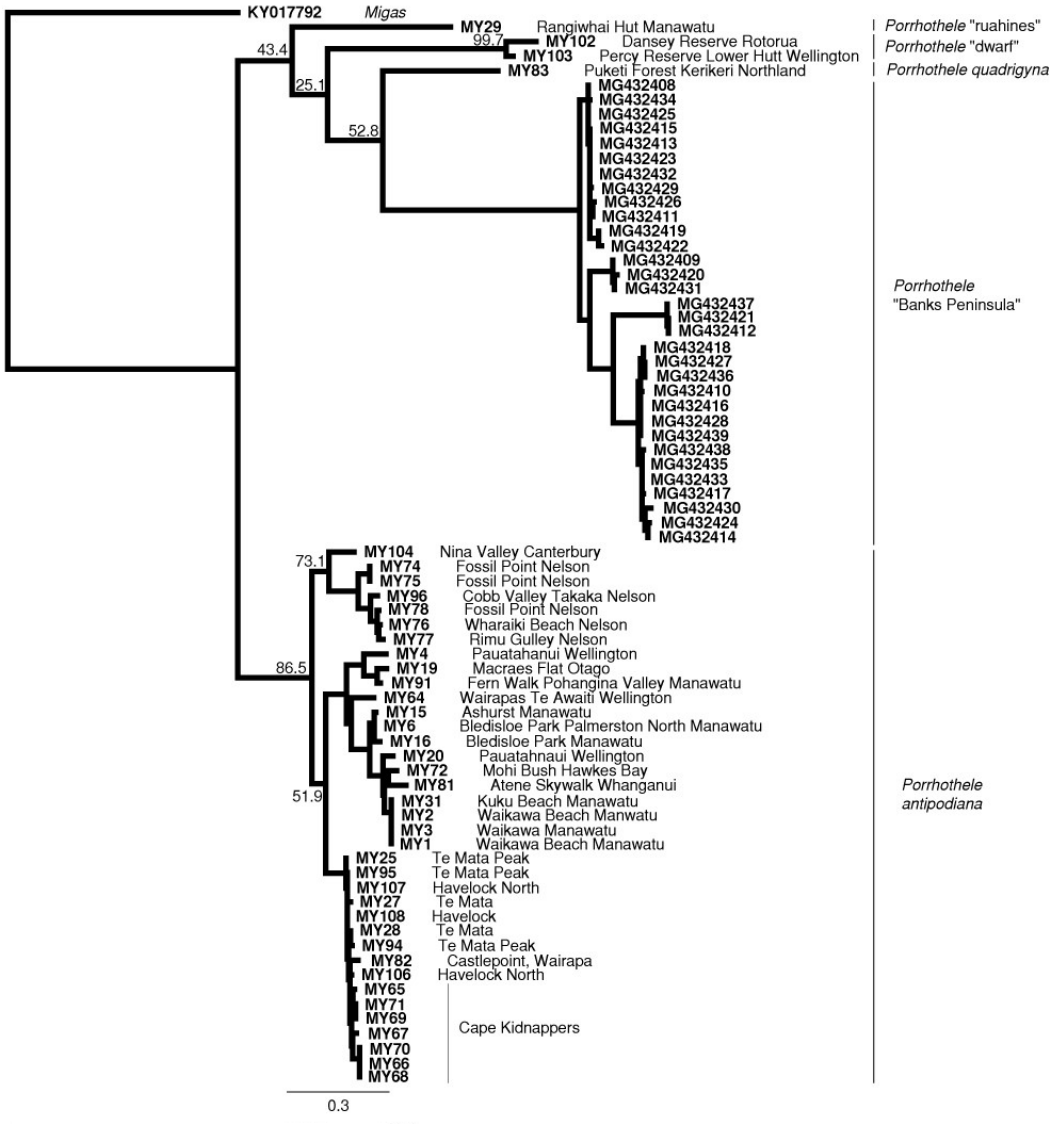

Figure 5. Maximum likelihood tree of *Porrhothele* with consensus values. Pattern suggests a strong separation of *Porrhothele antipodiana* from *Porrhothele* "Banks Peninsula"

Figure 6. Posterior probability tree of *Porrhothele* with bootstrap consensus values. Pattern suggests a strong separation of *Porrhothele antipodiana* from *Porrhothele* "Banks Peninsula"

Morphology

A total of 108 spiders were measured. From this dataset, all adult males and juveniles were removed leaving 48 adult female spiders. Several principal components of variation were successfully calculated using R studio. While the broken-stick test and the average eigenvalue rule suggest that only the first component should be considered significant, the scaled eigenvalue rule and shape of the scree plot suggest that the second component may also contain biologically meaningful variation (Fig. 7 & 8). Additionally, when PC1 and PC2 are plotted (Fig. 9), it appears that PC2 can separate *Porrhothele* specimens from most *Hexathele* specimens. Based on this evidence, PC2 is significant. All principal components beyond PC2 were found to be insignificant regardless of test used. The largest principal component (PC1) explained 82.5% of the morphological variation and can be summarized as a measure of specimen size. The second component of variation (PC2) explained 7.9% of variation and reveals a shape difference between *Porrhothele* and *Hexathele* specimens (Fig. 9). The traits contributing strongly to PC2 (and separating *Porrhothele* from *Hexathele*) were the metatarsus length, fovea position and tarsus length (Table 6). Neither principal components of variation were able to effectively separate the mtDNA lineages I had identified within *Porrhothele* (Fig. 10).

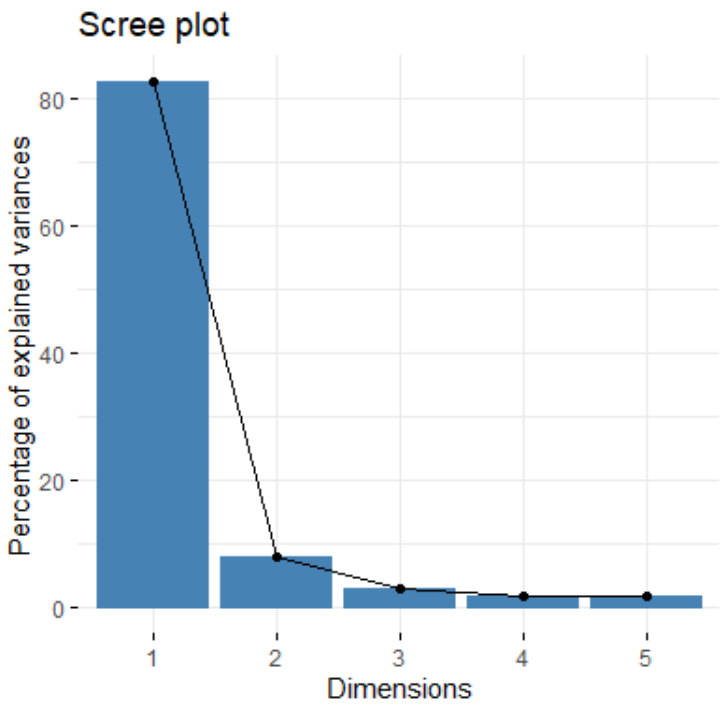

Figure 7. Scree plot of principal components. The elbow at PC2 suggests it may be informative.

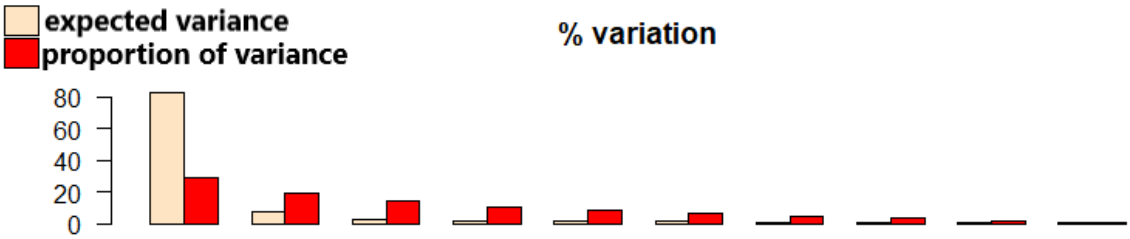

Figure 8. Broken stick test results of principal components analysis. The test suggests only PC1 is significant.

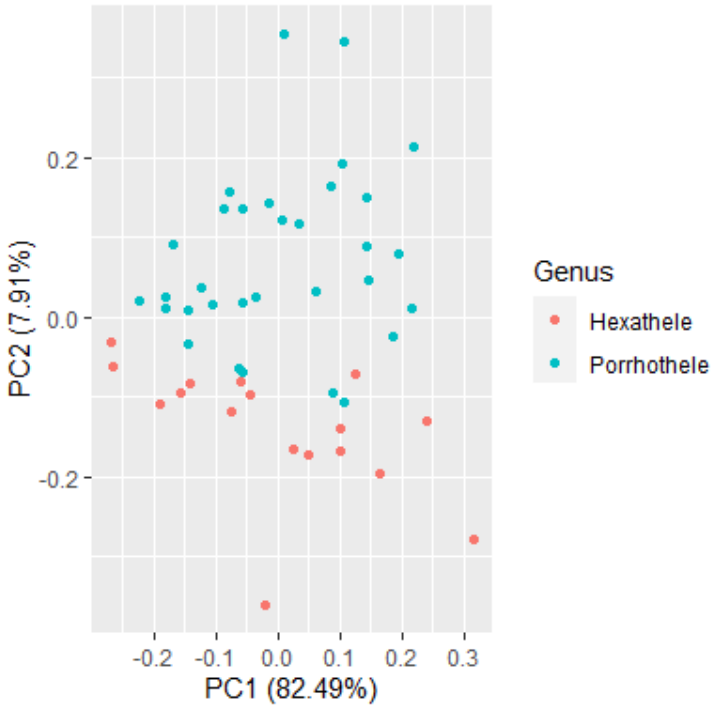

Figure 9. Principal components analysis of *Porrhothele* and *Hexathele*. PC2 appears to be able to separate *Porrhothele* from *Hexathele*

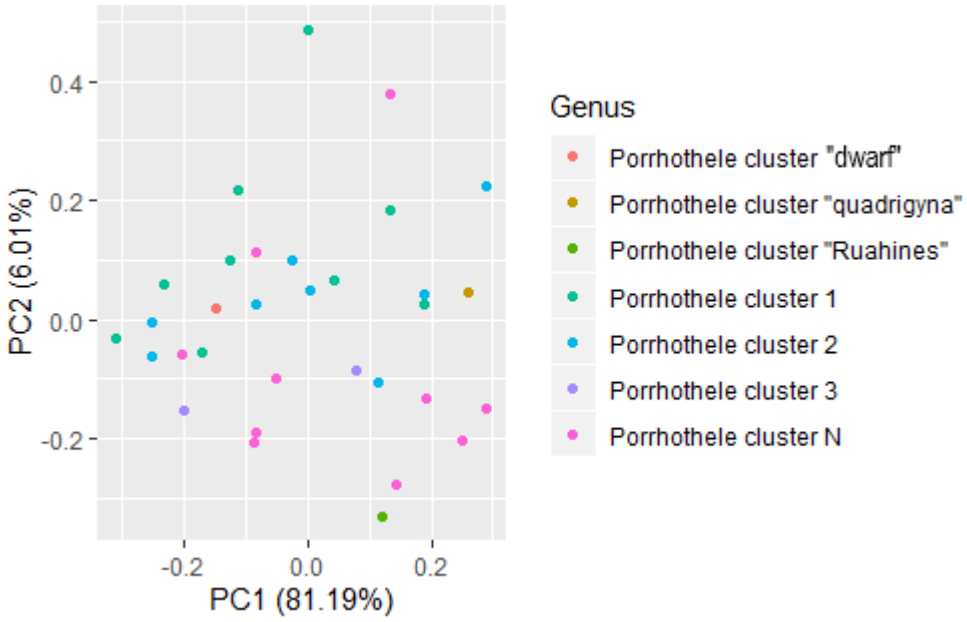

Figure 10. Principal components analysis of *Porrhothele* mtDNA clades. Neither principal component can separate any of the clusters.

Table 6. Contribution of morphological traits to each principal component. In PC2, metatarsus is the most informative trait.

Trait	PC1	PC2
Metatarsus	8.523186	23.8630100
Fovea position	9.040441	19.0598792
Tarsus	9.258428	17.0154772
Tibia	10.381095	10.7056671
Head length	10.551979	9.7878125
Eye group width	10.300295	8.2450341
Head width	10.509762	8.1980977
Femur	11.343435	2.7242299
Eye group height	9.913950	0.3482379
Patella	10.177428	0.0525544

Metatarsus, fovea position and tarsus means between *Porrhothele* and *Hexathele* were compared using T-tests. There was a significant difference in mean metatarsus length (p =0.00068), an insignificant difference in fovea means (p=0.7057) and an insignificant difference in tarsus means (p=0.1166). There was considerable overlap between genera for these traits (see boxplots; fig 11).

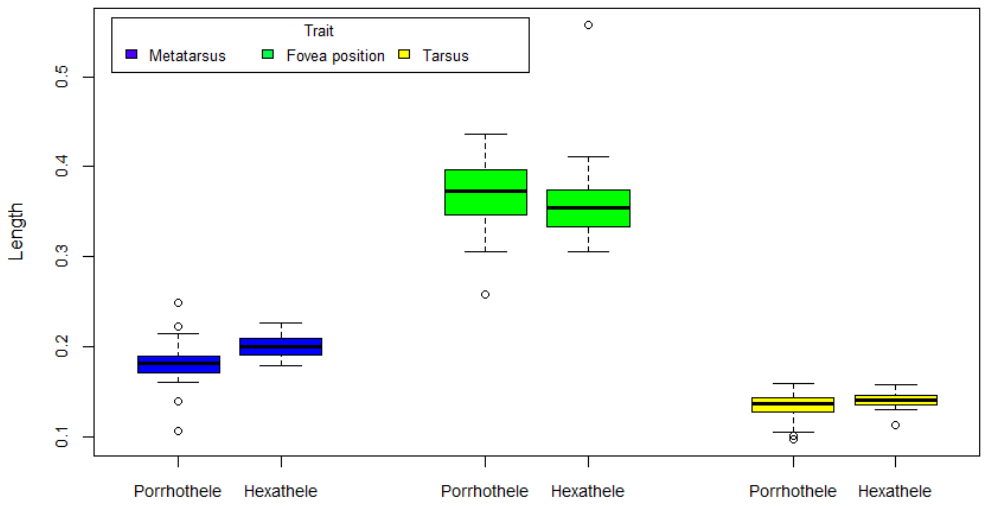

Figure 11. Boxplot of metatarsus, fovea position and tarsus measurements. All traits have significant overlap between genera and only metatarsus has a significantly different mean

Metatarsus length, fovea position and tarsus length were used in the cluster analysis (Fig. 12). The VEI (diagonal, equal shape) model was selected (Fig. 13) with a BIC value of 652.5273 and produced two clusters (Fig. 14). These two clusters did not correspond with the *Porrothele* and *Hexathele* groups, suggesting that the two groups could not be distinguished from one another. None of the mtDNA lineages were distinguished using these morphological traits. Metatarsus length and fovea position were combined to create a ratio which had differing means found to be significant using the Wilcoxon-Mann-Whitney test (p=0.004611).

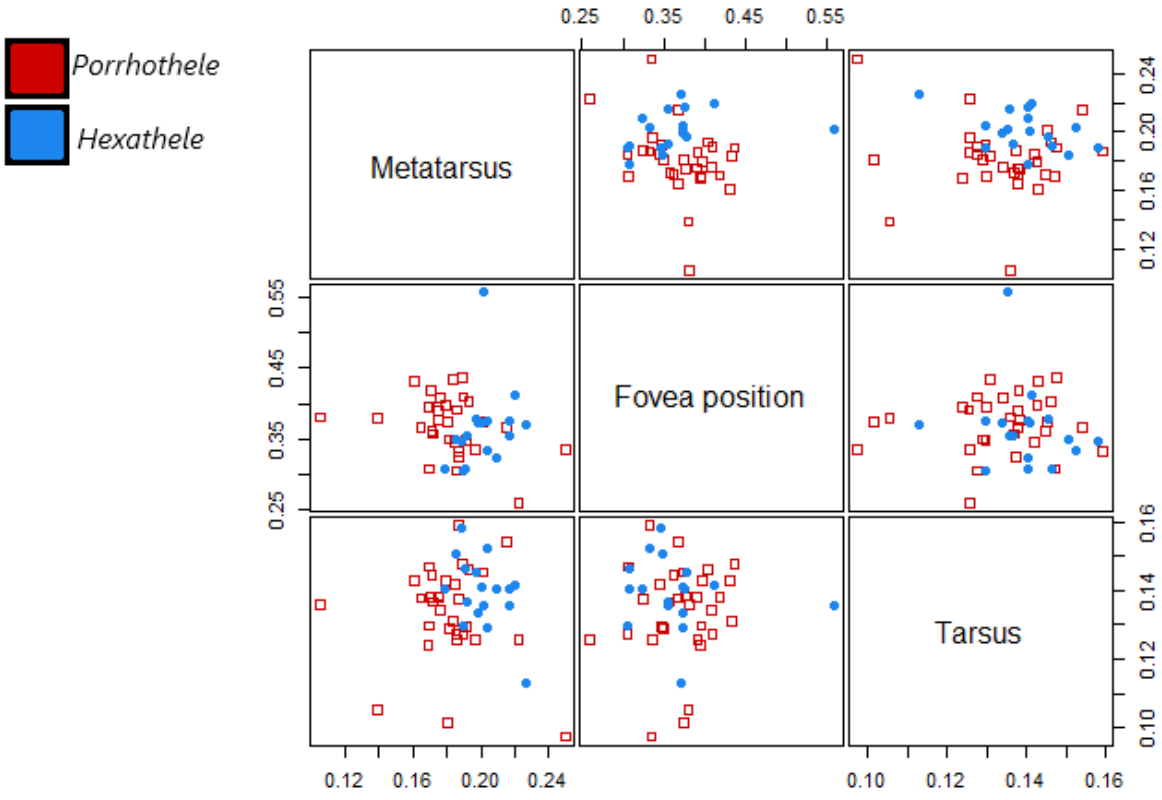

Figure 12. Cluster plot of metatarsus, fovea position and tarsus. In most groupings, the traits appear to be able to somewhat separate *Porrhothele* from *Hexathele.*

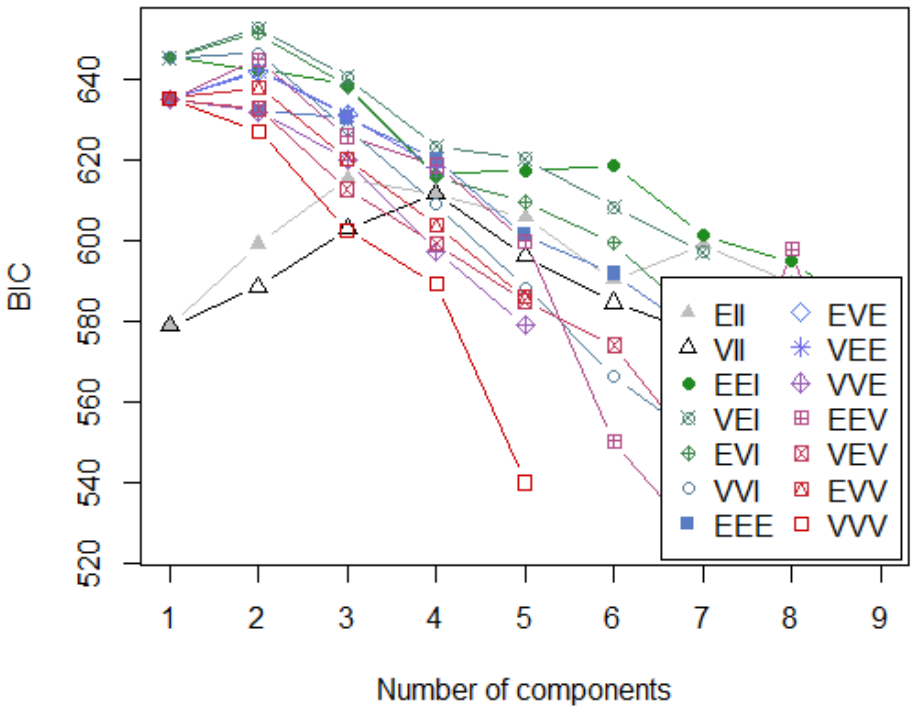

Figure 13. Plot of models for the cluster analysis. The VEI model was found to be the best choice.

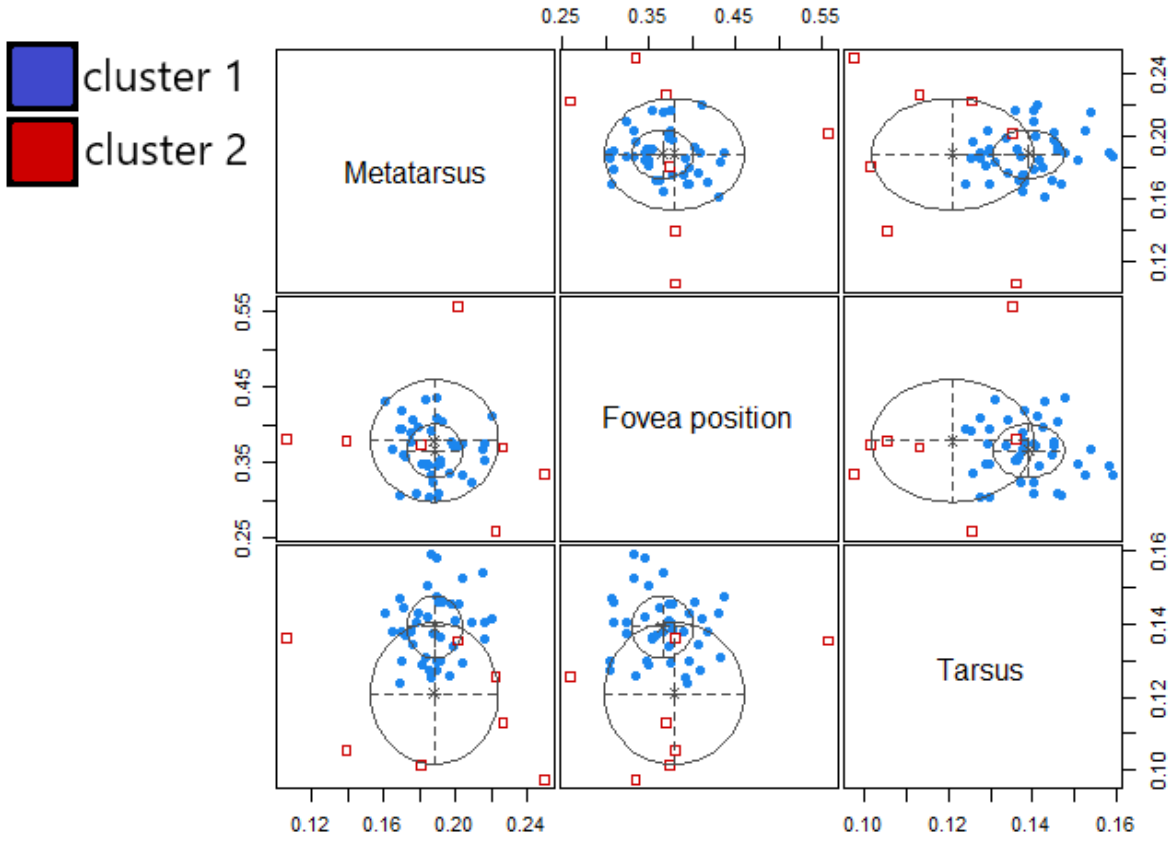

Figure 14. Unsupervised cluster analysis of metatarsus, fovea position and tarsus lengths. Two clusters were produced, but no measurements were able to separate the two genera.

A total of 21 adult female *Porrhothele* were successfully dissected (Table 7). Within the *Porrhothele antipodiana* clade, 18 of the dissected individuals had three lobed spermatheca, but there were three deviant individuals. Two of these deviants had two lobed spermatheca whereas one had three lobed and four lobed spermatheca. The single *Porrhothele quadrigyna* specimen had four lobes as described for this species (Forster & Wilton, 1968), and this was also seen in the single specimen from the new mtDNA lineage *Porrhothele* "Ruahine". The single female specimen belonging to the new mtDNA lineage *Porrhothele* "dwarf" was unusual in having five and seven lobed spermatheca. Neither *Porrhothele* "Ruahine" or *Porrhothele* "dwarf" match the descriptions and locality of previously described *Porrhothele*, so may belong to new species (Forster & Wilton, 1968).

Table 7. Reproductive lobe counts of adult female *Porrhothele*. Lobe counts are inconsistent among individuals of *Porrhothele antipodiana*.

MY	Lobe	mtDNA clade
1	3	*P. antipodiana*
3	3	*P. antipodiana*
4	3	*P. antipodiana*
5	3	*P. antipodiana*
19	3	*P. antipodiana*
20	3	*P. antipodiana*
28	3	*P. antipodiana*
29	4	*P.* "Ruahine"
31	3	*P. antipodiana*
64	3	*P. antipodiana*
65	2	*P. antipodiana*
67	2	*P. antipodiana*
70	3 & 4	*P. antipodiana*
71	3	*P. antipodiana*
78	3	*P. antipodiana*
79	3	*P. antipodiana*
83	4	*P. quadrigyna*
95	3	*P. antipodiana*
103	5 & 7	*P.* "dwarf"
106	3	*P. antipodiana*
107	3	*P. antipodiana*

Discussion

Endemic Mygalomorphae phylogenetics

A phylogenetic hypothesis including representatives of New Zealand's five endemic Mygalomorphae genera was produced using mtDNA CO1 data (Fig. 3). The phylogeny produced did not match the tree shape predicted in Figure 1. The phylogenetic tree produced suggests that the unnamed *Migas* species from Northland New Zealand (Wheeler et al. 2016) is more closely related to *Porrhothele* than any of the representatives from other genera within Mygalomorphae. A polytomy in my analysis between *Hexathele*, *Stanwellia* and the *Porrhothele*/*Migas* cluster means my data could not resolve this relationship. These results partially achieve my aim of providing a hypothesis describing how New Zealand's Mygalomorphae are related to one another but do not support previous hypotheses.

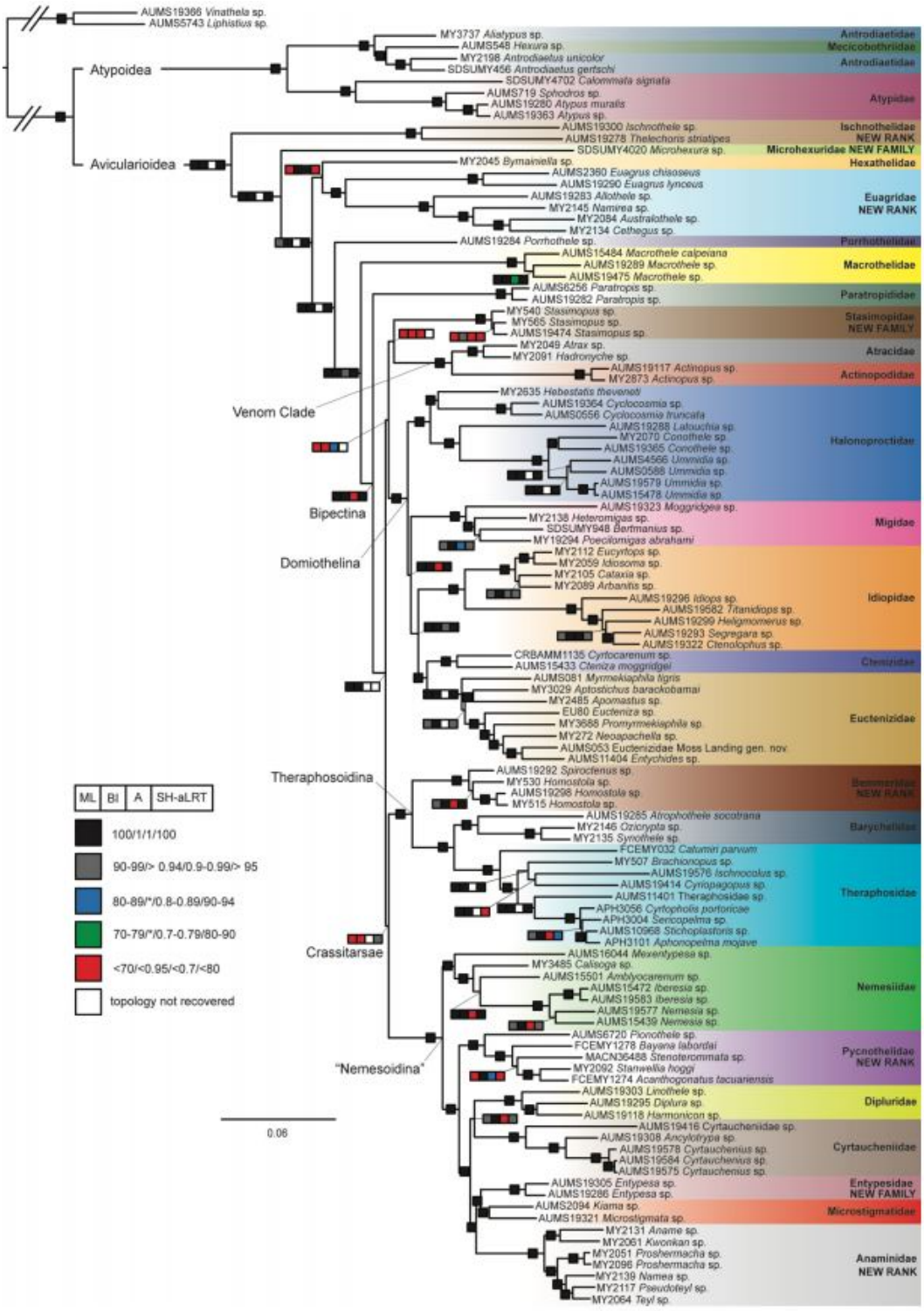

Figure 15. Figure from Opatova et al, (2019) depicting the hypothesized relationship of Mygalomorphae

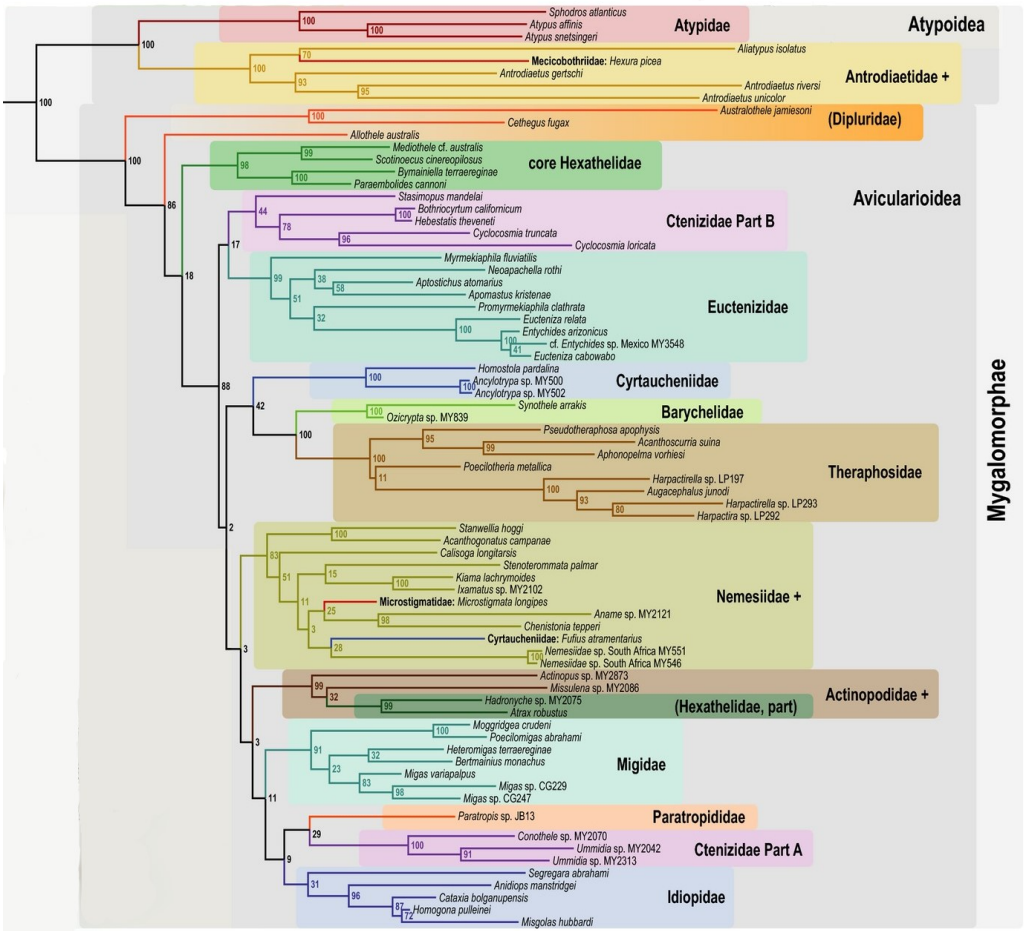

Figure 16. Figure 2 from Wheeler et al, (2016) depicting the hypothesized relationships of Mygalomorphae with minor editing

While the phylogenetic tree provided supports the monophyly of the five genera, it also conflicts with those previous hypotheses of phylogenetic relationships of Mygalomorphae seen in the wider literature as depicted in Figure 1. For instance, Opatova et al. (2019) found (Fig. 15) Migidae to be more closely related to Idiopidae than Porrhothelidae. This relationship would predict that *Migas* should be most closely related to *Cantuaria* in my analysis because these two genera belong to the Migidae and the Idiopidae ,respectively (Fig. 1), but this is not reflected in Figure 3, which suggests that *Migas* is more closely related to *Porrhothele*. However, except for *Porrhothele*, Opatova et al. (2019) used entirely different genera to represent the respective Mygalomorph families. This difference may indicate that genera are categorized in the wrong family, which may occur where traditional taxonomy is based on few morphological characters. Comparing the phylogenetic trees could therefore indicate a stable family level classification and suggest that characters used previously are similar due to convergence rather than shared ancestry. It is also worth noting that Opatova et al. (2019) used 111 specimens from 88 genera to represent all 20 families of Mygalomorphae, which is much broader sampling than conducted in

this study. Additionally, the authors extracted whole genomic DNA and were able to target and recover 472 loci. Because of this, the difference in scale may make comparison unreasonable.

Similarly, phylogenetic analysis of Mygalomorphae families (Fig. 16) by Wheeler et al. (2016) conflicts with my hypothesis (Figure 3). In this example Wheeler et al. (2016) targeted three mitochondrial and three nuclear genes, as opposed to the one mitochondrial gene targeted by my study. Like Opatova et al. (2019), Migidae appears to be most closely related to Idiopidae (of the families of interest). This (again) would predict that *Migas* will be most closely related to *Cantuaria*, but this was not resolved by my data. However, Wheeler et al. (2016) may not be a useful comparison. Firstly, as seen in Opatova et al. (2019), very few taxa are used to represent each family. This could make comparison problematic because families may be misrepresented if the genera are in the wrong family. This issue may even be visible in the phylogenetic tree because it appears that Hexathelidae has been split into two separate groups with several families between them. Secondly, the Porrhothelidae family is not represented in Wheeler et al. (2016). Obviously, this makes it impossible to compare how Porrhothelidae relates to the other families. Thirdly, many of the nodes have very low support and may be better represented as polytomies. The node that separates Pycnothelidae from the clade containing Migidae and Idiopidae has support as low as 3% whereas the node that separates "core" Hexathelidae from the clade that contains the rest of the relevant families only has support of 18%. Clearly this seems to indicate that the tree is poorly resolved and does not, therefore, conflict with my findings.

Although my phylogenetic hypothesis conflicts with previous studies on the relationships between New Zealand's Mygalomorphae genera, it does appear to support the separation of *Porrhothele* from the Hexathelidae family. Previously, *Porrhothele* was placed in the Hexathelidae alongside *Hexathele* (Forster & Wilton, 1968). However, in Hedin et al, (2018), evidence from ultra-conserved element sequences separated *Porrhothele* from the Hexathelidae family and placed into its own family, the Porrhothelidae. This taxonomic change was later supported by Opatova et al, (2019), which produced a phylogeny that clearly separates Porrhothelidae from the Hexathelidae (Fig. 15). In my results, *Porrhothele* clusters with *Migas*, which represents the Migidae family, and is separate from the *Hexathele*, which represents the Hexathelidae family (Fig. 3). This supports the separation of *Porrhothele* from the Hexathelidae family because if *Porrhothele* were to be included in the Hexathelidae, then *Migas* should also be Hexathelidae. This seems extremely unlikely due to previous molecular and morphological evidence (Wheeler et al, 2016). Therefore, it seems more likely that the separation of *Porrhothele* from Hexathelidae is justified.

Unfortunately, an issue with the use of COI for deeper level phylogenetic analyses, such as that done in this study, is that it may evolve too quickly to accurately depict the deeper structure of the tree. Because of how quickly COI evolves, the sequences are likely to have become saturated (Harrison et al, 2017). This is especially problematic because the Porrhothelidae family is hypothesized to have diverged from other Mygalomorphae in the Jurassic, which may mean the COI gene is even less well suited for this type of analysis (Opatova et al, 2019).

The phylogenetic tree produced by this study provides a hypothesis that partially resolves how New Zealand's five endemic genera of Mygalomorphae are related to one another. The phylogenetic tree produced conflicts with the wider literature in at least one component. However, comparisons between the literature and this study should be approached with caution because New Zealand's genera are absent from previous studies and because some Mygalomorph family level relationships in other studies are poorly resolved. The relationships between New Zealand's genera could be further resolved in future studies by sequencing a more varied set of genes such as ribosomal genes, and by including closely related overseas taxa.

Population genetics

The phylogenetic structure of the genetic diversity documented within *Porrhothele* indicates the presence of several distinct lineages that are separate from *Porrhothele antipodiana*. Most notably, *Porrhothele* specimens from Banks Peninsula formed a clade that is clearly separate from *P. antipodiana*. This mtDNA data suggests that the Banks Peninsula group might be a separate undescribed species. Similarly, two other previously unknown lineages are clearly separate from *P. antipodiana* and may also represent new species but are represented by only three specimens (Figure 4).

Clade support for both *Porrhothele antipodiana* and *Porrhothele* "Banks Peninsula" was very high. *P. antipodiana* had clade support of 99.9%, 86.5% and 99.9% when using the neighbour-joining, maximum likelihood and bayesian phylogenies, respectively. Similarly, *Porrhothele* "Banks Peninsula" had clade support of 100%, 100% and 97.6%. Because of this, the monophyly of these two groups is supported.

Porrhothele antipodiana was found to have a high (6.2%) level of intraspecific distance (Table 3.). Intraspecific divergence within species of Mygalomorphae have previously been observed in the range of 2 to 11%, so *P. antipodiana* appears to have a normal level of intraspecific

divergence for a Mygalomorph species (Hamilton et al, 2011; Castalanelli et al, 2014; Rix et al, 2018). This level of intraspecific divergence also appears to fit within the normal range of divergence for a New Zealand invertebrate (Trewick et al, 2011).

Previously, Mygalomorphs have been studied in DNA barcoding literature, which has produced estimates of a "barcode gap". A barcode gap is an estimated threshold of interspecific distance that could, in principle, be used to delimit species. Studies on Theraphosidae and Pycnothelidae species have suggested using barcode gaps of 5-6% using K2P/uncorrected distance (Hamilton et al, 2011; Hamilton et al, 2014; Leavitt et al, 2015). Similarly, interspecific distances of 12.7% to 17.5% have been recorded in genera of Migidae (Ferretti et al, 2019; Harvey et al, 2015). The proposed species groupings all have an inter-distance value of greater than 6%, which would appear to support the species groupings (Table 3.). However, it is worth noting that using this barcode gap would also divide *Porrhothele antipodiana* and *Porrhothele* "Banks Peninsula" even further. *P. antipodiana* for instance could be divided into three species (Figure 4), all of which have inter-clade distance values that exceed 6% whilst *Porrhothele* "Banks Peninsula" could be divided into four species (within 50km of one another), three of which exceed 6% whilst one is at 5.6% (Table 4.). However, DNA barcoding when used alone can be highly misleading due to confounding factors such as haplotype paraphyly and high error rates in groups with incomplete sampling (Trewick, 2008; Meyer & Paulay, 2005). I was unable to find any consistent morphological difference between the clades within *P. antipodiana* and as such have decided not to consider these groups separate species. As the diversity is strongly structured based on geography the variation may be explained by isolation by distance within a species, accentuated by the non-recombining nature of the mitochondrial genome. However, the diversity documented may indicate the presence of cryptic species within *P. antipodiana* and *Porrhothele* "Banks Peninsula". To verify this, there must be more sampling and sequencing using a variety of genes to see if these patterns of population genetic differentiation persist. Importantly, a morphological examination of spiders collected from Banks Peninsula should be undertaken to determine whether they can effectively be differentiated from the widespread *P. antipodiana* lineage.

Morphometrics

Using 10 morphological traits from adult female spiders and a principal components analysis I was unable to separate the mtDNA *Porrhothele* clades (Fig. 10). However, the second principal

component of variation revealed a shape difference between *Porrhothele* and *Hexathele* specimens. PC2 differences were associated with the metatarsus length, fovea position and tarsus length. Of these, only the length of metatarsus had a mean that significantly differed between the two genera. Furthermore, the cluster analysis was unable to separate the two genera from one another regardless of which trait was used. Overall, these results suggest that the mtDNA *Porrhothele* clades cannot be distinguished with simple size and shape differences. Although the morphological traits measured reveal differences among genera, they are not diagnostic for separating *Porrhothele* from *Hexathele*.

A limitation of this study is that some other morphological traits that were not used may have been more informative. The swollen tibia of male *Porrhothele* appears to be a promising a feature that has previously been used to distinguish species and thus may have been informative in morphometric analyses (Forster & Wilton, 1968). While using this feature was considered, it was not done due the difficulties in collecting enough male *Porrhothele*. The promarginal cheliceral teeth are also used for as a diagnostic character for the *Porrhothele* genus and have been used for distinguishing species of *Porrhothele* (Hedin et al, 2018; Forster & Wilton, 1968). This character may also be an informative trait to use in morphometrics and should be studied in future research.

The ineffectiveness of the selected traits may be due to the similar life histories of the mtDNA *Porrhothele* clades and of *Hexathele*. Both *Porrhothele* and *Hexathele* are burrow dwelling spiders that occur in holes in rock faces and under logs and rocks. They are also broadly distributed throughout New Zealand in a variety of habitats and are generalist predators that catch prey in an identical manner. Due to this similarity, it is likely that the two genera experience similar selective pressures. Frogs that live in identical microhabitats have the tendency to have similar morphology, which is expected due to similar selective pressures (Moen et al, 2013). Similar patterns have also been noted in scallops and river dwelling dolphins (Serb et al, 2011; Page & Cooper, 2017). The implication of this is that morphological traits which are not subjected to selective pressures may be the best traits to use for distinguishing Mygalomorphae. Further research that identifies morphological traits that are not subjected to selective pressures within *Porrhothele* and *Hexathele*, may be useful in identifying ways that mtDNA *Porrhothele* clades can be distinguished from one another and from *Hexathele*.

Female receptacles for species delimitation

Many species of Mygalomorphae are described using the shape and number of receptacles. For example, the *Raveniola* genus from India is separated from other members of the Pycnothelidae family by the presence of double or multiple receptacle lobes (Siliwal et al, 2015). Similarly, species among the *Ischnocolus* genus can be distinguished by the shape of the receptacles (Guadanucci & Wendt, 2014). Similarly, receptacle lobe number and structure has been used as descriptive features in a range of other Mygalomorph taxa (Gonzalez-Filho et al, 2012; Gargiulo et al, 2018; Perafan & Perez-Miles, 2010). This would appear to indicate that receptacles are a valuable morphological feature for delimiting species and genera. However, I have observed that within *Porrhothele antipodiana* there is some variation in shape and number of receptacle lobes (Table 7). In previous literature, the receptacles have been described as having only three lobes with some minor variation in the position of the medial lobe (Forster & Wilton, 1968). But what I have observed is that while the majority of specimens (15 of the 18 *P. antipodiana*) conform to this, there are some exceptions where the number of lobes may be two or four and where there may be considerable differences in shape of the lobes (Table 7). Another unusual feature with the receptacle lobes was observed in a single *Porrhothele* specimen. This specimen was unique in the data set because it had five lobed receptacles on one side of its body and seven lobed receptacles on the other side. Mismatched lobe numbers in *Porrhothele* have previously been observed in Court (1984), where lobe numbers of 5/4, 7/6 were observed in specimens collected from Poor Knights Islands. This provides evidence that variation among lobe numbers may even exist between pairs of receptacle lobe bunches.

The inconsistency in number and shape of receptacle lobes in *Porrhothele antipodiana* could be explained by continuous moulting once the female is fully matured (D.J. Court, personal communication, 2020). An unusual feature of the Mygalomorphae is that adults (or at least the adult females) will continue to molt and grow once they have matured (Costa & Perez-Miles, 2002; Herzig, 2010; Stewart & Martin, 1982). During moulting, the entire layer of cuticle surrounding the body is shed and replaced (Forster & Forster, 2005). In all spiders, the receptacles are layered with cuticle (Michalik et al, 2005), which is presumably shed with the rest of the cuticle. Deformities in the reproductive system produced from ecdysis have previously been recorded (Kaston, 1963), so it seems reasonable to predict that the receptacles of a female *P. antipodiana* could have their shape altered during molting. The implication of this is that Mygalomorphs may have greater variability in shape of receptacles when compared to other groups of spiders which do not moult after reaching maturity. This would also predict that the Liphistiidae, which appear to moult during adulthood, would also have greater variability in

shape of the receptacles (Platnick & Sedgwick, 1984). The implication of this is that receptacles may be less reliable for species delimitation than previously thought. Additionally, this hypothesis also predicts that young adult *P. antipodiana* should have less variable receptacle shapes whereas older adults will have greater variability in shape of the receptacles. However, this explanation has two flaws. Firstly, while a change in shape of the receptacles seems plausible, it seems less likely that this hypothetical process could generate additional lobes. Secondly, this process is speculative and would require further studies to prove.

In Forster & Wilton (1968), adult females of *Porrhothele antipodiana* are described as having trilobed genitalia with some minor variation in the positioning of these lobes. Due to the presence of *P. antipodiana* adult females with two lobed and four lobed genitalia found in this study, it appears likely that individuals *P. antipodiana* have been assigned to incorrect species. Because of this, *P. antipodiana* may need a taxonomic revision and needs to use different diagnostics traits.

Conclusion

This thesis has attempted to describe the phylogenetic relationships of New Zealand's native Mygalomorphae, and to reveal the phylogenetic structure of the Porrhothele genus, with a focus of solving whether *Porrhothele antipodiana* represents multiple species. Using the COI gene, a phylogenetic tree hypothesis was produced which partially resolved the relationship of New Zealand's Mygalomorphae and did appear to be consistent with previous literature. However, due to the saturation of the COI gene, the phylogenetic tree produced is likely to be unreliable. Further research using genes with lower substitution rates would be beneficial in resolving this problem. The phylogenetic tree of the *Porrhothele* genus revealed the presence of several new lineages, which may represent new species. One of the species was previously thought to be *P. antipodiana,* so this seems to indicate that the *P. antipodiana* species may indeed represent multiple species. The limitation of this result is that only the COI was used and only a limit geographical range was sampled. In future research, the usage of additional genes (particularly nuclear ones) and greater sampling throughout New Zealand could prove or disprove the findings of this study.

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