THE EVOLUTION AND DIVERSITY OF
PARASITES OF NEW ZEALAND LIZARDS

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DEDICATION

To my wonderful friends and family who have supported me throughout my university journey
ABSTRACT

Co-phylogenetic studies have been an indispensable tool to unravel the processes and patterns of evolution that occur between parasites and their hosts. Ecto-parasites, so far, have been the main subjects of these studies. However, in order to develop more general rules of evolution, we must study a broader range of parasitic associations. The present study focused on parasitic nematodes, a group that has received relatively little attention from a co-phylogenetic perspective. The aim of the study was to investigate the evolutionary history between New Zealand lizards and their nematode parasites in order to test the following hypothesis: host-nematode associations, in which the parasites have limited dispersal, will follow a mostly co-evolutionary pattern. This research also offered the opportunity to advance our knowledge of NZ reptile parasites by assessing the genetic diversity and phylogenetic relationships of parasitic nematodes, and by conducting a survey of mites parasitic on lizards.

Nematodes for this study were collected via searching faecal pellets released by wild lizards and recovering any expelled nematodes. The phylogenetic relationships between the nematodes were explored using three genetic markers: two nuclear (18S rRNA, 28S rRNA) and one mitochondrial (cytochrome oxidase subunit I: COI). Two algorithms, MrBayes and Maximum Likelihood, were used to build gene trees, and between-clade genetic distances were calculated for all markers. Finally, to explore the evolutionary history between these hosts and their parasitic nematodes, the co-evolutionary analysis program PACo was used.

The study produced several important findings. First, nematodes from NZ skinks were found to be of the genus Spauligodon rather than Skrjabinodon to which they had previously been assigned. The results also showed that both skink and gecko nematodes contain more diversity than has been previously detected by morphological examinations. This study provides evidence for several provisionally cryptic species. In relation to the main aim, the analysis provides evidence that both co-evolutionary processes (co-speciation) and host switching events have been important in the evolution of NZ lizard nematodes. The study indicates that Farhenholz’s rule does not
apply to NZ lizard-nematode associations. However, further sampling is required before we have sufficient evidence to support or reject the main hypothesis.

Finally, a survey of parasitic mites of NZ lizards, another parasitic group that has received little attention, was conducted, and complemented by a thorough literature review of existing host and location records. Mites were obtained from lizard hosts already being handled for the collection of parasitic nematodes. The survey produced both new host and locality records, and highlighted the need for future studies focusing on these small native parasites. Overall, the results of this thesis have important implications for biodiversity conservation and for taxonomy of these little-studied parasite groups.
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CHAPTER 1: INTRODUCTION

Understanding the processes and patterns of evolution is a fundamental goal of biological sciences. This includes understanding rates of evolution, drivers of speciation and adaptation (Brooks, 1991, Page, 2003). Over the last couple of decades, co-phylogenetic studies have proven to be a particularly useful tool for increasing our knowledge of these key evolutionary concepts. As the name suggests, co-phylogenetic studies focus on the evolutionary history between symbionts (i.e. ecologically related taxa) by comparing their reconstructed phylogenies (Page, 1993). Most often, the main goal of this type of analysis is to determine the level of co-divergence (the simultaneous split of symbiont populations or species) or co-speciation (the simultaneous speciation of symbionts) that has occurred in the history of the association (Banks and Paterson, 2005; de Vienne et al., 2013). This approach, therefore, is particularly appropriate for taxa involved in intimate and persistent interactions, where the interacting taxa are likely to exert strong reciprocal selective pressures on each other, such as the associations between parasites and their hosts (Barrett, 1986). Significant co-divergence is said to have occurred when the compared phylogenies are congruent. For example, the best-known case of significant co-phylogeny is that of pocket gophers and their parasitic chewing lice (Hafner and Nadler, 1988, Hafner and Nadler, 1990, Hafner and Page, 1995), see Fig.1.1 This case displays such convincing co-divergence it has become the ‘textbook’ example.
Figure 1.1. Phylogenetic comparison between pocket gophers and their parasitic chewing lice showing significant co-speciation. Phylogenies were constructed using nucleotide sequences from Hafner et al. (1994). Lines between the taxa show current host-parasite interactions. Solid circles indicate co-speciating events. Diagram is credited to Clayton et al. (2004).

Co-phylogenetic studies, however, provide much more information than just the level of co-divergence that has occurred. These studies can also provide an insight into the origin of associations, allow us to make inferences on the evolution of traits, and determine the micro-evolutionary events that have led to present day associations (i.e. host switching versus co-speciation) (Paterson and Banks, 2001, Page, 2003).

Furthermore, when paired with ecological and geographic knowledge this tool can be useful in studies of biogeography (Ronquist, 1997), explaining diversity (Johnson and Clayton, 2004) and determining the conditions under which certain macro-evolutionary events occur. Importantly, co-phylogenetic studies also provide a means to test fundamental evolutionary theories such as Fahrenholz’s rule, which states that host and parasite phylogenies mirror each other, i.e. hosts and parasites have undergone repeated co-divergence (Klassen, 1992).
Host-parasite associations are just one of many interactions that can be used within a co-phylogenetic framework. In fact, any ecological association, including mutualism, predator-prey interactions, competition, plant-herbivore interactions, etc., can be examined using these methods (Page, 2003). Yet, parasitic interactions have been at the forefront of co-phylogenetic studies (see reviews such as de Vienne et al., 2013 for examples) despite being largely ignored by many fields including ecology and even general evolutionary biology (Poulin, 2007). So why focus on host-parasite evolution? Co-phylogenetic studies can help understand many of the questions surrounding parasites that parasitologists strive to answer. This includes: why are some parasites more host-specific than others (Poulin, 2007), under which circumstances do host switches occur (Cooper et al., 2012), and do parasites evolve more slowly than their hosts? (Page, 2003). Answering these questions is central to understanding parasitic organisms, which are an intricate part of ecosystems (Hudson et al., 2006) influencing almost all aspects of host life history. For example, parasites can alter the ability of the host to reproduce (e.g. host castration Baudoin, 1975) influence behaviour (see Hughes, 2012) and alter survival rates (e.g. Lemaître et al., 2009).

Perhaps, though, one of the most compelling reasons to focus co-phylogenetic studies on host-parasite associations is because they are ideal candidates for testing fundamental evolutionary concepts. This is largely due to the often-tight associations they form with their hosts. For example, a major goal of co-phylogenetic studies is to understand the evolution of adaptations, and this is more discernible in host-parasite associations than in free-living species for two main reasons (Paterson and Banks, 2001). First, the evolution of a species is influenced by the interactions it has with the environment and the genomes it encounters. The tighter the association with one genome, the more likely they are to co-evolve, that is, in the narrowest sense, to employ reciprocal adaptations (i.e. The Red Queen arms race hypothesis) and have a shared evolutionary history (Barrett, 1986). Parasites, due to the nature of their lifestyle, mainly interact and are often tightly associated with one genome, that of the host. In contrast, free-living species interact with many genomes. For example, free-living species will regularly encounter the genomes of their predators, prey and competitors (Paterson and Banks, 2001). Thus, host-parasite associations are ripe for co-evolution.
Second, understanding adaptations requires knowledge of the ancestral environments under which traits have evolved (Paterson and Banks, 2001, Page, 2003). Reconstructing the ancestral environment for parasites is equivalent to reconstructing the ancestral host, a more tractable problem than reconstructing the entire ancestral ecosystem in which free-living species existed (Paterson and Banks, 2001, Page, 2003).

Another notable reason host-parasite associations are interesting under a co-phylogenetic framework relates to the fact that parasites are the cause of many human and agricultural diseases that we wish to prevent and/or cure. For example, parasitic nematodes alone are estimated to cause 118 billion dollars of damage annually to world agriculture (Atkinson et al., 2012). Emerging infectious diseases are also a serious concern of the twenty first century with human activities, including globalization, introduction of organisms to new habitats and farming practices increasing the risk of epidemics (Daszak et al., 2001, Anderson et al., 2004). It is, therefore, in our interest to understand how parasites evolve with their hosts and under which circumstances they jump to a new host (host switching). Indeed, in recent times, there has been a renewal of interest in these types of studies due to this need to better understand diseases and the factors that lead to emergent infectious diseases in order to develop effective control measures (de Vienne et al., 2013).

### 1.1 A HISTORICAL PERSPECTIVE

Host-parasite evolution has long intrigued biologists. The first mention of what is now often referred to as co-evolution, which in the broadest sense describes the pattern of congruent symbiont phylogenies, came from the father of evolution himself, Charles Darwin (Hoberg et al., 1997). In a letter to Henry Denny in 1844, Darwin wrote:

“I am much obliged for your note and have been greatly interested by the facts you mention of identical parasites on the same species of birds at immensely remote stations…what an interesting comparison would be the comparison of the parasites of the closely allied and representative birds of the two countries”
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Darwin realised that the tight associations between hosts and parasites could lead to a shared evolutionary history. However, it was not until several decades later that the evolutionary associations between hosts and parasites were included in formal scientific studies. The earliest researcher to do so was von Ihering (1891, 1902), even though he was not trained as a parasitologist. Rather, von Ihering was interested in former land connections and how biological data could support these connections in the absence of sufficient geological evidence (Klassen, 1992). His understanding of three important concepts, speciation by isolation, relative age of lineages and the host-specific nature of some parasites, led him to recognise that parasites were important biogeographical tools (Klassen, 1992). Subsequently, von Ihering used a group of flatworms that parasitise freshwater crayfish in both New Zealand and South America as one piece of evidence of a former land connection between the two countries (von Ihering, 1891). Von Ihering is recognised as the first researcher to use parasites as biogeographic indicators (Klassen, 1992).

Soon after von Ihering’s pioneering work, the field of host-parasite co-evolution began to rapidly develop. The fast progression of the field in these early years can be attributed to a number of key researchers, such as Kellogg (1896a, 1896b) Fahrenholz (1909), Harrison (1914), Metcalf (1920, 1929), Manter (1940), Szidat (1939) and Eichler (1941). Kellogg and Fahrenholz were the earliest of these biologists and inspired much of the research that was to follow. Despite being seemingly unaware of each other’s work in the earlier years and of von Ihering’s (1891, 1902) work, both of these biologists established co-evolutionary research programs around the same time. As with many of these earlier researchers concerned with host-parasite evolution, both focused on parasitic lice. Independently, these two researchers also came to similar conclusions regarding the causality of host-parasite associations.

Initiated by Kellogg’s and Fahrenholz’s early works, several hypotheses emerged, all important to the field’s development and our current understanding of host-parasite associations. The first came from Kellogg’s view that parasite speciation and variable specificity were a product of straggling. That is, parasites of the same species that existed on geographically distant hosts had not yet diverged as the host had. Kellogg, therefore, predicted that phylogenetic relatedness was more important in parasite
speciation than ecological interactions and adaptation (Hoberg et al., 1997). Manter (1940, 1966) later expanded Kellogg’s idea going on to suggest that parasite speciation lagged behind the host group and specificity was correlated with the duration of an association (Hoberg et al., 1997). The other hypotheses emerge from Fahrenholz’s ‘school of thought’. Unlike Kellogg, Fahrenholz placed more importance on the parasite’s adaptation to its host, reasoning that like free-living species that speciate in response to environmental changes, parasites speciate in response to host differences (i.e. the host is the parasite’s environment). Therefore, “…on hosts of the same species you will find parasites of the same species, and on hosts of different species the parasites will diverge from one another to the same degree that their hosts are related” (Fahrenholz, 1913). This work largely influenced Eichler (1942) who took Fahrenholz’s concept and narrowed it so that co-speciation was the only mechanism in parasite evolution. Eichler considered host specificity a precursor to co-evolution (Klassen, 1992).

In an attempt to summarise the current ideas on host-parasite evolution, Eichler (1942) developed these concepts into a set of ‘parasitological rules’, each attributed to who Eichler considered to be the originator of the idea (rules are as summarized by Hoberg et al., 1997):

1. **Fahrenholz’s Rule:** This rule states that parasite phylogeny mirrors the host phylogeny.

2. **Szidat’s Rule:** This rule states that the more primitive the host, the more primitive the parasites it harbours.

3. **Eichler’s Rule:** This rule states that diverse host groups will harbour greater numbers of parasites than less diverse taxa.

4. **Manter’s Rule:** This rule states that (a) parasites evolve more slowly than their hosts, and (b) the longer the association within a particular host group, the greater the specificity exhibited by the parasite.
The significance of these rules was that they provided a conceptual framework in which ideas of host-parasite evolution could be tested. However, at times these concepts have been treated as laws rather than hypotheses. This has led to assumptions that have persisted throughout co-evolutionary studies. Take for example the widespread belief that the parasites’ phylogeny mirrors that of the hosts (Page, 2003). This assumption has so deeply rooted itself within the field that it still forms the null hypothesis of co-phylogeny studies today (Paterson and Banks, 2001). This belief emerges from acceptance of Farhenholz’s rule in absence of sufficient evidence, which led to the reasoning that the host phylogeny could be used to deduce the parasite phylogeny and vice versa. Thus, co-evolution appeared to be the most common path to host-parasite associations (de Vienne et al., 2013). In more recent years the error of this assumption has been recognised. Phylogenies are now constructed independently of each other. This has become increasingly easier with the development of molecular techniques and sophisticated computer software, which has also increased their reliability (Page and Holmes, 1998). As a result, many host-parasite phylogenies have become available, allowing analysis of evolutionary trends and the parasitological rules to which they pertain.

1.2 Concepts of Co-Phylogeny

So what are the events and processes that produce congruent and incongruent phylogenies? As mentioned earlier, widespread co-speciation (shown in Fig.1.2) is the macro-evolutionary event that is largely responsible for phylogenetic congruence. If co-speciation were the only macro-evolutionary event to occur during the length of an association, then the topologies of the compared phylogenies would be the exact mirror image of each other, i.e. Farhenholz’s rule (Fig.1.2). In this case each extant parasite has come to be associated with the host via descent, i.e. the host has inherited its ancestor’s parasites (Brooks and McLennan, 1993). It is not hard to imagine how this might occur in a host-parasite association when the limited dispersal abilities of many parasites are considered. Geographical isolation of a subset of the original host population is likely to result in isolation of their parasites as well, which would eventually result in host divergence from its original population followed by the parasite (Paterson and Banks, 2001). However, it must be noted that not all congruent
phylogenies are the result of co-speciating taxa. False congruence could arise if a parasite consecutively colonises the close relatives of its host species and then speciates, by chance creating a phylogeny that mirrors that of the hosts (Brooks, 1991). This problem may somewhat be resolved if divergence times are tested, however this is not always possible and can be difficult without accurate rates of evolution.

Figure 1.2: The colour blue in these illustrations represents the host lineages and the red the parasite lineages. A. Single case of co-speciating taxa. B. Multiple co-speciating events (at each of the nodes) resulting in mirrored phylogenies as described by Farhenholz’s rule.

Perfect congruence, however, is rarely the case; even in the best examples of co-phylogeny there is almost always some degree of incongruence (Clayton et al., 2004). Incongruence between interacting host and parasite phylogenies has been attributed to four evolutionary processes (Johnson et al., 2003). First, incongruence can be produced by sorting events (also known as ‘loss events’), which is when the parasite fails to track one of the diverging hosts (Paterson and Banks, 2001, Charleston and Perkins, 2006). There are two ways in which this may occur. The first, commonly referred to as “missing the boat”, is when the host speciates via allopatric speciation but the parasite is not present in the colonising group (Fig.1.3) (Poulin, 2007). Similarly, the parasite may be present in the colonising group but subsequently goes extinct due to the absence of secondary/intermediate hosts (Charleston and Perkins, 2006). The second sorting event method is when the parasite goes extinct in one host lineage (Fig.1.3) (Johnson and Clayton, 2004).
Figure 1.3: The colour blue in these illustrations represents the host lineages and the red the parasite lineages. A. Missing the boat scenario. B. Parasite extinction in one lineage (indicated by the black line).

A second source of incongruence comes from the failure of the parasite to speciate with the host. That is, the host diverges but the parasite species occupying these now sister host species maintains genetic contact, preventing speciation in the parasite (Banks and Paterson, 2005). For failure to speciate to occur, parasite dispersal between the diverging host populations must remain possible so that parasite gene flow persists (Banks and Paterson, 2005). The opposite of failure to speciate, duplication, may also occur, representing the third process producing incongruence. Duplication is where the parasite speciates while the host does not (Fig.1.4). This can occur when the subsets of parasites develop preferences for different niches within the host, which may result from competition avoidance (Charleston and Perkins, 2006).
A fourth source of incongruence comes from an evolutionary event known as host switching, i.e. *association by colonisation* (Page, 2003). Host switching is when a parasite “jumps” to and colonizes a new host (Fig. 1.5). Host switching is typically divided into two categories: incomplete and complete. Incomplete host switching describes when the parasite jumps to and colonises a new host but does not go extinct in the host species from which it originated (Clayton et al., 2003). Complete host switching is when the parasite goes extinct in the host of origin after the switch has occurred (Clayton et al., 2003). Aside from macro-evolutionary events, several other factors may produce apparent incongruence. Incongruence may result from simply failing to detect all parasite species inhabiting a host through inadequate sampling, which using co-phylogenetic methods would be interpreted as a loss event (i.e. parasite extinction, missing the boat) (Charleston and Perkins, 2006). However, it is important to note here that loss events including failure to detect the parasites cannot be distinguished using co-phylogenetic methods (Charleston and Perkins, 2006).
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**Figure 1.5:** The blue colour represents the host lineage and the red the parasites. A. Incomplete host-switching event. B. Complete host switch where the parasites have gone extinct (indicated by the black line) in the host from which they originated.

### 1.3 **CO-SPECIATION VS. HOST SWITCHING**

When is co-speciation likely to occur? Co-speciation appears to dominate only when there is no opportunity for host switching (Barker, 1994, Clayton et al., 2004, Poulin, 2007). Take for example the model case of the pocket gophers and their chewing lice. Pocket gophers live fairly solitary lives in underground tunnels that are unshared between individuals of the same or different species (Hafner et al., 2003). Lice are usually transferred between mother and offspring (Rust, 1974). This solitary lifestyle combined with the louse’s limited dispersal ability and inability to survive for long periods of time off the host, has presented few opportunities for host switching. As a result co-speciation has played a large role in the co-evolution of these two groups (see Hafner and Nadler, 1988, Hafner et al., 1994). Here it is clear that the life histories of both the host and parasite are the driving force behind their co-evolution. Indeed, experimental studies have shown that chewing lice can colonize other species of gophers when given the opportunity (Hafner and Nadler, 1988).

In contrast to the gophers, cowbirds and the diversity of their parasitic lice wonderfully demonstrate a scenario where frequent host switching opportunities exist. These opportunities stem from the cowbird’s somewhat unusual chick rearing strategy where
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it places its eggs in the nests of other bird species, fooling them into raising cowbird offspring as their own (Hahn et al., 2000). The Brown-headed cowbird is particularly interesting as it is a brood-parasite generalist using multiple host species to rear its young within a single locality (Hahn et al., 2000). Lice require close contact to be transmitted which usually only occurs between birds of the same species. However, the cowbird nestlings are in the unusual situation where they have direct contact with members of a different species. As a result the Brown-headed cowbird nestlings have been found to harbour about as many lice species as their hosts combined (Hahn et al., 2000).

Laboratory experiments investigating host specificity have been useful to explore the importance of opportunity for host switching or alternative host use (Poulin and Keeney, 2008). In the laboratory, we can break down ecological barriers to host switching by transferring the parasite of focus to species it does not usually parasitise in the wild. Such studies have often shown that in the absence of ecological barriers, many parasites readily accepted the alternative host. For example, when Dick et al. (2009) experimentally transferred wingless bat bugs *Hesperoctenes fumarius* to a bat species, *Molossus molossus*, they do not naturally infect, the bugs not only infected this ‘new’ host but remained on it even in the presence of their natural host.

Implicit in the above examples is the role ecological factors play in the likelihood of host switching or co-speciation. Host behaviour and parasite dispersal mode are perhaps two of the most obvious factors that will limit or increase host switching opportunities. Host behaviour, such as whether the species lives in mixed species colonies or has close interactions with other species that may be suitable hosts (e.g. predator-prey associations), are likely to increase the chance of host switching. This is likely to be particularly true for parasites with limited dispersal abilities. For example, Clayton and Price (1990) showed that sharing nest holes might facilitate the transfer of a species of owl lice, *Strigiphilus*, to other species of birds, which are found to have the louse species in areas where they share nest habitat. This could occur if the nest holes, which are limiting resources, are soon inhabited by other bird species when they have been recently vacated. This also shows that parasites with limited dispersal, such as lice, will be constrained to hosts that share the same habitat as they can only survive a short
time off the host (Clayton et al., 2003). It should be noted though that species with limited dispersal abilities, although they can often only survive a short time off the host, do not necessarily need host-to-host contact to host switch. Clay (1949) and Timm (1983) suggested several ways in which lice could disperse between birds, including dispersal on detached feathers and via shared dust baths.

Of course making contact with a potential host is only one phase in a host switch and it does not necessarily mean the parasite will be able to become established (i.e. survive and reproduce on the host). Once in contact with the potential host, the parasite must be able to escape the host defences, feed and reproduce on that host. For these reasons, it has been suggested that host switches are more likely to occur between species that are closely related as host defences are likely to be similar, giving the parasite a better chance of avoiding them (Jackson, 1999, Cooper et al., 2012). However, host switches between unrelated taxa are reported often in the literature. For example, many of the emerging infectious diseases in humans can be traced back to organisms that are only distantly related; SARS, for instance, originated from a bat (see Pike et al., 2010).

Other ecological factors, such as parasite behavioural plasticity (Bush, 2009), abundance of the main host (Nieberding et al., 2010), degree of specialization (Clayton et al., 2003), competitive exclusion (Barker, 1994) and generation times of both host and parasite (Nieberding et al., 2010), are also likely to influence the chance of host-switching and co-speciation. Yet, despite the identification of factors that might promote host switching, our predictive power is still limited and conditions under which host switching occurs remain poorly understood. Further developments in this area are required, including methods using ecological factors that could reliably estimate the probability of host switching in different groups/situations.

1.4 Thesis motivation

Despite the long history of co-phylogenetic studies and the many phylogenies that are now available, our understanding of host-parasite associations in an evolutionary context is still relatively young. This can somewhat be attributed to the difficulties associated with producing reliable phylogenies and analysis methods, which have hindered the uncovering of true evolutionary patterns. However, even with the
available tools surprisingly few groups have undergone comprehensive analysis, with the majority of studies focusing on lice and other ectoparasites, especially of birds (e.g. Page et al., 2004, Weckstein and Johnson, 2004, Banks et al., 2006). The result of this is that the interactions between lice and their hosts form the basis for much of our understanding of how parasites evolve with their hosts. To develop more generalised models of host-parasite co-evolution, including general rules, as well as to understand the relative roles of factors, such as parasite life histories, we must investigate a broader range of host-parasite associations.

One such group that requires further investigation under a co-phylogenetic framework is parasitic nematodes. Nematodes are an extremely important and intricate part of the World’s ecosystems and greater attention should be directed towards these organisms and their evolution with their hosts. To give an idea of the significance of these organisms, it has been said that if we made the whole world, apart from the nematodes, transparent, we would be left with a hazy outline of the planet’s surface, from the fauna of nematodes living in soil and aquatic sediments, and of plants and animals as well, from their worm burdens (Buchsbaum, 1976). Yet, despite their importance we know relatively little about the majority of these species. The focus, so far, has mainly been on species that have negative impacts for society, such as those that cause harmful human diseases. For example, a large body of literature exists for the nematode Wuchereria bancrofti because it causes the iconic disease elephantiasis (e.g. Zhong et al., 1996, Kazura, 2010, Chauhan et al., 2015). Similarly, the spotlight has been on species that have serious economic implications such as parasitic nematodes (order Strongylida) of ruminants, which have received much attention in veterinary sciences because they cause weight loss and reduce milk production in cattle (Durie, 1962, Christensen et al., 1994, Tan et al., 2014). However, economically important or not, these studies have rarely examined host-nematode associations from a co-phylogeny perspective.

For these reasons, this study aimed to investigate the evolutionary history between New Zealand native lizards and their parasitic nematodes. This association is attractive for co-phylogenetic analysis for a number of reasons. First, it has the potential to provide an excellent model system for reptilian hosts and their nematodes due to the large
amount of host diversity while being monophyletic. Second, the nematodes of New Zealand lizards have so far received little attention and this study provides essential information on these native parasites.

While investigating the evolutionary history between New Zealand lizards and their nematode parasites was the main goal/motivation of this thesis, the opportunity to offer additional information on another group of their parasites, the mites, came to my attention. Initially, the idea was to investigate the evolutionary history between the mites and their lizard hosts and compare the similarities and differences between these two parasitic groups that are sharing the same hosts. This has seldom been done and would have provided an excellent opportunity to look at the relative roles the parasite life history plays in the evolution between host and parasite. Unfortunately, the mites collected during this study did not make this possible as not enough mites from a single genus were obtained (see chapter 3). However, the data obtained is still very useful for uncovering the diversity and distributions of these native mites so that in future the association may be analysed under an evolutionary context.

1.5 HOST-PARASITE STUDY SYSTEM

New Zealand (NZ) consists of three main Islands (North, South and Stewart) and a series of smaller offshore islands. All of these islands making up New Zealand are part of a larger submerged subcontinent, Zealandia, which originates from the southern part of the supercontinent Gondwana (Gibbs, 2006). Approximately 130mya Eastern Gondwana, containing the landmasses that would become Australia, Antarctica and Zealandia, split away from Gondwana (Gibbs, 2006). Zealandia remained connected to the Australia and Antarctica landmasses until approximately 80mya (Molnar et al., 1975). NZ’s long isolation since then has contributed to its high level of endemism (Gibbs, 2006). Particularly rich in species are the reptiles of NZ. The reptilian fauna belongs to three lineages: diplodactylid geckos, lygosomine skinks and Tuatara, all of which are endemic (Nielsen et al., 2011) with the exception of one invasive skink species, the rainbow skink, Lampropholis delicata, which arrived in NZ from Australia in the late sixties (Gill et al., 2001). This study focuses on two of these lineages: the lygosomine skinks and the diplodactylid geckos.
1.5.1 NEW ZEALAND SKINKS

NZ skinks are a monophyletic group belonging to the *Eugongylus* lineage (Smith et al., 2007). They occur in a wide range of environments including rocky shores, forests and grasslands throughout NZ and its offshore islands, with several species occurring in the same geographical area and even microhabitat (Jewell, 2008). However, current skink distributions have been reduced since the arrival of humans due habitat degradation and the introduction of predators (Chapple et al., 2009). The first descriptions of NZ skinks came from American and European taxonomists in the 1800s, then later by NZ herpetologists in the late 19th and early 20th century (see Hardy, 1977). However, early descriptions were inaccurate and type specimens were lost or located overseas, which resulted in species being described multiple times (Hardy, 1977). Consequently, there was much taxonomic confusion in the literature. McCann’s (1955) revision of the skink taxonomy provided some much needed clarity (Chapple et al., 2009). The author listed 17 species, all but one he put in the genus *Leiolopisma*. Since this revision many more species have been described. All NZ skinks are now identified as belonging to the genus *Oligosoma*. Currently 33 species are recognized with more yet to be described (Nielsen et al., 2011). The large number of species is attributed to the past geography of NZ and the lack of small mammals, which would have acted as competitors as well as predators (Hickson et al., 2000).

The arrival of skinks in NZ is generally agreed to result from post-Gondwanan dispersal (e.g. Hickson et al., 2000, Smith et al., 2007, Chapple et al., 2009). Current hypotheses propose that the skinks arrived in NZ from New Caledonia around 16-23mya (Hickson et al., 2000, Chapple et al., 2009). It is thought that the ancestral skink colonized the North Island, which was followed by an initial diversification. It appears the skink fauna then underwent further diversification into open habitat (clades 1-2), forest (clades 3-5) and coastal (clades 6-8) radiations (Chapple et al., 2009). Geographical events have also been important for the diversification of skinks. For example, Greaves et al. (2007) found genetic divergence within *O. lineoocellatum* between Nelson and Marlborough populations, which genetic calibrations place the timing to around same the time of the Southern Alps uplift.
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To date, only two phylogenies for New Zealand skinks have been published (Hickson et al., 2000, Chapple et al., 2009). The first study (Hickson et al., 2000) used 12SrRNA to construct the phylogeny of 25 skink species. Unfortunately, that study had poor phylogenetic resolution, which is probably due to insufficient taxon sampling (Chapple et al., 2009). The second study (Chapple et al., 2009) used a much larger range of species: the authors obtained tissue samples from 32 of the 33 extant species and analysed several molecular markers of both mitochondrial (ND2, ND4, cytochrome b, 12SrRNA, 16SrRNA) and nuclear (RAG-1) origin. This resulted in a phylogeny that had well resolved relationships between species within clades.

1.5.2 NEW ZEALAND GECKOS

Naturalists visiting NZ in the early 1800s provided the first descriptions of NZ geckos, as they did with skinks (McCann, 1955). Originally three genera were recognised to occur in NZ: Hoplodactylus, Naultinus and Gymnodactylus. However, McCann (1955) found no support for the latter genus and only considered Hoplodactylus and Naultinus to be present in NZ. In recent years, genetic analysis has further aided that classification of NZ geckos with the last major taxonomic work conducted by Nielsen et al. (2011). At the time of this study 20 species were recognised in NZ but 13-20 potentially cryptic species had been identified using preliminarily mitochondrial DNA sequencing (Daugherty et al., 1994, Chambers et al., 2001). Nielsen et al. (2011) supported the recognition of 16 new species as well as suggesting several modifications to the current classification of NZ geckos, which included the erection of several new genera. Today at least 40 species are recognised belonging to the genera: Dactylocnemis, Hoplodactylus, Mokopirirakau, Naultinus, Toropuku, Tukutuku and Woodworthia (Nielsen et al., 2011, Bell, 2014).

Several studies have tried to resolve the gecko phylogeny (Bauer, 1990, Chambers et al., 2001). Most of these attempts have resulted in poorly resolved or weakly supported phylogenies (Bauer, 1990) with the exception of Nielsen et al. (2011). These authors conducted the most comprehensive phylogenetic study to date using both nuclear and mitochondrial genes from 19 of the 20 recognized species. The result was a phylogeny with well-resolved relationships (Nielsen et al., 2011). This analysis also confirmed that
NZ *Diplodactylidae* is a monophyletic group, which had also been proposed by several other studies (Kluge, 1967, Chambers et al., 2001). The analysis also suggested that NZ geckos underwent diversification in the mid to late Miocene to form the current major clades. Major geological events have played roles in the diversification of geckos, such as the lateral displacement of the alpine fault, Pliocene mountain rise and Pleistocene glaciation (Nielsen et al., 2011). Today’s distribution, as with skinks, has been dramatically reduced due to human impacts (Chapple et al., 2009, Nielsen et al., 2011).

The origins of NZ geckos are not yet clear. Kluge (1967) hypothesised that Asian geckos dispersed to Australia, then later onto New Caledonia before arriving in NZ in the Miocene. The proposed timing of this particular hypothesis, however, does not fit with the fossil record (Nielsen et al., 2011). Others have hypothesised that the geckos originated from Gondwanaland vicariance (Bauer, 1990). However, in more recent years, hypotheses for dispersal after the breakup of Gondwanaland seem to have taken favour. The most convincing evidence so far comes from Nielsen et al. (2011). These authors found that NZ diplodactylids were sister taxa with the Australian diplodactylids, except for one Australian genus *Pseudothecadactylus*. This evidence favours dispersal from Australia post-Gondwanaland. Wind and ocean currents also would have made dispersal from Australia more likely (McDowall, 2008).

### 1.5.3 PARASITIC NEMATODES OF NEW ZEALAND SKINKS AND GECKOS

Nematodes (i.e. roundworms) are common parasites of lizards worldwide. Therefore, it is unsurprising that nematodes have also been documented to parasitise NZ native skinks and geckos, with records beginning over fifty years ago (e.g. Barwick, 1959). However, like many parasite species, since their discovery they have received remarkably little attention, despite decades of research focused on the life history of their hosts.

**DIVERSITY OF NEW ZEALAND NEMATODES**

The first record of nematodes parasitising the native lizards of NZ is credited to Barwick (1959) who became aware of the nematodes while examining the gut contents of the common skink (*Oligosoma polychroma*). The author identified these worms as belonging to the genus *Pharyngodon*. However, it has since become apparent that
Barwick most likely misidentified these nematodes as, to date, no New Zealand skink or gecko species have been recorded with infections by *Pharyngodon* nematodes. This view is shared by Ainsworth (1985), and Clark (1982) who is of the opinion that these nematodes belong to the genus *Parathelandros*. Ainsworth (1985) seems to agree with Clark’s assignment of *Parathelandros* as she found several lizards from the Wellington area that were infected with nematodes fitting Clark’s description.

In addition to nematodes belonging to the genera *Pharyngodon* and *Parathelandros*, several other nematode species have also been recorded from New Zealand skinks and geckos. These include a *Capillaria sp.* (Clark, 1982) and *Hedruris minuta* (Andrews, 1974). As with *Pharyngodon*, few records exist for these species. In fact, there is only one record for *Capillaria sp.*, which was found in the speckled skink on Stephens Island (Clark, 1982). Due to the lack of subsequent studies, further investigations should be undertaken to confirm Clark’s finding. *Hedruris minuta*, on the other hand, has received slightly more attention. The species was first identified by Andrews (1974), who formally described it based on eight specimens that were extracted from the short-tailed skink (*Leioplosima smithi*; now *Oligosoma smithi*). This species has since been recorded in *L. infrapunctatum* (*O. infrapunctatum*) (Clark, 1982) and skinks identified as *L. nigriplantare maccanni* (*O. maccanni*) Ainsworth (1985). A further possible record comes from Gill (1996) who reported nematodes which he considered likely to be *H. minuta*.

Several years after these initial species descriptions, Ainsworth (1992) conducted the most comprehensive study to date on the nematodes infecting NZ skinks and geckos. Despite this study being conducted over 20 years ago, Ainsworth remained the only author until the present study, to conduct a survey with the specific goal of recovering nematodes that parasitise NZ lizards (i.e. the nematodes were not ‘stumbled’ upon like previous studies). The study surveyed 33 of the 41 lizard species that were described at the time and revealed that many of these lizards were commonly infected with nematodes. For example, Ainsworth found 80% (32/40) of *Hoplodactylus maculatus* (*Woodworthia maculata*) from the Wellington area to be infected with nematodes. However, unlike past records, which report a number of genera present in NZ,
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Ainsworth identified eight nematode species all of which belonged to the genus *Skrjabinodon*.

The two most common and widespread species identified by Ainsworth were *Skrjabinodon trimorphi* and *S. poicilandri*, which the author has formally described (Ainsworth, 1990). The study revealed these two species to be widely distributed throughout the main islands of NZ, but *S. trimorphi* was confined to skink hosts and *S. poicilandri* to gecko hosts (Fig. 1.6). The additional six species, *Skrjabinodon* n. sp. PK, *Skrjabinodon* n. sp. HC, *Skrjabinodon* n. sp. FP, *Skrjabinodon* n. sp. NP, *Skrjabinodon* n. HO, *Skrjabinodon* n. sp. SU, named after their geographic location, structural characteristics or host (PK= Poor Knights, HC= Hen and Chickens, FP= five prong, NP= no prong, HO homalonotum, SU= suteri), tended to be restricted to small islands off the North East coast (Fig. 1.6). The author provides descriptions of all species as well as a key for their identification in her thesis, although formal descriptions were never published.

In relation to previous species reported in NZ, Ainsworth did not find any species matching *Hedruris minuta* or *Capillaria* sp., as mentioned above. However, on comparison to the specimens Clark (1982) identified as *Parathelandros*, Ainsworth found they fitted the description of several of the *Skrjabinodon* species. Ainsworth corrected Clark’s placement of these individuals in *Parathelandros* based on differences in caudal morphology between the two genera. Due to the comprehensiveness of Ainsworth’s work it is likely that *Skrjabinodon* is the only genus present in NZ. Therefore, *Skrjabinodon* is the only genus considered in the rest of this chapter.

CHARACTERISTICS/ LIFE HISTORY OF *SKRJABINODON*

The genus *Skrjabinodon*, Inglis 1968, belongs to the order Oxyurida, super family Oxyuroidea and to the pinworm family, Pharyngodonidae, Travassos 1919. This family mainly infects reptiles and amphibians in the lower gut region (Petter and Quentin, 1974). The lifecycle of all members of Oxyuroidea is strictly monoxenous (one-host life cycle, i.e. no intermediate host) and in most cases the egg of Oxyurid nematodes are the infective stage (Anderson, 2000). All Oxyurid nematodes are haplodiploid:
unfertilised eggs develop into males and fertilised eggs produce females (Adamson, 1981, Anderson, 2000, Jorge et al., 2014). However, differences exist between species regarding the stage of development at which the eggs are deposited. Some species deposit eggs in an early stage of development that only begin to mature once they have been expelled in the host faeces (Anderson, 2000). In other species, females migrate to the anus region and deposit eggs that quickly progress to an infective stage; from there, the eggs either enter the environment or are ingested by the host through grooming (Anderson, 2000). However, it is unclear which strategy Skrabinodon exhibits. The next host becomes infected when it accidently ingests material contaminated with eggs at the infective stage. Thus, Skrabinodon are reliant on the host for dispersal, as all other helminths with a similar lifecycle.

**Figure 1.6:** Visual representation of the localities in which Ainsworth (1990) found nematodes parasitic on New Zealand skinks (A) and geckos (B). The different colours represent the different nematode species. Nematodes from skinks: *S. trimorphi* (dark blue), *S. n. sp. HO* (lime green) and *S. n. sp. SU* (pink). Nematodes from geckos: *S. poicilandri* (red), *S. n. sp. PK* (purple), *S. n. sp. HC* (light pink), *S. n. sp. FP* (aqua), *S. n. sp. NP* (black). Diagrams re-drawn from Ainsworth (1992).
1.5.4 PARASITIC MITES OF NEW ZEALAND SKINKS AND GECKOS

Like nematodes, mites are common parasites of lizards worldwide. Often red, brown or orange in colour they are readily observed between the toes and around the armpits, tail, and eyes of many lizards (see Fajfer, 2012). In NZ, mites parasitic on native skinks and geckos are also common and anyone who has worked closely with these animals is likely to have observed bright patches where the mites aggregate on their host (Fig. 1.7). Currently, eight species have been described to infect NZ lizards: *Geckobia haplodactyi*, *G. naultina*, *Odontacarus lygosomae*, *Neotrombicula naultini*, *N. sphenodonti*, *Microtrombicula hoplodactyla*, *Ophionyssus galeotes*, *O. scincorum*. The descriptions of these mites started as early as the 1940s (Dumbleton, 1947) but the last description published was in the late 1980s (Goff et al., 1987), nearly thirty years ago. The lack of discovery of new species in the last thirty odd years reflects the little attention these parasites have received in the years following these first descriptions. As a result, basic knowledge on these mite species including their geographic distributions, the lizard species that harbour them and their genetic diversity is incomplete. Information such as the effect they have on their hosts, what determines the levels of infection and host specificity also still eludes us (Reardon and Norbury, 2004). It is surprising that these mites have been largely ignored given they are visually striking on their host (Fig 1.7).

![Figure 1.7: The arrow in the photograph is pointing out the clusters of small red mites that are attached to the rear legs of this NZ gecko species, Woodworthia sp (the image is credited to Sam Haultain).](image-url)
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The literature that is available on these NZ species is scattered throughout journals with no comprehensive reviews available. Although, the checklist of ecto- and endoparasites of New Zealand reptiles compiled by McKenna (2003) is a useful piece of work. In this document the author provides brief notes on host records, prevalence and distributions as well as a few notes on any other pieces of information the author thought were interesting or relevant. Chapter 3 of this thesis provides an updated review of the parasitic mites of NZ lizards, including new data collected as part of this research, and a comprehensive review of the information that is currently available for these parasites. Therefore, the background information presented here is kept brief; see chapter 3 for more detail.

1.6 Thesis aims and hypotheses

The main aim of this thesis is to investigate the evolutionary history of New Zealand skinks and geckos with their parasitic nematodes using co-phylogenetic methods. Encompassed in this main aim is determining the macro-evolutionary events have led to the present day associations. The central hypothesis being tested is that the nematodes, which have limited dispersal abilities and a direct life cycle, will mostly follow a co-speciation pattern with their hosts, having had few opportunities to host-switch. An additional hypothesis is that cases of host switching will be limited to host species that share habitats and occur in sympatry. However, in order to test these hypotheses, the thesis must achieve several smaller goals including:

- Genetically analyse the parasitic nematodes (using three markers 28S, 18S and COI) of native skinks and geckos of New Zealand to detect the presence of cryptic species
- Reconstruct the nematode phylogeny using three markers (28S, 18S and COI) to determine the structure of relationships within New Zealand nematode species

The second aim of this study was to review the literature on the mites that parasitise NZ skinks and geckos, and add to it new data on host-parasite records obtained from the
samples collected during the present research. This allowed me to collate the current information in order to provide a resource to aid future research and to build on the known distributions and hosts of the mites that parasitise New Zealand skinks and geckos.

These aims are addressed in the following two chapters. The first chapter investigates the diversity of Skrjabinodon nematodes within their skink and gecko hosts, while the second chapter focuses on the mites. The final chapter reviews the main findings of this study and provides suggestions for future research.
CHAPTER 2: CO-SPECIATION OR HOST SWITCHING: THE EVOLUTIONARY HISTORY OF LIZARD-NEMATODE ASSOCIATIONS

2.1 INTRODUCTION

Direct life cycle nematodes are an economically and ecologically important group but have received relatively little attention from a co-phylogenetic perspective (see Ch.1, section 1.5). Studies so far suggest that these associations generally do not display patterns of strict co-speciation (e.g. Mayer et al., 2009, Perlman et al., 2003, Chilton et al., 2011), and therefore Farhenholz’s rule is not likely to be the most frequent pathway to modern day host-nematode associations. But this result is hardly surprising given that the transmission mode of the parasite is considered to be one of the major factors influencing co-evolution (see Ch.1, section 1.4), and strict co-speciation does not seem to hold true for groups that are even more restricted in their transmission, such as lice (Perlman et al., 2003). In fact, a recent review of all studies reporting co-phylogenetic analyses found that convincing cases of co-speciation were rare, and those that were convincing most often came from mutualistic associations where the symbionts are vertically transmitted (i.e. mother to offspring) (de Vienne et al., 2013). So if not Farhenholz’s rule then what are the most common patterns of co-phylogeny in host-nematode associations? And what factors predict a history of host switching verses a history dominated by co-speciation, or in other words, which factors promote or inhibit these macro-evolutionary events from occurring?

Currently, too few studies have focused on host-nematode associations to be able to detect general patterns of co-phylogeny. However, what past studies do show us is that wide ranges of co-phylogenetic patterns are possible for direct life cycle nematodes. These patterns range from multiple host-switching events (e.g. Perlman et al., 2003) to a more co-speciation dominated history (e.g. Glen and Brooks, 1985). Consider, for example, one of the more extensively studied host-nematode associations, that between marsupials and the nematode subfamily Cloacininae. This subfamily is a diverse group
consisting of 36 genera and more than 256 species, which occur in the sacculated forestomachs and oesophagi of marsupials, such as kangaroos (Macropodidae), wallabies (Macropodidae) and rat kangaroos (Potoroidae) (Beveridge and Chilton, 2001). Several cloacinid genera have undergone co-phylogenetic analysis at different resolutions, indicating that the relative importance of host switching versus co-speciation events varies greatly in the evolution of this host-nematode association. For example, Beveridge and Chilton (2001) concluded that host switching appeared to be a significant mechanism in the evolution of the genera Cyclostrongylus, Macropostrongylus, Pharyngostongylus, Popovastrongylus, Rugopharynx, Thallostonema, Wallabinema and Zoniolaimus while co-speciation played a secondary role. A slightly different result was found for the tribe Labiostrongylinea, in which host switching events were still important but Chilton et al. (2011) concluded that co-speciation was broadly detectable. In contrast to both these studies, co-speciation appeared to have played a greater role in the diversification of the species complex Hypodontus macropi with four of the six taxa hypothesised to have arisen via co-speciation and the other two via host switching (Chilton et al., 1992). These studies indicate that both host switching and co-speciation events are important in the evolution of the subfamily Cloacininae.

If we consider the transmission mode of these parasites when trying to predict which macro-evolutionary event, host switching or co-speciation, has played a larger role in the evolution of host-nematode associations then we might expect host switching to be a more common event. This is because direct lifecycle nematodes usually spend the transmission phase of their lifecycle in the environment (i.e. as eggs or juveniles) providing opportunity for encounters with potential hosts. Drosophila flies and their Howardula nematodes (Perlman et al., 2003) are a perfect example of how this life history could promote host switching events. The Howardula nematodes parasitise Drosophila larvae by burrowing into their cuticle; when the adult fly emerges the female nematodes release their juveniles, which pass through the anus and ovipositor as the fly visits a mushroom. Many Drosophila species use the same mushroom species for breeding, providing ample host switching opportunities and as a result little
Chapter 2: Co-speciation or host switching: the evolutionary history of lizard-nematode associations

congruence was found between the phylogenies of this host-nematode association (Perlman et al., 2003).

Host switching is also a common feature in other host-nematode associations. For example, frequent host switching has been observed between diplogastrilid nematodes and their beetle hosts (Mayer et al., 2009). However, this group can be phoretic (attached to the body and using the beetle for transport) or endoparasitic, and due to limited information on these nematodes it is not clear which life cycle the genera in this study exhibit. In line with these studies, host switching has been the main driver of diversity for the genus Trichinella, a genus that is somewhat unusual in that it completes its entire lifecycle within a single vertebrate host and is transmitted via predation (e.g. humans become infected with T. spiralis when eating undercooked pork) (Zarlenga et al., 2006). But host switching events have not been the most common event in all host-nematode associations. In contrast to the above examples, co-speciation has been found to be the dominant pattern between strongylate nematodes (Oesophagostomum) and their primate hosts. Glen and Brooks (1985) found the host and parasite phylogenies to be highly congruent with very little evidence of host switching. Cases like this, which are perhaps the exception, are likely to be particularly informative of the factors that regulate host switching, just as the pocket gopher example (see Ch. 1, section 1.1) has highlighted the importance of host and parasite life histories in co-evolutionary processes.

The studies reviewed here demonstrate that every pattern of co-phylogeny is possible with direct life cycle nematodes and their hosts, although host switching, even at this early stage, appears to be a more frequent diversifying factor than was previously thought. However, the results of many of these studies must be interpreted with caution as many use morphological characteristics to build host and/or parasite phylogenies; this can underestimate diversity especially with nematodes which tend to be morphologically similar due to convergent evolution (Anderson et al., 1998). Many of these studies also suffer from unresolved phylogenies, use outdated analytical methods for building and comparing phylogenies and suffer from limited samples of host and parasite taxa. While we can still draw some conclusions from these studies, robust host-nematode models using updated molecular techniques and cutting-edge analytical tools
are required. More studies are also required in order to draw general conclusions about host-nematode evolution or identify the factors that contribute to certain patterns of evolution.

As introduced in chapter 1, this chapter investigates the evolutionary history between New Zealand skinks and geckos and their nematode parasites (family Pharyngodonidae). This association has the potential to be a good model for direct lifecycle parasitic nematodes because the host taxa are, in both cases, monophyletic (Chapple et al., 2009, Nielsen et al., 2011) and there is only one other non-native lizard species present in NZ, providing the simplest situation that could be hoped for. NZ lizards have rapidly diverged into different habitats with closely related species generally being isolated from each other; this study could, therefore, shed light on the role of both host and parasite life histories in patterns of co-phylogeny. The choice of lizard-nematode associations is also beneficial because this type of association has not been well explored in terms of evolutionary histories. Nematodes are important parasites of lizards and their interaction deserves greater attention, especially considering lizard species are in decline worldwide (Gibbon et al., 2000). Studies such as this could also inform conservation management because they can reveal the extent of former host ranges (the same species of parasites found in now isolated hosts show that the hosts were once in the same habitat) (Whiteman and Parker, 2005), which could either complement fossil records or provide new insights and be useful in selecting translocation sites for population restoration, etc.

The hypothesis tested here is that co-speciation will be the dominant mechanism in nematode-lizard associations where the parasites have a direct life cycle and the host has limited dispersion capabilities. Additionally, host switching should only occur between hosts that share habitats and occur in sympatry. A prerequisite to testing these main hypotheses is that the parasite phylogeny be resolved. This will allow the genetic diversity of NZ lizard nematodes to be assessed for the first time, and their phylogenetic relationships with other species of the same family to be determined. Due to the wide geographic distribution of the two main species currently described (Ainsworth, 1992), I hypothesise that the diversity of these nematodes is underestimated with cryptic species likely to be present. It is important to quantify the
diversity of nematode species because they contribute to biodiversity and they are as endangered as their hosts and thus at risk of co-extinction (Koh et al., 2004, Whiteman and Parker, 2005). Furthermore, knowledge of parasite diversity is also important for conservation reasons (Whiteman and Parker, 2005). For example, parasites affect how a host performs; future host switches could alter the competitive ability of the novel host (e.g. Tompkins et al., 2000).

2.2 METHODS

2.2.1 NEMATODE COLLECTION

The method most commonly used to obtain internal parasites is to dissect the host to search for the parasite(s) of interest. Yet, sacrificing the host is undesirable and arguably unethical when the host species is declining and/or endangered, which is the case with many of New Zealand’s native skinks and geckos. Fortunately, a non-invasive method for obtaining lizard intestinal nematodes has previously been identified. This method involves searching the host faecal pellets to recover any nematodes that have been expelled (e.g. Fenner et al., 2011, Jorge et al., 2011, Gyawali et al., 2013). The obvious benefit of this method is that it allows the detection of these nematodes at a range of lifecycle stages (adults or juveniles) with no harm inflicted on the host. The trade-off, however, is that additional information on the nematode loads of individual hosts can not be calculated or estimated because the number of nematodes that an individual harbours does not correlate with the number found within faecal pellets (Jorge et al., 2013a). The likelihood of detecting the parasite is also much lower as a negative sample does not indicate that the host is free from infection, whereas dissection would provide an accurate estimate of the parasite burden.

The collection of faecal pellets from New Zealand native lygosomine skinks and diplodactylid geckos took place over two sampling periods (December 2012 to June 2013, and October 2013 to May 2014) that were mainly focused in the spring and summer months when lizard activity and digestion rates were high. To enhance the number of samples obtained from different species at different geographic localities, in the time permitted, two strategies for faecal sample collection were employed. First, researchers that had planned and already received approval for field studies involving
Chapter 2: *Co-speciation or host switching: the evolutionary history of lizard-nematode associations*

Wild lizards were asked to collect faecal pellets spontaneously released by the lizards during handling. This provided the only source of faecal pellets in the Dec. 2012–June 2013 sampling period. The second strategy involved joining other researchers working with wild populations and either collecting pellets that were released spontaneously or using a method that involved gently massaging the lizard’s abdomen to induce the expulsion of pellets from the intestines (Jorge et al., 2013a). Permits for this exercise were obtained from the Department of Conservation (permit numbers: 38672-FAU, 38674-FAU, 38678-FAU) and were approved by the University of Otago Animal Ethics Committee (protocol 36/14).

For both sampling strategies, expelled pellets were collected in 1.5ml Eppendorf tubes prefilled with 75-96% ethanol to preserve any nematodes. Each tube held faeces from a single lizard individual. The criteria for faecal pellet collection were: a) pellets had to be freshly released when collected in order to prevent desiccation of the specimens, and b) the collector had to be certain of the host identity (which most often required the animal to be handled) because correctly identifying the host is essential for co-phylogenetic studies. In some cases, samples were collected from captive populations, when a) the species could not be sampled in the wild for conservation reasons, or b) samples could not be obtained directly from the wild because no researchers worked with wild populations or they declined to collect samples for this study. Avoiding samples from captive populations was important because captive animals may harbour nematodes that do not occur naturally in the locality from which they were collected. Such cases would obscure the true evolutionary patterns between the hosts and their parasitic nematodes. For these reasons, samples from captive animals were only used if they were collected from wild-born animals kept in single-species colonies so that contamination was unlikely.

In total, 729 faecal pellets were obtained from 26 lizard species (14 skink and 12 gecko species) from a wide range of localities around New Zealand including several offshore islands (Table 2.1). A full list of the species from which faecal pellets were obtained, the area of collection and number of samples from each species at a particular locality are shown in Table 2.1. The large variation in the number of samples obtained from each species reflects the opportunistic nature of the sample collection method.
Chapter 2: Co-speciation or host switching: the evolutionary history of lizard-nematode associations

The majority of faecal samples collected were obtained from wild lizards with the exception of a small subset collected from captive populations. Exact co-ordinates for each locality are not listed as it is well recognised among NZ herpetologists that poaching is a major threat to NZ lizards, and information given in scientific documents could be utilised for these illegal activities. However, exact localities can be provided upon request.

Table 2.1: Lizard species from which faecal pellets were obtained. Listed are the number of samples that were obtained at each location (numbers in bold indicate the total of number of samples collected from the species), the general geographic location where the lizards were sampled, and whether the lizards were sampled in the wild (W) or captivity (C). The location listed for captive populations is where the animal(s) were originally collected.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of samples</th>
<th>Location of collection (or origin of captive lizards)</th>
<th>Captive (C), wild (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skinks:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oligosoma aenuem</em></td>
<td>29, 11, 24</td>
<td>Limestone Is. / Great Barrier Is. / Auckland</td>
<td>W</td>
</tr>
<tr>
<td><em>Oligosoma grande</em></td>
<td>3, 6, 21</td>
<td>Central Otago / Macraes flat</td>
<td>W</td>
</tr>
<tr>
<td><em>Oligosoma infrapunctatum</em></td>
<td>4</td>
<td>Stephens Is.</td>
<td>W</td>
</tr>
<tr>
<td><em>Oligosoma lineoocellatum</em></td>
<td>3, 16</td>
<td>Stephens Is. / Canterbury</td>
<td>W</td>
</tr>
<tr>
<td><em>Oligosoma maccanni</em></td>
<td>97</td>
<td>Macraes flat</td>
<td>W</td>
</tr>
<tr>
<td><em>Oligosoma moco</em></td>
<td>20, 18</td>
<td>Limestone Is. / Great Barrier Is.</td>
<td>W</td>
</tr>
<tr>
<td><em>Oligosoma polychroama</em></td>
<td>4, 1, 25, 26, 5</td>
<td>Stephens Is. / Maud Is. / Macraes flat / Wellington / Christchurch</td>
<td>W</td>
</tr>
<tr>
<td><em>Oligosoma ornatum</em></td>
<td>8, 2, 5, 1</td>
<td>Great Barrier Is., Wellington, Auckland, Limestone Is.</td>
<td>W</td>
</tr>
<tr>
<td><em>Oligosoma otagense</em></td>
<td>3, 16</td>
<td>Central Otago / Macraes flat</td>
<td>C / W</td>
</tr>
<tr>
<td><em>Oligosoma smithi</em></td>
<td>6, 5, 8, 10</td>
<td>Limestone Is. / Whangarei / Gisborne / Motouki Is.</td>
<td>W</td>
</tr>
<tr>
<td><em>Oligosoma suteri</em></td>
<td>8</td>
<td>Rangitoto Is.</td>
<td>W</td>
</tr>
</tbody>
</table>
## Table 2.1 continued:

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of samples</th>
<th>Location of collection (or origin of captive lizards)</th>
<th>Captive (C), wild (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligosoma whitakeri</td>
<td>11</td>
<td>Wellington</td>
<td>C</td>
</tr>
<tr>
<td>Oligosoma zelandicum</td>
<td>4</td>
<td>Stephens Is.</td>
<td>W</td>
</tr>
<tr>
<td><strong>Geckos:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dactylocnemis pacificus</td>
<td>8</td>
<td>Great Barrier Is.</td>
<td>W</td>
</tr>
<tr>
<td>Mokopiriakau granulatus</td>
<td>1, 1, 1 (3)</td>
<td>Taranaki / Limestone Is. / Great Barrier Is.</td>
<td>C / W / W</td>
</tr>
<tr>
<td>Mokopirirakau sp. ‘Southern North Island’</td>
<td>8</td>
<td>Wellington</td>
<td>W</td>
</tr>
<tr>
<td>Naultinus elegans</td>
<td>4</td>
<td>Upper North Island</td>
<td>W</td>
</tr>
<tr>
<td>Naultinus punctatus</td>
<td>2</td>
<td>Wellington</td>
<td>C</td>
</tr>
<tr>
<td>Naultinus gemmeus</td>
<td>90</td>
<td>Dunedin</td>
<td>W</td>
</tr>
<tr>
<td>Naultinus manukanus</td>
<td>3</td>
<td>Stephens Is.</td>
<td>W</td>
</tr>
<tr>
<td>Naultinus rudis</td>
<td>1</td>
<td>Canterbury</td>
<td>C</td>
</tr>
<tr>
<td>Tukutuke rakiurae</td>
<td>8</td>
<td>Stewart Island</td>
<td>W</td>
</tr>
<tr>
<td>Woodworthia brunnea</td>
<td>67</td>
<td>Canterbury</td>
<td>W</td>
</tr>
<tr>
<td>Woodworthia chryosiretica</td>
<td>9</td>
<td>New Plymouth</td>
<td>W</td>
</tr>
<tr>
<td>Woodworthia ‘Central Otago’</td>
<td>28</td>
<td>Central Otago</td>
<td>W</td>
</tr>
<tr>
<td>Woodworthia ‘Otago large’</td>
<td>43</td>
<td>Macraes flat</td>
<td>W</td>
</tr>
<tr>
<td>Woodworthia maculata</td>
<td>3, 11, 1, 10, 28 (53)</td>
<td>Stephens Is. / Maud Is / Crusoe Is. / Great Barrier Is. / Wellington</td>
<td>W</td>
</tr>
</tbody>
</table>
RECOVERY, IDENTIFICATION AND MORPHOLOGICAL MEASUREMENTS

In the laboratory, faecal pellets were dissected by gently teasing them apart under an Olympus SZ30 dissection microscope. Pellets were kept moist with 70% ethanol while being examined to prevent any nematodes within the pellet from desiccating. Nematodes extracted from the samples were cleaned of debris using a fine paintbrush and then stored in Eppendorf tubes containing 90% ethanol. In total, 44 nematodes were recovered from 31 of the 729 faecal samples (see Table 2.2, Fig. 2.1). Where possible, three nematodes per locality/host species were selected for genetic analysis (specimens used in the genetic analysis are indicated in Table 2.2). A maximum of one nematode per faecal sample (1 per host individual) was used. Female worms were always chosen over males for sequencing because they are generally larger and, therefore, contain more DNA. When a sample contained more than one female, the ‘cleaner’ female was selected. That is, the female with the least amount of faecal pellet debris stuck to her in order to prevent contamination.

Table 2.2: Faecal samples from which parasitic nematodes were recovered. * Indicates samples included in the genetic analysis. † Indicates samples with nematodes in condition too poor to be used for genetic analysis (i.e. only the cutical/body wall remaining).

<table>
<thead>
<tr>
<th>Host species</th>
<th>Sample ID</th>
<th>No of nematodes</th>
<th>Location of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skinks:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oligosoma aenuem</em></td>
<td>RP1008</td>
<td>1 Female</td>
<td>Great Barrier Is.</td>
</tr>
<tr>
<td><em>Oligosoma grande</em></td>
<td>0g5</td>
<td>1 Male</td>
<td>Macraes Flat</td>
</tr>
<tr>
<td><em>Oligosoma grande</em></td>
<td>EuL.2.T</td>
<td>1 Juvenile Male</td>
<td>Macraes Flat</td>
</tr>
<tr>
<td><em>Oligosoma maccanni</em></td>
<td>RP1369</td>
<td>1 Female Sub-adult</td>
<td>Macraes Flat</td>
</tr>
<tr>
<td><em>Oligosoma polychroma</em></td>
<td>RP1818</td>
<td>2 Females; 1 Male</td>
<td>Canterbury</td>
</tr>
<tr>
<td><em>Oligosoma polychroma</em></td>
<td>W7</td>
<td>1 Female</td>
<td>Wellington</td>
</tr>
<tr>
<td><em>Oligosoma otagense</em></td>
<td>W28R</td>
<td>1 Male</td>
<td>Macraes Flat</td>
</tr>
<tr>
<td><strong>Geckos:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dactylocnemis pacificus</em></td>
<td>RP999</td>
<td>1 Juvenile Female</td>
<td>Great Barrier Is.</td>
</tr>
<tr>
<td><em>Naultinus punctatus</em></td>
<td>RP317</td>
<td>1 Male</td>
<td>Wellington (C)</td>
</tr>
</tbody>
</table>
Table 2.2 continued:

<table>
<thead>
<tr>
<th>Host species</th>
<th>Sample ID</th>
<th>No of nematodes</th>
<th>Location of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woodworthia brunnea *</td>
<td>RP1688</td>
<td>1 Female</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Woodworthia brunnea</td>
<td>RP1690</td>
<td>1 Female</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Woodworthia brunnea *</td>
<td>RP1691</td>
<td>1 Female</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Woodworthia brunnea</td>
<td>RP1717</td>
<td>1 Juvenile Female</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Woodworthia brunnea</td>
<td>RP1752</td>
<td>1 Juvenile- Sex undetermined</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Woodworthia brunnea *</td>
<td>RP1775</td>
<td>1 Female</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Woodworthia brunnea *</td>
<td>RP1758</td>
<td>1 Female</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Woodworthia brunnea</td>
<td>RP1843</td>
<td>1 Juvenile Male</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Woodworthia brunnea *</td>
<td>RP1863</td>
<td>1 Female; 1 Male</td>
<td>Canterbury</td>
</tr>
<tr>
<td>Woodworthia ‘Central Otago’ *</td>
<td>RP1140</td>
<td>1 Juvenile Female</td>
<td>Central Otago</td>
</tr>
<tr>
<td>Woodworthia maculata *</td>
<td>GB10</td>
<td>1 Male</td>
<td>Great Barrier Is.</td>
</tr>
<tr>
<td>Woodworthia maculata *</td>
<td>RP974</td>
<td>2 Juvenile Males</td>
<td>Great Barrier Is.</td>
</tr>
<tr>
<td>Woodworthia maculata *</td>
<td>RP986</td>
<td>1 Juvenile Male</td>
<td>Great Barrier Is.</td>
</tr>
<tr>
<td>Woodworthia maculata</td>
<td>RP84</td>
<td>1 Juvenile Female</td>
<td>Maud Is.</td>
</tr>
<tr>
<td>Woodworthia maculata</td>
<td>BH3</td>
<td>3 Female</td>
<td>Wellington</td>
</tr>
<tr>
<td>Woodworthia maculata</td>
<td>W1</td>
<td>1 Female</td>
<td>Wellington</td>
</tr>
<tr>
<td>Woodworthia maculata</td>
<td>W82</td>
<td>1 Female</td>
<td>Wellington</td>
</tr>
<tr>
<td>Woodworthia ‘Otago large’ *</td>
<td>RP1377</td>
<td>1 Female</td>
<td>Macraes Flat</td>
</tr>
<tr>
<td>Woodworthia ‘Otago large’ *</td>
<td>RP330</td>
<td>1 Female; 7 Male</td>
<td>Macraes Flat</td>
</tr>
<tr>
<td>Woodworthia ‘Otago large’ *</td>
<td>RP945</td>
<td>1 Female Subadult; 1 Juvenile Female</td>
<td>Macraes Flat</td>
</tr>
</tbody>
</table>
Figure 2.1: Distribution of New Zealand skinks (black) and geckos (blue) from which nematode parasites were recovered. Locations marked starting from the top of the North Island are: Great Barrier Island (orange), Wellington (purple), Maud Island (pink), Canterbury (red and blue), Macraes Flat (yellow), Central Otago (green).
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Prior to genetic analysis, nematodes selected for sequencing were photographed at a range of magnifications using an Olympus CX41 compound microscope with attached Olympus DP25 camera. First, photographing each nematode allowed measurements of key morphological features such as spines, vagina and excretory pore position, for species identification. Second, the photographs provided a record of the nematodes that underwent genetic analysis (the DNA extraction process requires digestion of the whole nematode) in case morphological features had to be re-checked after sequencing. The measurements taken are provided in appendix A as there were not enough specimens to carry out a comparative morphological study. To photograph each nematode, a temporary slide was made using a 1:1 ratio mixture of glycerol and water (Foitová et al., 2008). The glycerol acts as a clearing agent so that the internal organs become visible without the nematode cuticle obscuring the internal structures. Photographs and measurements were taken of key features including: body length and width, tail, oesophagus, bulb, excretory pore position etc following measurement guidelines in Jorge et al. (2013b). The number of spines on the tail was also counted (see appendix A).

2.2.2 DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

Three partial gene fragments were selected for amplification, two nuclear genes: 18S rRNA and 28S rRNA, and one mitochondrial gene: cytochrome oxidase subunit I (COI). These markers were selected because they have been identified as useful for nematode phylogenetic classification and identification (see Blaxter, 2013). Furthermore, these fragments are available for species that belong to the same family as Skrjabinodon (e.g. Jorge et al., 2011, Falk and Perkins, 2013), allowing the different species to be compared. The 18S fragment was amplified using the primers Nem 18S F and Nem 18S R designed by Floyd et al. (2005) and the 28S fragment with the primers 28S rD1.2a and 28S B described by Whiting (2002). The COI fragment, however, proved to be difficult to amplify; as a result several primers and primer combinations were trialled including primers LCO and HCO described by Folmer et al. (1994), LCO1490 and HCO2198 designed by Geller et al. (2013) and C-NemF1_t1 and C_NemR1_t1 (a primer cocktail with each final primer consisting of a mix of three primers) designed by Prosser et al. (2013). However, the only primers that were
successful in amplifying any of the COI fragments were the C-NemF1_t1 and C_NemR1_t1 of Prosser et al. (2013) and even so only three samples were successfully amplified. It is unclear why there was such a poor success rate with these primers, especially primers by Prosser et al. (2013), which successfully amplified this DNA fragment in nematodes belonging to the same family as *Skrjabinodon*.

Due to the low success of existing COI primers, it was necessary to design new primers in order to obtain these remaining sequences. The computer software Geneious 8.0.3 {http://www.geneious.com Kearse, 2012 #210} was used to design these new primers. A consensus sequence created from the few successful sequences was used as a template. The selected primer combination was then analysed by NetPrimer (http://www.premierbiosoft.com/NetPrimer/AnalyzePrimer.jsp) to check primer-primer interaction. The primers were named SKR_F for the forward and SKR_R for the reverse, SKR standing for the focal genus, *Skrjabinodon*. The forward primer was 20 base pairs (bp) long: 5’- TTT TTA TGG TGA TAC CTA TT- 3’ and the reverse sequence 21bp: 5’- TAG TAT TAA AAT TAC GAT CAA -3’. The Tm (50mM NaCl) of these primers was 41.9 °C. A temperature gradient PCR using the sample W1 revealed the annealing temperature that produced the highest yield of DNA to be around 47.6°C. These primers, however, amplified a smaller fragment in comparison to the other primers trialled in this study, with the total fragment length only being around 430bp in length.

All PCRs were performed in a total volume of 20µL. This volume consisted of 4µL of MyTaq™ Red reaction buffer (Bioline, Bioline (Aust) Pty, Alexandria, NSW, Australia), 1µL of each primer at 0.5mM, 0.2µL of 0.4mg/mL BSA (bovine serum albumin), 0.1µL of MyTaq™ DNA polymerase and 1.5- 5µL of DNA template. All reactions were performed on an Mastercycler pro S. Cycle conditions were typically as follows: denatured at 95°C for 3 minutes followed by 35 iterations of: 40 seconds at 95°C, 40 seconds at 47.6-54°C (depending on primers), 1 min at 72°C and ending with a final extension at 72°C for 10 min. Amplified products were cleaned using ExoSAP-IT™ (USB, Cleveland, Ohio, United States) and sent to the Department of Anatomy at
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the University of Otago, Dunedin, New Zealand, for sequencing. Sequences were obtained for both directions using the same primers as in the PCR.

2.2.3 PHYLOGENETIC ANALYSIS

For each pair of sequences (forward and reverse), a contiguous (contig) sequence was assembled using Geneious (version 8.0.3, Biomatters Ltd). This allowed any uncertainty in base identities to be eliminated, i.e. if a base identity was not clear in one sequence it was clarified using the complimentary strand. Each contig was uploaded to BLAST (Zhang et al., 2000) to confirm the sequences were indeed nematode DNA and not a contaminant. All sequences were then aligned in Geneious using the MUSCLE algorithm (default settings) to confirm any base positions that were still unclear. The final alignments consisted of 764, 1152, and 369 base pairs for the 18S, 28S and COI genes respectively. All COI sequences were translated to confirm codons corresponded to amino acids.

All phylogenetic analyses were performed on the online phylogenetic tree tool CIPRES Science Gateway V. 3.3 {http://www.phylo.org Miller, 2010 #254}. Evolutionary models were selected for each of the three amplified markers using a jModel test (jModelTest 2 Darriba et al., 2012) with the AIC criterion. Outgroups and all additional sequences for all analyses were obtained from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) (Table 2.3). For consistency, all additional sequences across markers were obtained for the same individual (i.e. the 18S, 28S and COI were obtained from one individual). Phylogenetic analyses were preformed for each dataset using Bayesian inference and maximum likelihood methods. Bayesian analyses were performed in MrBayes 3.2.3 (Ronquist et al., 2012) and run for 10 x 10⁶ generations with random starting trees, sampling every 100 generations. Two independent runs were performed each with one cold and three heated chains. The first 25% of the trees were discarded as burn-in and the remaining trees pooled. Mixing and convergence of each run was monitored by the statistics provided in MrBayes (values of standard deviation of partition frequencies (<0.01), potential scale reduction factors (PSRF) (1.00) and effective sample sizes (EES) (>200)). A 50% majority-rule consensus tree was used to summarise the trees sampled from the post-burn-in trees.
Maximum likelihood analyses were performed using Randomized Accelerated Maximum Likelihood (RAxML) (Stamatakis, 2014). Branch support was estimated by bootstrap analysis (Felsenstein, 1985) with 1000 replicates. Trees with the best support are presented in the results and all other trees can be found in appendix B.

First explored was the 18S marker. This marker contains limited phylogenetic information but is useful for assessing the deeper relationships at the family level. As this was the first time New Zealand nematodes have undergone genetic analysis, the 18S marker was useful to confirm their taxonomic identification. Representative sequences from all other available Pharyngodonidae genera were selected to investigate this relationship (Parapharyngodon echinatus, P. cubensis, Spauligodon anolis, S. atlanticus, S. nicolauensis, Thelandros scleratus, T. tinerfensis). The species Thelestoma gueyei, T. krausi and Skrjabinema kamosika, which belong to the same order but a different family were used as outgroups (Table 2.3). The jModel test revealed the best evolutionary model for this data set to be GTR + I + G. The data was explored using both RAxML and MrBayes algorithms. For each analysis the parameters according to the selected evolutionary model were implemented.

Table 2.3: GenBank accession numbers for additional nematode sequences used in the phylogenetic analyses in this study. DS stands for direct submissions to GenBank.

<table>
<thead>
<tr>
<th>Species</th>
<th>18S</th>
<th>28S</th>
<th>COI</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parapharyngodon cubensis</td>
<td>KF029168</td>
<td>-</td>
<td>-</td>
<td>(Falk and Perkins, 2013)</td>
</tr>
<tr>
<td>Parapharyngodon echinatus</td>
<td>JF829224</td>
<td>JF829241</td>
<td>-</td>
<td>(Jorge et al., 2011)</td>
</tr>
<tr>
<td>Spauligodon anolis</td>
<td>KF029057</td>
<td>-</td>
<td>KF029396</td>
<td>(Falk and Perkins, 2013)</td>
</tr>
<tr>
<td>Spauligodon atlanticus</td>
<td>JF829235</td>
<td>JF829261</td>
<td>JF829306</td>
<td>(Jorge et al., 2011)</td>
</tr>
<tr>
<td>Spauligodon nicolauensis</td>
<td>JF829226</td>
<td>JF829243</td>
<td>JF829265</td>
<td>(Jorge et al., 2011)</td>
</tr>
<tr>
<td>Thelandros scleratus</td>
<td>KC335146</td>
<td>-</td>
<td>-</td>
<td>DS</td>
</tr>
<tr>
<td>Thelandros tinerfensis</td>
<td>KJ778073</td>
<td>-</td>
<td>-</td>
<td>(Jorge et al., 2014)</td>
</tr>
<tr>
<td>Thelestoma gueyei</td>
<td>AM260939</td>
<td>-</td>
<td>-</td>
<td>DS</td>
</tr>
</tbody>
</table>
The model selected to analyse the 28S data was TIM3 + I + G, the parameters for both RAxML and MrBayes are the same as those for the 18S. Due to the presence of poorly aligned regions in the 28S alignment the data was further explored by removing those regions using the program Gblocks (Castresana, 2000). Two Gblock analyses, one more stringent than the other, were run using the server http://molevol.cmima.csic.es/castresana/Gblocks_server.html (Copyright Jose Castresana). The more stringent test was run using the default parameters while the second less stringent test allowed gap positions within the final blocks and allowed less strict flanking positions. The model selected for the more stringent test was TPM3uf+G and that for the less stringent test was TIM3+G. MrBayes was then used with the same parameters as described above. The species *Thelandros tinerfensis* and *Paraphayngodon echinatus* were used as outgroups in all 28S analyses. The available *Spauligodon* sequences were also included in the analysis to confirm the relationship between these species and the New Zealand lizard nematodes that was indicated in the 18S analysis.

To explore the COI sequences, the skink and gecko data were first separated due to the 18S and 28S analyses revealing that the skink and gecko nematodes belonged to different genera (see results, section 2.3). The first and second codons and the third codon were checked for substitution saturation for both the skink and gecko data using Xia’s method (Xia et al., 2003) in the software DAMBE (Xia, 2013). Substitution saturation, although often overlooked, must be verified because if saturation is complete then the phylogenetic signal will be lost and similarities between sequences will occur through chance rather than common ancestry (Xia, 2009). Saturation had not occurred for the COI first and second codons in the skink or the gecko nematodes as the Iss > Iss.c in both cases, which indicates that there is little saturation. No significant saturation was detected for the third codon for the skink nematodes (Iss > Iss.c). The gecko nematodes, however, showed some saturation (Iss was close to the Iss.c value). The problem of saturation of the third codon can be overcome by excluding the position (Xia, 2009). However, when it is not fully saturated it is often left in the analysis because this position should follow the neutral theory of molecular evolution more closely than the other two codons (Xia et al., 2003). Therefore, the COI data was first
explored including the third position with a codon partition model using MrBayes and maximum likelihood algorithms. To prepare the COI data for these analyses, the alignment was translated to amino acids in order to determine the reading frame, and visually examined for mistakes. The rationale for a codon partition model is that codon positions do not evolve homogeneously; for example, the third codon position is subject to substitutions and reversions more readily than the first and second positions because changing the third position is less likely to affect the coded protein (Salemi, 2009). By dividing the data into subsets (partitions), a different independent Markov model of nucleotide substitution can be applied to each partition (Nylander et al., 2004).

Finally, because there was no significant incongruence between the 28S and COI phylogeny, the 28S and COI genes were combined (again keeping the skink and gecko nematodes separated) to obtain a more robust phylogeny. However, with one fragment being rDNA and the other mtDNA, they are most likely subject to different evolutionary pressures, therefore a mixed model analysis was conducted. That is, the data was partitioned so that it had independent substitution model parameters while assuming a shared topology. The data was partitioned by gene (i.e. 28S and COI) and by codon position and then aligned using the MrBayes algorithm, using the same parameters as described for the other analyses above. This analysis was run twice: once including the third codon and once excluding it to determine the influence of the third codon position on the overall support of the tree branches.

2.2.4 GENETIC DISTANCES

To determine the genetic differences between and within each of the clades, which were based on the combined 28S and COI inference trees, pairwise uncorrected p-distances were calculated in MEGA (version 6.06) (Tamura et al., 2013). First, a pairwise calculation was conducted with the COI marker (separately for the skink and the gecko nematodes). Genetic distances were then calculated between and within clades at both the COI and 28S markers. Specimens that were not included in this joint analysis because of missing data at the 28S marker were omitted for the group distance measurements for a more conservative analysis, but were included in the pairwise distance calculations to give an idea of their relationships to the other specimens from
the same and different localities. To give an idea of the relationships between New Zealand skink and gecko nematodes and their relationship to *Spauligodon* nematodes, the closest related group of available sequences, genetic distances were calculated between these groups.

**CO-PHYLOGENY ANALYSIS**

To explore the possible evolutionary history between the New Zealand geckos and skinks and their respective nematode parasites, the distance based co-evolutionary analysis program PACo was used (Balbuena et al., 2013). PACo is a global fit method based on Procrustes analysis (a form of shape analysis used to find the optimal superimposition of multivariate datasets) (Balbuena et al., 2013). Global fit methods are more appropriate than the alternative event-based methods given the data obtained in this study. Indeed, while event-based methods are attractive for a number of reasons, such as determining which events could have produced a given data set, the analysis requires fully resolved phylogenies, which was not the case in this study. In contrast, distance-based methods like PACo do not require fully resolved phylogenies. What sets PACo apart from other distance-based methods such as Parafit and HCT is that PACo tests the dependence of the parasite phylogeny on that of the hosts using a Procrustean superimposition in which the parasite matrix is rotated and scaled to fit the corresponding host matrix (Balbuena et al., 2013). The null hypothesis of this method is that the arrangement of the parasite phylogeny does not depend on the hosts (Balbuena et al., 2013). The PACo analysis was carried out in R Console version 3.1.2, the code files are listed in appendix C. Host sequences used were from the 16S gene and acquired from GenBank, accession numbers are listed in Table 2.4. These sequences were aligned as described for the nematodes.
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Table 2.4: GenBank accession numbers for the 16S sequences obtained from NZ lizard species. All gecko sequences were obtained by Nielsen et al (2011) and skink sequences by Chapple et al (2009). Letters in () represent the location the host in this study came from: GB - Great Barrier Island, M - Maud Island, W - Wellington. Numbers in () represent the clade the O. polychroma species belonged to.

<table>
<thead>
<tr>
<th>Gecko species</th>
<th>16S</th>
<th>Skink species</th>
<th>16S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dactylocnemis pacificus</td>
<td>GU459993</td>
<td>Oligosoma aeneum</td>
<td>Eu567866</td>
</tr>
<tr>
<td>Naultinus gemmeus</td>
<td>GU459959</td>
<td>Oligosoma maccanni</td>
<td>EU567883</td>
</tr>
<tr>
<td>Naultinus punctatus</td>
<td>GU459956</td>
<td>Oligosoma polychroma&lt;sup&gt;(1)&lt;/sup&gt;</td>
<td>EU567890</td>
</tr>
<tr>
<td>Woodworthia brunnea</td>
<td>GU460035</td>
<td>Oligosoma polychroma&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>EU567894</td>
</tr>
<tr>
<td>Woodworthia maculata&lt;sup&gt;(GB)&lt;/sup&gt;</td>
<td>GU460047</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Woodworthia maculata&lt;sup&gt;(M)&lt;/sup&gt;</td>
<td>GU460044</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Woodworthia maculata&lt;sup&gt;(W)&lt;/sup&gt;</td>
<td>GU460048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Woodworthia ‘Otago large’</td>
<td>GU460118</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.3 RESULTS

2.3.1 MORPHOLOGY

Morphological examination of the adult nematode specimens prior to genetic analysis revealed three distinct species according to available morphological descriptions. The first two aligned with the formally described species S. poilicandri (Fig. 2.2) and S. trimorphi (Fig. 2.3) (Ainsworth, 1990). All S. trimorphi specimens came from skink hosts and all S. poicilandri specimens came from gecko hosts. The third species was identified as the informally described species S. ‘five prong’ (Ainsworth, 1990) (Fig. 2.4). Although only one S. ‘five-prong’ specimen was found and it was a juvenile specimen, it was easily assigned to this species because of the five large basal spines after which the species is named. This individual was collected from a Pacific gecko (Dactylocnemis pacificus) host on Great Barrier Island, thus expanding the known geographical range of this species, which has previously only been reported from the Coromandel Peninsula, Hen and Chicken island group Alderman Island and Karewa Island (Ainsworth, 1992). A few specimens were not well matched morphologically to any available descriptions: GB10 had a greater body length, longer oesophagus, smaller tail size and fewer spines than S. poicilandri male morph 1 (see appendix A). However, this is the first time specimens from geckos have been collected on Great Barrier Is. RP1688 was also larger in body size, which does not necessarily mean that it does not
fit *S. poicilandri* descriptions as these are based on relatively few specimens. However, genetic analysis suggests that there may be more diversity than morphological descriptions have previously identified (see below).

### 2.3.2 GENETIC ANALYSIS

Of the 23 specimens that underwent genetic analysis, fragments for all markers were successfully obtained for 17 specimens. The 28S marker could not be amplified for the samples BH3 (*Woodworthia maculata*, Wellington), RP1377 (*Woodworthia* ‘Otago large’, Macraes Flat) and RP315 (*Woodworthia* ‘Otago large’, Macraes Flat) despite the successful amplification of both the 18S and COI fragments for these samples. The DNA from these samples may have been slightly degraded, which would explain why this larger fragment could not be successfully sequenced. Other specimens from the same hosts and localities as these failed samples were obtained and, therefore, they were not critical for the subsequent analyses. Unfortunately, amplification was not successful for any marker for the samples W28R (*O. Otagense*, Macraes Flat), Eul.2.T (*O. grande*, Macraes Flat) and Og5 (*O. grande*, Macraes Flat); therefore, no nematode representatives from the Otago (*O. otagense*) or Grand (*O. grande*) skinks were included in the phylogenetic analysis. The failure of these samples is also likely to be due to DNA degradation. These samples were collected in 2004 and donated to this study; their storage conditions were unknown. The final lengths of the 18S, 28S and COI markers used in the genetic analysis were 764, 1052 and 369 base pairs, respectively.

### 2.3.3 PHYLOGENETIC ANALYSIS

In all analyses (18S, 28S and 28S Gblock analyses), nematodes parasitising skink hosts and those parasitising gecko hosts grouped into separate monophyletic clades (Fig. 2.5). These two major clades were supported with 99-100% posterior probability values. Interestingly, the nematodes from skinks previously identified as *Skrjabinodon* form a clade with *Spauligodon* nematodes, indicating that these nematodes are more closely related to *Spauligodon* than *Skrjabinodon* worms from the New Zealand gecko hosts. Therefore, the nematodes infecting New Zealand skinks do not belong to the genus *Skrjabinodon* but rather *Spauligodon*. In terms of topologies, all 18S, 28S and
associated Gblock analyses were very similar with the 28S trees, showing slightly higher resolution in the within clade relationships. However, the within clade and between clade relationships were not always well supported and hence a combined 28S and COI analysis was conducted to improve this resolution. Therefore, only the 18S tree with the highest support is presented (Fig. 2.5) (all other trees can be viewed in appendix B).
Figure 2.2: Series of images showing some of the key features of *Skrabinodon* nematodes. A- adult female worm, B- three spines (or prongs) found at the base of the body near the tail, C- head of the worm showing the oesophagus and bulb, D- head of worm showing the lips, E- eggs, F- tail spines (left corner is part of the body spines), G- male morph 1, H- side view of male showing one of the caudal papilla.
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Figure 2.3: Series of images showing some of the key features of *Spauligodon* nematodes. A- adult female worm, B- tail region, C- head region of male worm, D- head region of female showing vagina, E- eggs, F- spines on tail, G- male worm, H- male showing caudal papillae
Figure 2.4: Sample RP999 that was identified as the undescribed species *Skrabinodon* ‘five prong’. A- juvenile worm, B- tail region showing prongs.
Figure 2.5: MrBayes inference tree for the 18S nuclear gene. The tree shows the phylogenetic relationships of the nematodes recovered from New Zealand gecko and skink lizard species with other representatives from the order Oxyurida. Numbers at nodes are posterior probabilities.
At the finer resolution (i.e. 28S, COI and their combined analyses), all gecko *Skrjabinodon* nematodes formed a monophyletic clade, which excluded outgroups, indicating that the sampled nematodes belong to the same genus as also supported in the 18S trees (Fig. 2.5). Tree topologies for *Skrjabinodon* nematodes were very similar with the exception of the COI maximum likelihood tree, but many branches in the latter tree were unsupported and, therefore, it was not a reliable estimate of phylogenetic relationships. The MrBayes analysis of the COI data differed from the combined COI and 28S analysis in that it could not resolve the relationship of RP84 whereas the latter analysis grouped RP84 (*W. maculata*, Maud Island) into a clade with the nematodes of *W. maculata* geckos from Wellington. The position of the sample RP330 (*W. ‘Otago large’, Macraes Flat) also slightly differed between these two analyses; in the joint data analysis, RP330 was the sister taxon to RP945 (*W. ‘Otago large’, Macraes Flat) (70% posterior probability) with this clade sharing a common ancestor with the *W. brunnea* gecko nematodes clade (59% posterior probability), while in the MrBayes bipartition model RP330 was basal (99% posterior probability) to the larger Brown gecko and Otago large clade (86% posterior probability).

The tree with the best support was the combined 28S and COI data tree that was partitioned by gene and position (Fig. 2.6) (see appendix B for all other trees). Specimens from the same locality and host formed well-supported clades (Fig. 2.6) with posterior probability values exceeding 71%. However, the between clade relationships were not well resolved, which is likely due to the incomplete data set. An interesting feature of this tree topology is the divergence of specimen RP999 (*D. pacificus*, Great Barrier Is.), which was morphologically identified and *Skrjabinodon* ‘five prong’, from the rest of the specimens. This split is well supported with a posterior probability of 100%. Specimens that were not included in this analysis because information was missing at the 28S marker (BH3, RP1377, RP315) also formed clades with other specimens from the same host and locality based on the COI marker. The sample RP315 (*Naultinus gemmeus*, Canterbury) formed a clade with the only other green gecko, *N. punctatus*, nematode (RP317).
Figure 2.6: MrBayes inference tree for the combined nuclear 28S gene and mtDNA COI gene data obtained from nematodes that were recovered from New Zealand geckos. The data was partitioned by gene and by position. The numbers at the branch nodes are the bootstrap values (%). The locality each sample was collected from is indicated on the map; Macraes Flat (yellow), Canterbury (red), Maud Island (pink), Wellington region (purple), Great Barrier Island. (orange).

The topologies of the skink nematode phylogenies at the finer resolution (28S, COI and combined analyses) were identical. However, the combined 28S and COI analysis that was partitioned by gene and position had the greatest support (Fig. 2.7). As in the 18S analysis, New Zealand skink nematodes formed a monophyletic clade with *Spauligodon* nematodes, thus confirming that these species belong to the genus *Spauligodon* and not *Skrjabinodon* where they have been placed until now. Unlike the gecko specimens, too few skink nematodes were collected to have multiple specimens per locality within the analysis.
2.3.4 GENETIC DISTANCES

The COI marker was highly divergent between gecko clades (Fig. 2.8, Table 2.5). The largest between group genetic distances were seen between group 6, the nematode morphologically identified as *S*. ‘five prong’ from the host *Dactylocnemis pacificus*, and the other five groups. These distances ranged from 0.248 (group 3: *Woodworthia maculata*, Great Barrier Is.) to 0.276 (group 1: *Woodworthia* ‘Otago large’, Macraes Flat). Group 4, the nematode from the host *N. punctatus*, and group 5, the Wellington/Maud Island *Woodworthia maculata* nematodes, had the lowest between-groups genetic distance at just 0.167. In terms of within group distances, these were generally low, with group 2 (*Woodworthia brunnea*, Canterbury) having an average within group distance of 0.01 and group 3 (*Woodworthia maculata*, Great Barrier Is.) 0.001. Group 1
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(Woodworthia ‘Otago large’, Macraes Flat) had the largest within group distance at 0.154. While sample RP1377 was not included in the group analysis because of missing 28S data, pairwise distance analysis showed that at this marker it was identical to RP945. The genetic distance of RP330 to these other two samples suggests that more than one nematode species is present at this location within the same host. For the 28S marker, genetic distances were smaller than the faster evolving COI marker and ranged from 0.003 to 0.068 (Table 2.5). RP999 was the most distant genetically to the other specimens with an average distance of 0.068, in contrast to all other clades which were less than 0.010 distant to each other.

![MrBayes inference tree for the combined nuclear 28S gene and mtDNA COI gene data obtained from nematodes that were recovered from New Zealand geckos. The tree is being used to depict the groups, represented by a number, that were used for genetic distance analysis. The numbers at the branch nodes are the posterior probabilities. Species names refer to the host the nematodes were collected from.](image)
Table 2.5: Genetic distances (uncorrelated p-distance) calculated for the COI and 28S gene between *Skrjabinodon* nematode groups, which are defined in Fig 2.9.

<table>
<thead>
<tr>
<th>Groups</th>
<th>COI</th>
<th>28S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.170</td>
<td>0.005</td>
</tr>
<tr>
<td>2</td>
<td>0.216</td>
<td>0.201</td>
</tr>
<tr>
<td>3</td>
<td>0.183</td>
<td>0.191</td>
</tr>
<tr>
<td>4</td>
<td>0.187</td>
<td>0.216</td>
</tr>
<tr>
<td>5</td>
<td>0.276</td>
<td>0.262</td>
</tr>
</tbody>
</table>

Based on the COI marker, skink nematodes were less diverse than the gecko *Skrjabinodon* nematodes (Table 2.6), but this conclusion is constrained by the sample size. The largest genetic distance was between the specimen RP1008 (*O. polychroma*, Great Barrier Island) and RP1818 (*O. polychroma*, Canterbury) at 0.136 (Table 2.6), and the smallest between W7 (*O. polychroma*, Wellington) and RP1369 (*O. maccanni*, Macraes Flat) (Table 2.6). For the 28S marker, genetic distances were less than 0.023. At the slowest evolving marker, the 18S, the genetic distances between skinks and geckos was 0.043 while the genetic distance between skinks and *Spauligodon* representatives used in this study was 0.018, thus confirming that the nematodes that parasitise New Zealand skinks belong to the genus *Spauligodon* rather than *Skrjabinodon*.

Table 2.6: Genetic distances (uncorrelated p-distance) between *Spauligodon* nematodes collected from New Zealand skinks at the COI and 28S genetic markers.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>COI</th>
<th>28S</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP1008</td>
<td>0.136</td>
<td>0.022</td>
</tr>
<tr>
<td>RP1818</td>
<td>0.111</td>
<td>0.023</td>
</tr>
<tr>
<td>RP1369</td>
<td>0.125</td>
<td>0.023</td>
</tr>
</tbody>
</table>

2.3.5 CO-PHYLOGENETIC ANALYSIS

The PACo analysis gave a residual sum of squares ($m^2_{XY}$) of 0.0074 with a permutational value of $P<$0.00001 for the overall global fit of the *Skrjabinodon* nematodes with their NZ gecko hosts. The contribution of each parasite-host link to the global fit can be visualised in the Procrustean superimposition plot in Fig. 2.9. The plot shows four main groups of associations; one is formed by *W. brunnea* hosts and associated nematodes, a second contains the *W. maculata* hosts, the third was the single
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*D. pacificus* host and the fourth was formed by the two *Naultinus* hosts. The bar plot in Fig. 2.10 shows that the associations between *W. brunnea* contribute little to \(m_{XY}^2\) and, therefore, likely represent co-evolutionary links. Similarly, the majority of the *W. maculata* and *W. ‘Otago large’* associations had low \(m_{XY}^2\) but the confidence intervals were large making it difficult to assess their contribution to co-phylogenetic patterns. Those with the highest \(m_{XY}^2\) were the *Naultinus* host associations.

*Figure 2.9*: Procrustean superimposition plot showing the fit of the parasite, *Skrabinodon*, to the New Zealand gecko host tree configuration after the association had been subjected to procrustes analysis (parasite configuration is rotated to fit the host configuration to minimise the squared differences between the two). The dots represent the parasite while the arrow tips are the host, which scientific names are abbreviated (full names can be found in Table 2.2). The arrow lengths represent the residual sum of squares (\(m_{XY}^2\)). Therefore, the shorter the arrows the more likely they are to represent co-evolutionary links.
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**Figure 2.10:** Jacknife residual plot resulting from applying PACo analysis to New Zealand gecko-*Skrjabinodon* associations. The dotted line represents the mean residual value. The () following W_mac represent the area the host was sampled from: G- Great Barrier Island, M- Maud Island and W- Wellington. The error bars are the upper 95% confidence intervals. This plot allows us to identify which associations are contributing to the squared residual value and thus identify the potential co-evolutionary links (i.e those that contribute little to squared residuals).
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The PACo analysis for the New Zealand skink hosts and their associated *Spauligodon* nematodes yielded an $m^2_{XY}$ of 0.0008 with a permutational value of $P<0.1118$. Overall, the host ordination does not predict the parasite ordination and the null hypothesis can be accepted for this data set. The links that contribute most to the $m^2_{XY}$ are *O. maccani* and *O. polychroma* (Clade 1) links while *O. aeneum* and *O. polychroma* (Clade 5) contribute very little to $m^2_{XY}$ (Fig. 2.11, Fig. 2.12).

**Figure 2.11** Procrustean superimposition plot showing the fit of the parasite, *Spauligodon*, to the New Zealand skink host tree configuration after the association had been subjected to procrustes analysis (parasite configuration is rotated to fit the host configuration to minimise the squared differences between the two). The dots represent the parasite while the arrow tips are the host, which scientific names are abbreviated (full names can be found in Table 2.2). The arrow lengths represent the residual sum of squares ($m^2_{XY}$). Therefore, the shorter the arrows the more likely they are to represent co-evolutionary links.
Figure 2.12: Jackknife residual plot resulting from applying PACo analysis to New Zealand skink-*Spauligodon* associations. The dotted line represents the mean residual value. The number following O_pol represents which clade the host belongs to. The error bars are the upper 95% confidence intervals. This plot allows us to identify which associations are contributing the to the squared residual value and thus identify the potential co-evolutionary links (i.e. those that contribute little to squared residuals).
2.4 DISCUSSION

The findings of this study allow, among other things, a re-evaluation of the diversity and taxonomy of nematodes parasitic in New Zealand lizards, an analysis of their phylogenetic relationships, and an assessment of their co-phylogenetic patterns. The following discussion addresses each of these issues in turn.

2.4.1 TAXONOMY

Morphology used to be the only source of taxonomic information; now, molecular data is easily obtainable to either complement morphological data, or offer new insights into the organisation of species. This is especially true for nematodes, which are notoriously difficult to identify based on morphological characters due to very minor structural differences being key to their identification (Abebe et al., 2011). One of the first aims of this study was to use molecular data to confirm the current taxonomic classification of the nematodes that parasitise NZ native skinks and geckos, which previously has solely been based on morphological characters (e.g. Barwick, 1959, Ainsworth, 1985, Ainsworth, 1992). To date, Ainsworth’s (1992) morphological survey of the nematodes parasitising native lizards remains the most comprehensive morphological study. The author identified all recovered nematodes to the family Pharyngodonidae, which agreed with several other authors who had previously recovered parasitic nematodes from these reptiles (e.g. Barwick, 1959, Clark, 1982, Ainsworth, 1985). The genetic data from this study confirms that nematodes parasitic in both skinks and geckos belong to the family Pharyngodonidae. The evidence comes from the 18S gene trees in which the sampled taxa nested within a larger clade containing other genera from this family. Furthermore, the specimens in this study were also more closely related to these genera than to the outgroup Thelastoma gueyei (Oxyurida, Thelastomatidae). Bootstrap and posterior probability values well supported these relationships.

At the level of genus, previous studies have identified Pharyngodonidae nematodes of NZ skinks to the genera Skrjabinodon (Ainsworth, 1990, Ainsworth 1992), Pharyngodon (Barwick, 1959) and Parathelandros (Clark, 1982, Ainsworth, 1985). Contrary to these studies, the nematodes collected in this study aligned with the genus Spauligodon; in the 18S and 28S gene trees the sampled taxa formed a clade with
Spauligodon nematodes rather than the Skrjabinodon nematodes from NZ geckos (Fig. 2.8). This begs the question: are these other genera reported to parasitise NZ skinks really present, or have they simply been misidentified because of the difficulties associated with morphologically distinguishing members of these genera?

Morphologically, the nematodes in this study matched descriptions provided by Ainsworth (1990) and Ainsworth (1992) suggesting that the same ‘species’ were examined in this study. Therefore, given the fit to Ainsworth’s definitions, the position of the nematodes recovered in the 18S and 28S gene trees, and the fact that genetic distance data showed a smaller genetic distance at the 18S gene to Spauligodon than to Skrjabinodon nematodes, it is recommended that all nematodes parasitising NZ skinks matching Skrjabinodon descriptions be reclassified into the genus Spauligodon. This recommendation may seem bold when only a few specimens were recovered in this study and representatives from many skink hosts and localities were missing. However, the facts that (i) the specimens collected all belonged to the same genus despite coming from a wide range of locations (e.g. Wellington, Great Barrier Is. and Macraes Flat) and (ii) the two other species, in addition to S. trimorphi, that parasitise skinks identified by Ainsworth (1992) have the general characters of S. trimorphi, support this move. Nevertheless, it is highly recommended that skink nematodes recovered in future, either by accident (i.e. in diet analyses of hosts) or through targeted studies, be sequenced at the same markers as this study and their sequences be added to the public database GenBank to further elucidate the diversity of nematodes present in NZ lizards.

In terms of the other genera reported from NZ skinks (e.g. Pharyngodon, Parathelandros) Ainsworth (1992) ruled out the possibility of these genera parasitising NZ lizards, concluding they were incorrectly identified. Thus, Spauligodon may be the only Pharyngodonidae nematodes present in NZ lizards. There are two other genera that have been reported to parasitise NZ skinks outside of the family Pharyngodonidae, Hedruris and Capillaria; no nematodes fitting these genera were recovered in this study and, therefore, their presence in NZ skinks cannot be confirmed.
Revisiting morphology, it is easy to understand why Ainsworth (1992) placed NZ skink nematodes in the genus *Skrjabinodon*. Solely based on morphological features, it is difficult to distinguish between members of the family Pharyngodonidae due to the morphological similarities between genera (Jorge et al., 2014). Females are often too similar in terms of morphology and genera are usually distinguished based on morphological characters of the males. This applies to the genera *Spauligodon* and *Skrjabinodon*, which are very closely related. The features that set these two genera apart are that *Spauligodon* males have caudal alae, which are not supported by the last pair of genital papillae while *Skrjabinodon* males generally do not have caudal alae (Gibbons, 2010). The genital papillae are also often reduced and sessile in the latter (Gibbons, 2010). Ainsworth (1990) found no caudal alae present in either of the male morphs which, based on the characters that currently define the genera, would place these nematodes in *Skrjabinodon*. Therefore, Ainsworth (1990) placement of these nematodes in this genus cannot be faulted. The present results, however, indicate that these characters are not reliable for discriminating between the genera and may be the product of either an ancestral state or convergent evolution. The lack of taxonomically informative morphological traits is likely to extend beyond these two genera within the family Pharyngodonidae. For example, in the 18S data trees *Thelandros* and *Parapharyngodon* did not form a monophyletic clade. Thus, a genetic revision of the Pharyngodonidae family should be conducted.

For the NZ gecko nematodes, this study confirms that all specimens here belong to the same genus. Morphologically they fit into the genus *Skrjabinodon*, determined by Ainsworth (1992). As there is currently no other available sequence for *Skrjabinodon*, the relationship of NZ specimens to other *Skrjabinodon* nematodes could not be examined. However, the NZ specimens did not group with any of the other genera, confirming that these fall outside the genera *Spauligodon, Thelandros* or *Pharyngodon*. This data supports Ainsworth (1990) whose survey suggests that this is the only genus present within NZ geckos. Comparing NZ *Skrjabinodon* nematodes to other congeners should be a future goal so the monophyly of this genus can be assessed.
2.4.2 PHYLOGENY, DIVERSITY AND CRYPTIC SPECIES

One of the major aims of this study was to reconstruct the phylogenies of the nematodes parasitic in NZ skinks and geckos. Unfortunately, due to the small number of specimens recovered, a complete phylogeny could not be assembled, which is most likely the reason why the between-clade relationships for Skrjabinodon could not be resolved. Despite these limitations, this study yielded some interesting and informative results. The first major point of interest in terms of phylogenetic relationships was that nematodes from NZ skinks are not of the genus Skrjabinodon but Spauligodon (see detailed discussion above). Another point of interest is that the results indicate that Skrjabinodon and Spauligodon are sister genera (100% posterior probability support, 18S tree). This is the first time this relationship has been investigated using genetic data. This result is in disagreement with Ainsworth (1992) who performed a cladistic analysis of this family, which at the time was split into four genera (Pharyngodon, Parathelandros, Skrjabinodon, Spauligodon), and came up with two possible phylogenies (Fig. 2.13), neither of which agrees with the outcome of this study. However, it is difficult to compare the present findings with Ainsworth’s analysis because of ongoing changes to the classification of this group. The most effective way to achieve overview analyses of families like this is the ongoing commitment from all researchers to add new sequences to public databases such as GenBank. If Spauligodon and Skrjabinodon are sister genera, it may help explain the morphological similarities between them. Morphological characters that were previously considered to be genus-specific are rather likely to be ancestral traits.

Figure 2.13: The two phylogenies of the family Pharyngodonidae hypothesised by Ainsworth (1992). Images credited to the author.
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SKRJABINODON:

On a finer level (i.e. the 28S and COI tree), several conclusions can be drawn about NZ Skrjabinodon nematodes. First, there is greater genetic structure present within these nematodes than has previously been detected; nematodes from each location grouped into individual clades and were separated from other clades by large genetic distances. For example, at the COI marker the smallest distance between Skrjabinodon clades was over 16% (N. punctatus and W. maculata) (see Table 2.5). These distances are comparable to interspecific distances obtained for other nematode species within the same genus such as between the cryptic species Spauligodon occidentalis and S. atlanticus (12.8%) (Jorge et al., 2011, Jorge et al., 2013b), or within the genera Oesophagostomum (11.5-13.7%) (de Gruijter et al., 2002), Ancylostoma (4.8-11.1%) (Hu et al., 2002) and Pellioditis (Derycke et al., 2005). Blouin et al. (1998) suggests that if two mtDNA sequences differ by 10%, we should consider whether these are truly conspecifics. Even among the few specimens that were sampled in this study, there are likely to be several provisionally cryptic species. This data gives us a snapshot of the diversity present within these nematodes and indicates that they may contain as much diversity as their hosts.

Cryptic species are not an uncommon finding within parasitic nematodes (e.g. Grillo et al., 2007, Tan et al., 2012, Karpiej et al., 2013) and the COI gene has been highly useful and popular for their identification because of its fast mutation rate (Ballard and Whitlock, 2004, Frézal and Leblois, 2008). However, genes do not necessarily reflect the evolution of a species. Each gene may have a unique evolutionary history: they are trees within the species tree (Szöllosi et al., 2013). Therefore, multiple genes should be used to delimitate species and build species trees (Dasmahapatra et al., 2010, Fujita et al., 2012, Collins and Cruickshank, 2013). For this reason, I only go as far as calling these genetically diverse groups provisionally cryptic. Further surveys should be carried out to investigate if there are detectable morphological differences between these groups and to determine their geographical ranges.

Within clades, nematodes from sympatric hosts of the same species always grouped within the same clade and had relatively low genetic distances. These genetic distances
are comparable to those found intra-specifically in other nematode species (e.g. de Gruijter et al., 2002, Hu et al., 2002) and suggest that only one ‘species’ per host/locality was detected. The one exception was *Spauligodon* specimens collected from *W. ‘Otago large’* at Macraes Flat. The sample RP330 was 15% different to the other two samples sequenced from that area (RP945 and RP1377) which is comparable to interspecific distances and suggests that there are potentially two nematode species parasitising this host in that general locality. RP330 was collected on a different property to the other two specimens, separated by >10 kilometres, so it would be interesting to further assess this apparent small-scale genetic variation.

The possibility that human error (e.g. mislabelled tubes, recording error, etc.) is responsible for the diversity detected at Macraes Flat is very small, as a careful system was used to process samples and the original tubes were double-checked. Furthermore, if this sample had been mislabelled and came from another location or host, it would be unlikely to form a clade with the *W. ‘Otago large’* samples. *Woodworthia ‘Otago large’* is the only gecko species found within this area. Further sampling at this locality and of this host would shed light on the genetic structure of these nematode populations. Two sympatric nematode species is not a particularly unusual result. Ainsworth (1992) recorded this from several NZ offshore islands, where there appears to be high diversity in *Skrjabinodon* nematodes (Ainsworth, 1992). Two nematode species within a single host population may occur at more localities around NZ but remains undetected because of limited samples. The question of interest in situations like this is whether this diversity arose through co-evolution or whether one of the species became associated with the host through a host-switching event (see 2.4.5).

Another point of interest relates to the *S. ‘five prong’* species. This species was basal to all other NZ *Skrjabinodon* samples in the joint 28S and COI analysis. However, this does not indicate that this species truly occupies this position; it could rather be an artefact of the small sample size in this study. Still, it does show that this specimen is genetically very different from all others. The chromatogram for this sample was good and therefore the distances are not a result of errors in the data. This data supports Ainsworth (1992) conclusion that *S. ‘five prong’* is indeed a different species and
requires a formal description. The genetic diversity of this species would be interesting to investigate because it occurs on several islands in several species of host.

The genetic distance between RP999 (S. ‘five prong’) and the other nematodes sampled is very interesting. This species is morphologically quite different from the other Skrjabinodon species parasitising NZ skinks, having five basal body spines rather than three, so some genetic differences were expected. A number of factors could explain why the S. ‘five prong’ specimen is so genetically distinct from all the other specimens; the smallest distance between S. ‘five prong’ and the other clades was 25% at the COI marker and 6% at the 28S. One explanation could be that this species underwent rapid evolution upon colonisation of a new host (Bromham and Penny, 2003). Alternatively, the ancestral five prong species could have colonised NZ geckos in a separate event to the ancestor of the other Skrjabinodon species, or along similar lines it could have arrived in NZ with the original lizard colonisations but already have diverged from the ancestor of the NZ Skrjabinodon nematodes in its native range. An interesting note is that Skrjabinodon nematodes with three large basal body spines are limited to NZ geckos and three other gecko species from Australia (Jones, 2013), where the ancestral gecko of NZ species is thought to have originated (Nielsen et al., 2011). This could indicate that Skrjabinodon arrived in NZ with the colonisation of the host and three base ‘prongs’ is an ancestral state. So, either S. ‘five prong’ evolved its additional ‘prongs’ post-colonisation or it arrived independently of other Skrjabinodon nematodes. Further sampling needs to be conducted to uncover the evolutionary history of these species.

SPAULIGODON:

Only a handful of Spauligodon specimens were recovered from NZ skinks and so the associated results need to be interpreted with caution. Fewer specimens obtained from skinks despite similar numbers of faecal samples to geckos most likely indicate lower infection prevalence in skinks (Ainsworth, 1992). The combined 28S and COI tree had very good general support, although for better phylogenetic accuracy a much wider taxon sampling needs to be conducted. The pairwise genetic distances at the COI marker were lower than for Skrjabinodon nematodes despite similar geographical
distances; 14% was the highest genetic distance between *Spauligodon* specimens while 17% was the lowest between-clade distance. This could be due to differences in the mutation rate of this marker between the two genera (Spradling et al., 2001). Alternatively, it could reflect differences in the evolution of these species, i.e. populations of *Spauligodon* may have remained connected for a longer period of time and therefore diverged later than those of *Skrabinodon*.

2.4.3 CO-PHYLOGENY AND PHYLOGEOGRAPHY

The main motivation behind this study was to test the fundamental evolutionary scenario captured by Farhenholz’s rule: that parasite phylogeny mirrors that of its hosts, in a lizard-nematode association. The goal was to test it using direct life-cycle endoparasites in order to advance our understanding of co-evolutionary processes. In particular, the main aim of this study was to determine which macro-evolutionary event, host switching or co-speciation, has been the most common path to modern day associations within NZ lizard-nematode associations. The working hypotheses were (i) that co-speciation played a major role in the evolution of associations between NZ skinks and geckos and their nematode parasites, and (ii) that where host-switching events occurred they should only be between hosts that share habitats and occur in sympatry. Although we were unable to obtain full data sets, the analysis revealed a significant global fit of the *Skrabinodon* tree to that of the gecko hosts. In other words, host phylogeny significantly predicted parasite phylogeny, a result that provides preliminary support for this first hypothesis.

The gecko-*Skrabinodon* links in the PACo analysis that showed the strongest evidence of co-evolution (i.e. that contributed the least to the squared residual values) were those involving *W. brunnea* and *W. ‘Otago large’*. These two gecko species are part of a larger clade of brown geckos that radiated into the central and east coast of the lower South Island (Nielsen et al., 2011). The hosts’ radiation into these different areas clearly separated them long enough to genetically isolate them and form these different species. Assuming the ancestral parasite was present in the ancestral species of this radiation, the isolation also would have caused the nematode parasite to become isolated because they lack dispersal mechanisms. Interestingly, today *W. ‘Otago large’*
frequently occurs in the same or nearby sites as the other species that form the lower South Island *Woodworthia* clade (*W.* ‘Cromwell’, *W.* ‘Central Otago’ and *W.* ‘Southern Alps’) but the other three species remain largely separated from each other (Jewell, 2006). Unfortunately, I was unable to obtain any nematode specimens from these other species; getting these samples would be important to uncover the colonisation patterns of these nematodes and determine whether the host-nematode associations examined here are truly a result of co-evolution. Testing divergence times once the parasite phylogeny is complete will also be essential to assess whether these modern day associations are the outcome of co-speciation.

Species of the *Woodworthia* clade that occur in sympatry will be particularly interesting for testing the second hypothesis: that host switches should only occur between sympatric hosts. It is well recognised that host switches are more likely to occur between species that are closely related (Jackson, 1999). Thus, this complex is primed for host switching events. Could this explain the provisionally cryptic species found in *W.* ‘Otago large’? The specimen RP330 was most closely related to the other two *W.* ‘Otago large’ samples (RP945, RP1377) and, therefore, could indicate that the ancestor of RP330 originated from a close relative of *W.* ‘Otago large’ from neighbouring regions and became associated with *W.* ‘Otago large’ through host switching events.

For example, *Woodworthia* ‘Central Otago’ would be a likely candidate for the origin of this ‘species’. Further extensive sampling could unravel the relationship between this host clade and its *Skrjabinodon* nematodes.

The associations between the *W. maculata* hosts and their nematode parasites from both Great Barrier Island and Wellington are slightly more difficult to assess in terms of their potential co-evolutionary relationships because of the large confidence intervals in the jackknife residual plot (Fig. 2.14). The sample RP84 is interesting; its relatively large contribution to the squared residual values indicates that co-speciation cannot account for this relationship. The *Skrjabinodon* combined 28S and COI trees indicate that this sample is most closely related to those from Wellington *W. maculata* geckos. This relationship is, therefore, hard to explain with the current data, although geography may be more important in explaining this relationship than co-speciation events. For example, there might be a cline of diversity across the range of *W. maculata*,
stretching from the top of the South Island, across the lower North Island and reaching
up the East Coast to Bay of Plenty and Gisborne regions as well as extending to Great
Barrier Island (Jewell, 2008).

The two specimens collected from captive green geckos were large contributors to the
squared residuals indicating that these associations were not the result of co-speciation
events. Although I tried to select captive populations whose nematode fauna most
likely represented the parasites from the ‘native’ range, contamination within captivity
may have occurred. The main reason behind this conclusion is that there is no
difference in COI sequences between these specimens even though *N. gemmeus* hosts
originated from the Canterbury region and *N. punctatus* from the Wellington region.
Given the distance between these localities, we would at least expect some genetic
divergence on this geographical scale.

Captive populations can provide tests of host specificity for parasites as the ecological
barriers between species are removed. In the present case, it appears that given the
chance *Skrjabinodon* nematodes are able to exploit other host species. These two green
gcko species are very closely related (average sequence divergence of only 2.4%),
with the radiation of *Naultinus* occurring approximately 5.4-13.9mya (Nielsen et al.,
2011). It is therefore not surprising this nematode is able to parasitise both species.
However, it would be interesting to see if the nematodes from narrow toed geckos
(which include green geckos) can infect broad toed geckos, which begun diverging
approximately 15.5-33.8mya, if given the opportunity. Infection of novel hosts in the
absence of geographical barriers has also been found in other species. For example,
Perlman and Jaenike (2003) were able to infect taxonomically diverse species of
*Drosophila* flies with five different species of nematode, although to variable extent,
and with greater success in close relatives of the native host.

In contrast to the *Skrjabinodon*-geckos associations, the skink phylogeny did not
significantly predict the ordination of *Spauligodon* nematodes. However, this study has
such a small representation of the nematode diversity that this result is not informative
about the overall co-evolutionary pattern between NZ skinks and their *Spauligodon*
nematodes. The results indicate that the association between RP1008 (Great Barrier Is.)
and its *O. aenenum* host could be the result of co-speciation. The *O. maccanni* and *O. polychroma* Clade 5 and their nematodes do not appear to be associated through co-evolutionary processes. There is no clear link between geography and the distribution of diversity either, with specimens from Canterbury being basal, and specimens from Wellington and Macraes Flat forming sister species.

Overall, these results provide evidence that strict co-speciation, i.e. Fahrenholz’s rule, is not the case for *Skrjabinodon* or *Spauligodon* nematodes and their associated hosts. Most other studies investigating associations between hosts and direct life cycle nematodes have also found that Fahrenholz’s rule does not apply (e.g. Zarlenga et al., 2006, Perlman et al., 2003, Beveridge and Chilton, 2001). Even one of the better examples, that of primates and their Strongylate nematodes, in which it was concluded that co-speciation was the main path to modern day associations, still required several host switching events and incomplete host switches to explain the current distribution of the parasite species (Glen and Brooks, 1985). Thus, the present study adds to the mounting evidence which suggests that associations that are exemplars of Fahrenholz’s rule are a rarity rather than a common theme, and not just for nematode associations but all parasite groups including mites, lice and trematodes (de Vienne et al., 2013).

Although a larger sample of the nematodes parasitic in NZ skinks and geckos needs to be obtained before the hypothesis that co-speciation is a dominant mechanism in direct life cycle nematode-host associations can be accepted or rejected, this study provides preliminary evidence that co-evolution events are likely to be important in the evolution of modern day associations between *Skrjabinodon* nematodes and their gecko hosts, with occasional host switching events. This result is comparable to other studies on direct life cycle nematodes. For example, co-speciation and host switching events have both been important in the evolution of the subfamily Cloacininae (Beveridge and Chilton, 2001, Chilton et al., 2011), mentioned in the Introduction of this chapter. However, the role of co-speciation appears to be more important in the evolution of *Skrjabinodon* nematodes than it has been for other host-nematode associations. For example, Perlman et al. (2003), Zarlenga et al. (2006) and Mayer et al. (2009) found host switching to be the dominant mechanism in the evolution of the associations between *Drosophila* flies and their *Howardula* nematodes, vertebrates and *Trichinella*,

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and beetles and their diplogastrid nematodes, respectively. As the relationship between NZ lizards and their nematode parasites is further examined, the factors influencing the occurrence of different macro-evolutionary events will become clearer.

2.4.4 CONCLUSIONS

This research has been important for advancing our knowledge of the small native nematodes parasitising NZ reptiles. Importantly, this work has revealed that the nematodes parasitising NZ skinks are not of the genus *Skrjabinodon* but instead *Spauligodon*, and these two genera are likely to be sister taxa. It has also highlighted that there is much more diversity present within these nematodes than has previously been recognised, potentially as much diversity as there exists within the reptile hosts. I provide evidence for provisionally cryptic species, although further sampling of these nematodes is required to uncover their full diversity and should be a priority of future studies. It is important to understand their biodiversity so conservation managers can make informed decisions on how to manage both parasite and host populations.

The number of specimens recovered limits the conclusions we can draw about the co-evolution between New Zealand lizards and their nematodes and the range of species they came from. Except for *D. pacificus* and the two green gecko specimens (which are not informative of wild parasite evolution), all nematodes that were obtained from geckos were parasites of broad-toed species. To gain a better understanding of the overall evolutionary patterns exhibited between geckos and their *Skrjabinodon* nematodes, ideally parasites would be obtained from all gecko clades. Different patterns may apply to green geckos, which are arboreal unlike many of their brown relatives in the broad-toed clade. What we do see is that Fahrenholz’s rule is not the norm in the two studied associations, and that host switching and co-speciation are both likely to be important events in the evolution of these parasites. Completing the phylogeny of these parasites is essential for gaining a better understanding of how they have evolved with lizards over evolutionary time.
3.1 INTRODUCTION

Mites (Acari) may be small in size but they are a remarkably diverse group of organisms that are an intricate part of the Earth’s ecosystems. Currently, more than 50,000 mite species have been described but their true diversity is yet to be uncovered, with the group estimated to contain upwards of one million species (Walter and Proctor, 2013). Their small size has allowed them to inhabit virtually every environment, from the soil of the forest floor to the rocky shore of the intertidal zone; they occur practically anywhere that is capable of supporting life (Walter and Proctor, 2013).

Mites exhibit a wide variety of life histories and may be free-living or involved in symbiotic relationships. Parasitic species occur on a diverse range of both invertebrate and vertebrate hosts, and their success at this mode of life can be measured by their occurrence on virtually every vertebrate taxon (Krantz, 2009). Like any true parasite, mites negatively impact their hosts’ health, although the degree to which they harm the host varies widely. Some of the reported effects on the hosts include weight loss (e.g. Giorgi et al., 2001), anaemia (e.g. Kilpinen et al., 2005), tissue damage (e.g. Curtis, 2004) and, more seriously, host death when mite densities are exceptionally high (e.g. Kilpinen et al., 2005). In addition to the damage mites themselves inflict, they are also vectors for a range of other harmful organisms, such as viruses, rickettsiae, spirochetes and protozoans (Krantz, 2009).

Just as they are on other vertebrates, mites are a common feature on reptiles, with species belonging to 15 families known to parasitise these cold-blooded hosts (Fajfer, 2012). Unlike birds and mammals where the feathers, hair or fur of the animal provides a protected environment, mites of reptiles, such as snakes and lizards, have to attach between the scales. Some families have adapted to live completely hidden beneath the host’s scales (e.g. Pterygosomatidae & Trombiculidae), whereas others are only partially covered by the scales (e.g. genus Ophiomegistus) but tend to attach in areas...
that are difficult to scratch (Fajfer, 2012). Interestingly, members of the families Trombiculidae and Pterygosomatidae often occur in what are known as mite pockets, which are pocket-like structures where the mites aggregate, located either in unprotected sites around the host’s body or in skin folds and joints (Fajfer, 2012). The role of these pocket structures has been widely debated; two of the main hypotheses are that they are an adaption of the host to concentrate the mites and reduce their damage (Arnold, 1986) and that mites make use of existing structures without any benefit to the host (see Bauer et al., 1993). The direct impacts mites have on their lizard hosts are currently unclear but some of the reported effects are similar to those reported for other vertebrates and include anaemia, skin damage and reduced body weight (e.g. Stahl, 2003, Klukowski and Nelson, 2001). Certain species of lizard mites have also been identified as vectors for a range of other pathogens, such as hemogregarines (e.g. Lewis and Wagner, 1964, Smallridge and Paperna, 1997). However, there is still much to learn about lizard-mite associations and the effects the mites have on their hosts.

As stated in chapter 1, this chapter focuses on the parasitic mites of New Zealand skinks and geckos. In the past few decades, only a small number of researchers have turned their attention to these native parasites and as a result there exists very little information on their distributions, their range of host species and their effects on those hosts. These elements are vital to understanding the mite biodiversity present in NZ and the host specificity of each mite species. This type of data is also important for efforts related to conserving both the parasite and host species. If conservation managers are aware of the mites’ diversity and distributions, then informed decisions can be made regarding when to treat hosts involved in translocations and when to move the parasite with the host. For these reasons, the aims of this chapter were first to review existing knowledge of the diversity of mites known to parasitise NZ native skinks and geckos, second, to add new host and location data from the samples collected as part of this thesis’ research, and finally to discuss these results and the biology of NZ mites and impacts they have on their hosts.
3.2 CURRENT STATUS OF NEW ZEALAND MITE DIVERSITY

3.2.1 METHODS

The search engine ‘Google Scholar’ was used to search for records of mites that parasitise NZ native lizard species. Following McKenna (2003) species list, each parasitic mite species was individually searched using its current name and then any past synonyms. Additional records were retrieved using combinations of the search terms: ‘New Zealand’, ‘lizard’, ‘mites’, ‘skinks’ and ‘geckos’. The reference lists of all articles containing mite records were also searched for additional literature. Records were only included if the mites were identified to genus or species by the author(s).

3.2.2 NZ LIZARD-MITE ASSOCIATIONS

The first record of mites parasitising NZ native lizards came from Hutton (1872) in the late 1800s, although the author only mentioned his awareness of their existence and made no investigation into the mites’ species identities. It wasn’t until several decades later that the first formal descriptions of NZ lizard mites emerged, the first of which were provided by Womersley (1941). On the examination of one captive Hoplodactylus duvauceli specimen and one gecko specimen belonging to the genus Naultinus later recorded as *N. elegans* by Clark (1982), Womersley (1941) discovered two different species of mites (Fig. 3.1). The author placed both of these species in *Geckobia* (*Geckobia haplodactyli* and *G. naultina*), a genus that belongs to Pterygosomidae (synonymous with Pterygosomatidae) (order Prostigmata), a family of scale mites that almost exclusively parasitise lizards (Wall and Shearer, 2008).

Since the original description, few new records for *G. haplodactyli* and *G. naultina* exist. Only two new records could be located for *G. naultina*. The first comes from van Winkel (2008) who reported the parasite to be present on *H. duvauceli* geckos that were translocated from Korapuki Island to Tiritiri Matangi and Motuora Islands. The second comes from a study that was reporting on the blood parasite of *H. duvauceli* (Barry et al., 2011), and also found these geckos to be parasitised by *G. naultina*. The geckos in this later study also originated from Korapuki Island and appear to have been part of the same translocation studied by van Winkel (2008). Two additional records were also all that could be located for *G. haplodactyli*. The first comes from Ainsworth
(1985) who conducted a study on the parasites of lizards in the Wellington region. Ainsworth (1985) recorded *G. haplodactyli* parasitising common geckos from that region. The other record comes from Whitaker (1968) who recorded the species to be parasitising *Dactylocnemis pacificus* from the Poor Knights Islands.

**Figure 3.1.** These illustrations were created by Womersley (1941) to aid his descriptions of the two *Geckobia* species. On the left is Womersley’s drawing of the species he named *G. haplodactyli*. The parts drawn are as follows: A. dorsal view of the mite, B. palp, C. tip of mandible, D. scutal seta, E. dorsal seta, F. right coxae, G. tip of tarsus with claws, H. tip of tarsus from above without the claws. The group of illustrations on the right is the species Womersley described as *G. naultina*. The parts drawn are as follows: A. dorsal view of mite, B. dorsal scutum, C. scutal seta, D. right coxae. No scale bars were included in these drawings.

Several years after the first descriptions of NZ lizard mites, Dumbleton (1947) added two additional species to the list. The first, named *Acomatacarus lygosomae*, came from a *Oligosoma grande* skink in the Oamaru area. This species has since been reassigned to the genus *Odontacarus*, a genus belonging to the family *Leeuwenhoekiidae*, Womersley 1945, which is one of several families commonly referred to as “chiggers”. *O. lygosomae* has since been recorded to infect *O. zelandicum*, *O. lineoocellatum* and *O. polychroma* skinks from the Wellington area (Ainsworth, 1985). Further host records include *O. otagense*, *O. polychroma* and *O. maccanni* skinks from Macraes Flat (Reardon and Norbury, 2004).
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The other species described by Dumbleton (1947) was *Trombicula naultini*, which he labelled as coming from the host *Naultinus elegans* collected from Invercargill. However, given the location of this specimen it is most likely that the host species was *N. gemmeus* (Hardy, 1972). This mite species has since been redescribed by Goff et al. (1987) and placed in the genus *Neotrombicula* (Fig. 3.2), a genus belonging to the family Trombiculidae (order Prostigmata), which is also included in the group labelled chiggers. Goff et al. (1987) recorded this mite species to parasitise the hosts *Woodworthia maculata* from Stephens Is., *Mokopirirakau granulatus* from an unknown locality and *D. pacificus* geckos from the Wairarapa and Mt St Bernard, Craigieburn Canterbury. However, *D. pacificus* have never been known to occur at Mt St Bernard, Craigieburn and it is likely this host was misidentified. The host specimen is more likely to be *W. maculata* but this cannot be confirmed unless the original specimens are examined. Other host records include *O. otagense* (Clark, 1982) and *H. duvaucelii* (Clark, 1982) and a species Clark (1982) noted as *Leioloopisma nigriplantare maccanni*. However, post-1990 this species has been split into several species including *O. maccanni*, *O. inconspicuum*, *O. microlepis* and *O. polychroma* (Freeman, 1997). As none of these records from Clark (1982) have data on locality we cannot ascertain which species this is likely to be. The tuatara species, *Sphenodon punctatus*, has also been reported as a host species for *N. naultini* (Clark, 1982).
As well as redescribing *N. naultini*, Goff et al. (1987) also added two new species to the list of known mites in New Zealand. The first also belongs to the genus *Neotrombicula* and is named *N. sphenodonti* (Fig. 3.3). While this species is named after its tuatara host, Ainsworth (1985) also recorded the mite parasitising the skinks *O. zelandicum* and *O. infrapunctatum* from the Wellington area and *O. lineocellatum* from Stephens Island. This author also recorded the species on the host originally listed as *Leioplotes nigriplanta maccanni*, which is most likely to be the skink now known as *O. n. polychroma* (agrees with McKenna, 2003). The second species described by Goff et al. (1987) was *Microtrombicula hoplodactyla* (Fig. 3.3) that was found on *W. maculata* geckos from Wellington. No other records since this description could be found. *Microtrombicula*, like *Neotrombicula*, belongs to the family Trombiculidae, Ewing 1944 (order Prostigmata).
Figure 3.3: These illustrations were drawn by Goff et al. (1987) and show the key distinguishing features (A. scutum, B. gnathosoma- dorsal, C. palpal tibia and tarsus-ventral) of *Neotrombicula sphenodonti* (left) and *Microtrombicula hoplodactyla* (right).

The last two mites that have been described to parasitise New Zealand lizards so far, bringing the total to eight species, belong to the genus *Ophionyssus* (family: Macronyssidae). The first of these two mite species, *O. galeotes* (Fig. 3.4), has so far been recorded on two host species: *H. duvaucelii* from Wellington, on which the specimens for the description were found (Domrow et al., 1980), and *W. maculata*, which Ainsworth (1985) identified from Stephens Island. The second species, *O. scincorum* (Fig. 3.4), was originally described from specimens obtained off the host Domrow et al. (1980) tentatively listed as *Leiolopisma otagense*. Prior to 1985 this species was considered to be a form of *O. otagense* but later was recognised as a distinct species now known as *O. waimatense*. Domrow et al. (1980) also recorded the mite species to parasitise the hosts *O. otagense* (Middlemarch), *O. moco* (Northern North Is.), and *O. polychroma* (originally listed at *L. nigriplantare*, Foxton). However, *O. polychroma* were being held captive with *O. moco* therefore makes these two records somewhat unreliable because we cannot know whether these lizards would usually be parasitised by this mite in the wild or whether this is an artefact of captive conditions. Interestingly, the author also recorded protonymphs of the mite from the skink species *Niveoscincus pretiosum* from Tasmania. Perhaps mites recorded from these two countries shared a common ancestor but are now unlikely to be the same species given the large distance and the Tasman Sea that separates the two. The species has since been recorded at Macraes Flat infecting the host species *O. grande*, *O.*
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*Otagense, O. polychroma* and *O. maccanni* (Reardon and Norbury, 2004) as well as *O. lineoocellatum* skinks from Wellington (Ainsworth, 1985). Thus conclude the current records of mites that parasitise NZ lizards. Table 3.1 provides a summary of the records of NZ lizard-mite associations described above.

Figure 3.4: These illustrations were drawn by Domrow et al. (1980). The top row is a dorsal (left) and ventral (right) view of the mite *Ophionyssus galeotes* and the bottom row shows a dorsal (left) and ventral (right) view of the mite *O. scincorum*. 
Table 3.1: A summary of the mite species currently known to parasitise NZ lizards. Listed are the known host species, the geographic locations of these hosts and associated references. For completeness, tuatara records are also included. * Indicates records in which the host species is likely to have been misidentified or is recorded differently to what the original author states; refer to text (3.2.2) for more details.

<table>
<thead>
<tr>
<th>Mite species</th>
<th>Host records</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Geckobia haplodactyi</em></td>
<td><em>Hoplodactylus duvaucelii</em> Captive-origin unknown (Womersley, 1941)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Woodworthia maculata</em> Wellington (Ainsworth, 1985)</td>
<td></td>
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<tr>
<td></td>
<td><em>Dactylocnemis pacificus</em> Poor Knights Islands (Whitaker, 1968)</td>
<td></td>
</tr>
<tr>
<td><em>Geckobia nautilina</em></td>
<td><em>Naultinus sp.</em> Auckland (Womersley, 1941)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hoplodactylus duvaucelii</em> Korapuki Island (Barry et al., 2011; van Winkel, 2008)</td>
<td></td>
</tr>
<tr>
<td><em>Odontacarus lygosomae</em></td>
<td><em>Oligosoma grande</em> Oamaru (Dumbleton, 1947; Reardon &amp; Norbury 2004)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oligosoma otagense</em> Macraes Flat (Reardon and Norbury, 2004)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oligosoma maccanni</em> Macraes Flat (Reardon and Norbury, 2004)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oligosoma polychroma</em> Macraes Flat (Reardon and Norbury, 2004)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oligosoma zelandicum</em> Wellington (Ainsworth, 1985)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oligosoma lineoocellatum</em> Wellington (Ainsworth, 1985)</td>
<td></td>
</tr>
<tr>
<td><em>Neotrombicula nautilini</em></td>
<td><em>Naultinus gemmeus</em> Invercargill (Dumbleton, 1947)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Dactylocnemis pacificus</em> Lake Pounui, Wairarapa (Goff et al., 1987) Mt St Bernard, Craigieburn* (Goff et al., 1987)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Woodworthia maculata</em> Stephans Island (Goff et al., 1987, Clark, 1982)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Woodworthia ‘Otago large’</em> Macraes Flat (Reardon and Norbury, 2004)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Mokopirirakau granulatus</em> Location unknown (Goff et al., 1987)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Sphenodon punctatus</em> Location unknown (Clark, 1982)</td>
<td></td>
</tr>
</tbody>
</table>
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- **Hoplodactylus duvaucelli** Location unknown (Clark, 1982)
- **Oligosoma otagense** Location unknown (Clark, 1982)
- **Oligosoma sp.** Location unknown (Clark, 1982)

**Neotrombicula sphenodonti**
- **Sphenodon punctatus** Stephens Island (Goff et al., 1987)
- **Oligosoma infrapunctatum** Stephens Island (Goff et al., 1987, Ainsworth, 1985)
- **Oligosoma polychroma** Wellington (Ainsworth, 1985)
- **Oligosoma zealandicum** Wellington (Ainsworth, 1985)
- **Oligosoma lineooecellatum** Wellington (Ainsworth, 1985)

**Microtrombicula hoplodactyla**
- **Woodworthia maculata** Wellington (Ainsworth, 1985, Goff et al., 1987)

**Ophionyssus galeotes**
- **Hoplodactylus duvaucelli** Wellington (Domrow et al., 1980)
- **Woodworthia maculata** Stephens Island (Ainsworth, 1985)

**Ophionyssus scincorum**
- **Oligosoma otagense** Middlemarch (Domrow et al., 1980)
- **Oligosoma waimatense** Molesworth (Domrow et al., 1980)
- **Oligosoma moco** Northern North Is. (Domrow et al., 1980)
- **Oligosoma polychroma** Foxton (Domrow et al., 1980)
- **Oligosoma maccanni** Macraes Flat (Reardon and Norbury, 2004)
- **Oligosoma grande** Macraes Flat (Reardon and Norbury, 2004)
- **Oligosoma lineooecellatum** Wellington (Ainsworth, 1985)

**Ophionyssus sp.**
- **Dactylocnemis pacificus** Poor Knights Islands (Whitaker, 1968)
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3.3 **SURVEY OF NZ LIZARD MITES**

### 3.3.1 METHODS

Parasitic mites were collected from four localities around New Zealand: Great Barrier Island, Wellington, Macraes Flat and Christchurch (see chapter 2 for a map showing the locations). Mites were collected from all parasitised lizard species handled at these locations; refer to Table 3.2 in the results section (3.3.2) for a complete list of lizard species sampled. All lizards sampled in this study were being handled for other research purposes and therefore permits for handling the animals were held by the respective company/researchers. In the field, watchmaker forceps were used to gently pluck the mites off the host. Mites were stored in Eppendorf tubes pre-filled with 75-96% ethanol. Each tube contained mites from a single lizard. Only a few mites per individual host were collected to minimise handling time and therefore stress on the lizard as the animals were already being handled for other purposes. In the laboratory, the mites were individually mounted on temporary slides and examined under an Olympus CX41 compound microscope for key features using a range of magnifications. All mites collected from each host were identified because multiple species can occur on a single host (e.g. Reardon and Norbury, 2004). Characteristics given in the original species descriptions were used to identify the mites recovered in this survey.

### 3.3.2 RESULTS

In total four mite species were recovered: *N. naultini* (Fig. 3.5), *N. stephondonti* (Fig. 3.6), *M. hoplodactyla* (Fig. 3.7) and *O. lygosomae* (Fig. 3.8), were collected from 10 lizard species at five different localities throughout NZ. *Woodworthia brunnea* from the Christchurch region was both a new host and location record for *M. hoplodactyla*. Christchurch was also a new location record for *Odontacarus*. Great Barrier Island and Boundary stream (Hawke’s Bay) were new location records for the species *N. naultini*. All other locations and species recorded in this study matched existing records. Table 3.2 summarises the data found in this study. In some cases *Neotrombicula* mites could not be identified to species and were conservatively listed as *Neotrombicula* spp. This was usually because I could not get a clear image of the scutum, the shape of which is the main difference between the two *Neotrombicula* species (Fig. 3.5, Fig. 3.6).
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**Table 3.2:** A list of the mite species collected in a small survey of NZ skinks and geckos. Listed are the host species and the location of collection. * Indicates new host or location records

<table>
<thead>
<tr>
<th>Location</th>
<th>Mite species</th>
<th>Tube code</th>
<th>Host species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neotrombicula</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Great Barrier Is.*</td>
<td><em>N. naultini</em></td>
<td>RP979</td>
<td><em>D. pacificus</em></td>
</tr>
<tr>
<td></td>
<td><em>N. naultini</em></td>
<td>GB2</td>
<td><em>D. pacificus</em></td>
</tr>
<tr>
<td></td>
<td><em>N. spp</em></td>
<td>GB5</td>
<td><em>D. pacificus</em></td>
</tr>
<tr>
<td></td>
<td><em>N. spp</em></td>
<td>RP973</td>
<td><em>W. maculata</em></td>
</tr>
<tr>
<td></td>
<td><em>N. naultini</em></td>
<td>RP981</td>
<td><em>M. granulatus</em></td>
</tr>
<tr>
<td>Wellington</td>
<td><em>N. stephondonti</em></td>
<td>W11</td>
<td><em>O. polychroma</em></td>
</tr>
<tr>
<td></td>
<td><em>N. stephondonti</em></td>
<td>W80</td>
<td><em>O. polychroma</em></td>
</tr>
<tr>
<td></td>
<td><em>N. stephondonti</em></td>
<td>RP96</td>
<td><em>O. polychroma</em></td>
</tr>
<tr>
<td></td>
<td><em>N. spp</em></td>
<td>W2</td>
<td><em>W. maculata</em></td>
</tr>
<tr>
<td></td>
<td><em>N. naultini</em></td>
<td>W59</td>
<td><em>W. maculata</em></td>
</tr>
<tr>
<td>Macraes Flat</td>
<td><em>N. naultini</em></td>
<td>M60</td>
<td>*W. ‘Otago large’</td>
</tr>
<tr>
<td></td>
<td><em>N. naultini</em></td>
<td>M59</td>
<td>*W. ‘Otago large’</td>
</tr>
<tr>
<td>Boundary Stream*</td>
<td><em>N. naultini</em></td>
<td>BSHg</td>
<td>*M. ‘southern North Island’</td>
</tr>
<tr>
<td></td>
<td><em>N. naultini</em></td>
<td>BSHg</td>
<td>*M. ‘southern North Island’</td>
</tr>
<tr>
<td><strong>Microtrombicula</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wellington</td>
<td><em>M. hoplodactyla</em></td>
<td>W53</td>
<td><em>W. maculata</em></td>
</tr>
<tr>
<td>Christchurch*</td>
<td><em>M. hoplodactyla</em></td>
<td>RP1873</td>
<td><em>W. brunnea</em></td>
</tr>
<tr>
<td></td>
<td><em>M. hoplodactyla</em></td>
<td>RP1817</td>
<td><em>W. brunnea</em></td>
</tr>
<tr>
<td><strong>Odontacarus</strong></td>
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<td></td>
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</tr>
<tr>
<td>Macraes Flat</td>
<td><em>O. lygosomae</em></td>
<td>RP1388</td>
<td><em>O. maccanni</em></td>
</tr>
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<td></td>
<td><em>O. lygosomae</em></td>
<td>Og112</td>
<td><em>O. grande</em></td>
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<td></td>
<td><em>O. lygosomae</em></td>
<td>Oo8-AL</td>
<td><em>O. otagense</em></td>
</tr>
<tr>
<td>Christchurch*</td>
<td><em>O. lygosomae</em></td>
<td>RP1139</td>
<td><em>O. lineoocellatum</em></td>
</tr>
<tr>
<td></td>
<td><em>O. lygosomae</em></td>
<td>RP1124</td>
<td><em>O. lineoocellatum</em></td>
</tr>
</tbody>
</table>
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- *O. lygosphasmae*  RP1054  *O. lineoocellatum*
- *O. lygosphasmae*  RP1122  *O. lineoocellatum*
Figure 3.5: Series of images showing some of the key features of the mite *Neotrombicula naultini*. A- dorsal view of head and legs, B- scutum, C- ventral view of gnathosoma, D- dorsal view of mite, posterior dorsal setae, F- another view of the scutum.
Figure 3.6: Series of images showing some of the key features of the mite *Neotrombicula stephendonti*. A- dorsal view body, B- ventral view of leg arrangement, C- ventral view of gnathosoma, D- ventral view of mite with host tissue attached, E- scutum, F- dorsal setae.
Figure 3.7: A series of images showing some of the key features of the mite *Microtrombicula hoplodactyla*. A- Dorsal view showing a clear view of the scutum, B- head and leg positions, C- ventral setae, D- whole body, E- close up showing the scutum, F- dorsal setae.
Figure 3.8: A series of images showing some of the key features of *Odontacarus lygosomae*. A- Scutum, B- head, C- ventral setae, D- gnathosoma (circled), E- dorsal setae, F- second and third legs.
3.4 Discussion

The review of the known lizard-mite associations, performed in the first part of this chapter, achieves two important things. First, it provides a comprehensive resource for future lizard-mite studies so that new host and location records can easily be identified. Secondly, it highlights how little attention these native parasitic mites have received in the past 100 plus years. Our documentation of the lizards these mites parasitise and their distributions is limited and incomplete. To complicate matters further, many of the hosts reported in old records, before the modern classification of NZ lizards, are difficult to match with modern names because of the many synonyms, name changes and reshuffling of the species in the days prior to their genetic assessment (see Chapple et al., 2009, Nielsen et al., 2011). To date, there have been no widespread surveys exploring the NZ mite fauna; rather, we have relied on opportunist descriptions and occasional reporting of the species observed. Therefore, it is highly likely that we are not currently aware of the full extent of diversity of the NZ lizard mite fauna.

The modest survey of the NZ lizard mite fauna conducted as part of the present research was able to expand our current records. Four of the eight species of mites known to parasitise NZ skinks and geckos were collected and of these four species the known geographic range for three of these were expanded. This study also provides a new host record for the mite *M. hoplodactyla*, which was previously only known from *Woodworthia maculata* in the Wellington region; now we know it to also occur on *W. brunnea* from the Christchurch area. The widespread geographical range of these mites raises questions about the diversity present within these species and the possibility of cryptic species. Ainsworth (1985) noted some differences between her specimens and the original description of *O. galeotes*, which may be indicative of more diversity yet to be uncovered. However, as pointed out by Ainsworth, the small number of specimens makes it hard to know if these differences indicate undescribed species or natural intraspecific variation. Genetic studies will be useful for detecting cryptic species or highlighting diversity that may not be recognised by non-mite specialists or morphological studies. Vargas (2006) has given us the first glimpse of the diversity still
to be discovered in NZ mites, finding possible cryptic species within *Odontacarus* mites, between the two locations Macraes Flat, Otago and Birdings Flat, Canterbury.

Given the few investigations into the species of mites parasitic on NZ reptiles, a large amount of diversity has already been revealed. Surprisingly, these species have very different evolutionary histories. Unlike the parasitic nematodes of NZ native skinks and geckos, which belong to one family but two different genera (see chapter 2), the eight mite species known to occur in NZ belong to four different families: Pterygosomatidae, Leeuwenhoekiidae, Trombiculidae and Macronyssidae. These families exhibit varying life cycles and also differ in the stage at which mites are parasitic. For example, mites belonging to Pterygosomatidae are permanent parasites (Fajfer, 2012) while Leeuwenhoekiidae and Trombiculidae are only parasitic in the larval stages and free-living as adults (Shatrov and Kudryashova, 2006). The diversity and the success of these parasites on NZ lizards clearly demonstrate that these mites form an important association with the NZ reptile fauna. Like other lizard-mite associations, these mites are likely to have varying negative impacts on their hosts. These impacts may be associated with feeding and attachment of the mite on the host and/or by acting as vectors for other pathogen species. These two points are discussed in further detail below.

The effect of mites on NZ lizards has only been investigated once. Hare et al. (2010) tested the impact of *Odontacarus* mites on reproductive success of the McCann’s skink (*Oligosoma maccanni*). The study found that in the year mites were removed, the skinks had an increased pregnancy success compared with the year in which the mites were left on the skinks. However, the study linked this result with captive rearing and concluded that it was one of multiple stressors associated with captivity that probably tipped the balance; in the wild, this apparent effect of mites is probably less pronounced. The impacts of mites on their lizard hosts, in general, are poorly understood. Some mite species have been shown to have from little to no effect (e.g. Brennan et al., 2009), while others have been reported to have much larger negative impact such as reducing weight gain (e.g. Klukowski and Nelson, 2001). In captivity, mites have been known to cause anaemia, dysecdysis (skin shedding problems) and ulcerative dermatitis (Stahl, 2003) as well as increased mortality (Sorci and Clobert, 1995).
Intracellular apicomplexan parasites belonging to the hemogregarine group (family: Haemogregarinidae) are the most common blood parasites infecting lizards (García-Ramírez et al., 2005, Maia et al., 2011). Those that parasitise reptilian hosts usually belong to the genus *Hepatozoon* (Vilcins et al., 2009). Parasitic mites of lizards have been identified as vectors for these blood parasites (e.g. Ramanandan Shanavas and Ramachandran, 1990). In NZ the genus *Ophionyssus* has also been recognised as a vector of hemogregarine parasites. Dore (1919) was the first to describe NZ lizard hemogregarine as *Haemogregarina lygosomarum* though the species was later moved to the genus *Hepatozoon* (Allison and Desser, 1981). Allison and Desser (1981) were the first to provide experimental evidence showing NZ *Ophionyssus* mites were vectors for this blood parasite; mature gametocytes were seen to be emerging from the host blood cells in the gut of mites that had engorged on skinks heavily infected with the parasite. The only other study that has examined *Hepatozoon* parasites in NZ lizards found a significant relationship between individuals infected with *Hepatozoon* and the level of infection with the mite *O. scincorum* (Reardon & Norbury 2004). The authors found all individuals infected with *Haemogregarina* were also infected with *O. scincorum* and concluded that *O. scincorum* was the main vector for the blood parasite. It is not yet known what effects this blood parasite has on NZ lizard hosts although (Allison and Desser, 1981) noted that *H. lygosomarum* did not seem to affect the health of the host or its young. There have been some reports of mild anaemia (see Telford, 1984), however few other investigations have been made into the effects they have on their lizard hosts. In general the effects *Hepatozoon* has on reptile hosts is very poorly understood.

### 3.5 Conclusion

This small-scale study on the parasitic mites of NZ lizards has highlighted that mites are widespread and abundant associates of lizards. In addition it has shown just how much there is still to learn about these small but significant organisms. Future large-scale attempts to uncover the diversity of mites parasitising NZ lizards need to be carried out. This study was able contribute in several ways to enhancing our knowledge of these parasites by revealing a number of new localities for three of the four species recovered. I hope that this work will encourage other researchers to focus on these
important members of our fauna and continue to expand our understanding. This knowledge can be included when tackling issues surrounding the continual decline of the NZ reptile fauna.
CHAPTER 4: CONCLUSIONS

The intimate associations between hosts and their parasites have long intrigued biologists. Naturally, this curiosity has led them to raise questions about the origins of such associations, which ultimately gave rise of the field of co-phylogenetics (Page, 2003). Although this field has a long history, it remains a relevant and active area of research (Brooks and McLennan, 1993). Today co-phylogenetic studies continue to help uncover the processes and patterns of evolution that have led to modern-day associations between hosts and their parasites. This is not only important for understanding fundamental concepts of evolution but is particularly relevant for understanding diseases and their emergence. However, the factors that predict a history of host switching versus one dominated by co-speciation events are still not well understood and many parasitic groups, such as endoparasites, need further investigation under a co-phylogenetic framework in order to identify general rules of evolution.

This thesis set out to further our understanding of how parasitic nematodes and their lizard hosts evolved over evolutionary time, nematodes being a group that has received relatively little attention from a co-phylogenetic perspective. The aim was to investigate an association that had the potential to be a good model system in order to test the following hypothesis: host-nematode associations, in which the parasites have limited dispersal, will follow mostly a co-speciation pattern (chapter 2). New Zealand lizards (skinks and geckos) and their nematode parasites were identified as an ideal candidate for co-phylogenetic analysis because the hosts are monophyletic and there is only one introduced species of lizard in NZ. This system was also of interest because nematodes parasitic in New Zealand lizards have received very little attention in the past and this study offered the opportunity to advance our knowledge of these small native parasites. Therefore, this thesis also aimed to achieve several secondary goals, including assessing the diversity of the parasitic nematodes of NZ lizards (i.e. detecting cryptic species) and reconstructing the nematodes’ phylogenies in order to determine the structure of their relationships.
Chapter 4: Conclusions

The two latter aims listed above, i.e. uncovering the diversity of nematodes of NZ lizards and phylogeny re-construction, were achieved in chapter 2, with several important results emerging from this research. First, the study found that there is more diversity present within skink and gecko nematodes than has been previously recognised, and evidence for provisionally cryptic species of *Skrjabinodon* nematodes was provided. Second, the phylogenetic relationships of the parasites were determined, which revealed that nematodes from skinks and nematodes from geckos form monophyletic groups. Importantly, the nematodes from NZ skinks were reclassified from the genus *Skrjabinodon* to *Spauligodon*. However, the finer scale relationships between clades were difficult to resolve because of the limited number of nematode specimens recovered, which was an obvious limitation of this study. Nevertheless, the above results have important implications for both NZ conservation and wider nematode taxonomic studies.

The first implication, which is associated with the reclassification of NZ skink nematodes, is that the morphological characteristics that are used to separate *Spauligodon* and *Skrjabinodon* are probably ancestral traits rather than specific to the genera. Because so few phylogenetic studies have been conducted within Oxyuridea nematodes, it is highly likely that many species as they are currently classified do not represent monophyletic taxonomic groups. The second implication of these results relates to the discovery of cryptic species. The continual uncovering of cryptic species in light of molecular methods demonstrates that there is still much diversity within nematodes, both in New Zealand and worldwide, that is currently unrecognised. It also shows the importance of integrating molecular techniques into nematode taxonomy.

The last implication relates more directly to NZ conservation management. Following the recognition of new units of diversity, conservation managers can use this information to make informed decisions on how to manage both parasite and host populations, which may be particularly relevant when the host is involved in translocations.

The main aim of this study was also addressed in chapter 2. This study gave us the first insights into the co-phylogenetic relationships between NZ lizards and their nematode parasites. However, because too few nematodes were recovered in this study, I did not
have enough evidence to convincingly support or reject the main hypothesis tested here: i.e. that host-nematode associations, which are characterised by limited dispersal, should follow mostly a co-speciation pattern. The results of the PACo analysis, however, provided preliminary evidence that co-evolutionary processes have been important in the evolution of *Skrjabinodon* nematodes. The analysis also provided evidence that Fahrenholz’s rule (i.e. strict co-speciation) is probably not the dominant pattern and thus added to the growing body of evidence that suggests this rule is, ironically, the exception (de Vienne et al., 2013). Despite the fact that Fahrenholz’s rule may not be the most common path to modern-day associations, the rule is still useful to test in order to uncover the events that have been important in the evolution between hosts and parasites. However, our attention should now switch from whether or not the rule applies to focusing on identifying the factors that promote various macro-evolutionary events.

The final part of this thesis (chapter 3) took the opportunity to conduct a small survey and review of the parasitic mites of NZ skinks and geckos, another group of parasites that have received very little attention. The aim of that chapter was to review the existing knowledge of the diversity of mites known to parasitise NZ native lizards and to add new host and location data. Furthermore, I aimed to discuss the impact these mites have on their hosts. In addition to providing a comprehensive review of mite distributions and effects on hosts, this part of my work added several new location records and a new host record. The findings of that chapter are important for several reasons. First, providing new host-parasite records is important for understanding basic aspects of the ecology of these parasites, such as their host specificity. Second, reviewing current knowledge will hopefully encourage new research on this group. NZ reptiles continue to decline and therefore these mites are also at risk; we should strive to understand this group in order to decide best how to manage them.

Overall, this study has brought new insights to the parasitic nematodes and mites of NZ skinks and geckos. This thesis highlights how little we know about these small natives and I hope it will encourage other researchers to take an interest in these forgotten but important members of our fauna. The insight this study has given into the evolutionary history of the host-nematode associations examined provides another step in
uncovering the larger patterns of host-nematode evolution. However, further research will be required to uncover general evolutionary patterns. Thus, it is important to continue to focus on understudied groups to come to a better understanding of general host-parasite evolutionary processes.

So what should come next? High on the priority list should be studies that focus on the basic biology and ecology of both of these parasitic groups. Some suggested and important questions to ask are: what are the infection levels in natural populations (mean number of nematodes or mites per host)? What are the sex ratios of nematodes within the hosts? And do infection levels differ between males and females or young and old hosts? Further research is also needed to investigate the genetic diversity contained within both mite and nematode species, both at the population level and on a larger scale. This is important for understanding the biodiversity in NZ and is necessary for implementing appropriate management plans. With more intensive sampling of nematodes focused on the more common lizard species, it should be possible to resolve the genetic structure and phylogeography of the more widespread parasite species.

Another aspect that requires further investigation is host specificity. Host specificity is considered to be one of the most fundamental properties of parasitic organisms (Poulin and Keeney, 2008, Poulin et al., 2011), but it remains poorly understood for these parasites. Field surveys are required to determine the hosts each species parasitises, however laboratory experiments, investigating host specificity by exposing multiple host species to multiple nematode species, would be especially interesting. In particular, attempting to infect a host species with the nematodes from either a sympatric or an allopatric host, would reveal much about the role of geographic separation in restricting host specificity (and host switching).

Completing the phylogenies of the parasitic nematodes of NZ skinks and geckos should also be a goal of future studies. This will not only help to further understand the phylogenetic relationships within these groups, but also help to uncover the colonisation patterns of these parasites. It would be very useful to include sequences from nematodes belonging to the same genera from other countries, when these become available, in future phylogenetic assessments to help uncover the origin of NZ nematodes but also to assess the monophyly of these genera. Importantly and perhaps
most relevant to the major aims of this study, completing the phylogenies of these groups is necessary for further investigating the co-phylogenetic patterns of NZ nematodes with their lizard hosts. This study showed that these two nematode genera and their respective hosts have good potential to be model systems for host-nematode studies because of their reasonably uncomplicated pattern of evolutionary diversification (i.e. both hosts and parasites form monophyletic groups).

On a final note, I would like to again emphasise the importance of studying a larger range of host-parasite associations in order to identify the factors that promote certain evolutionary histories, and to further explore the nature of host-parasite associations. This in turn could be used to increase our predictive power of host switching events in certain species/situations. It is an exciting time for this field as new technologies and more powerful analyses continue to become available and help us understand the intricate associations between hosts and their parasites.
APPENDICIES

APPENDIX A: NZ LIZARD NEMATODE MORPHOLOGICAL MEASUREMENTS

Table A1: Measurements of key features used to identify parasitic nematodes collected from skinks and geckos native to New Zealand. Abbreviations are as follows: Loc- location of host, BL- body length, BW- body width, TL- tail length, TW- tail width, OW- oesophagus length, OL- oesophagus length, OBL- oesophagus bulb length, OBW- oesophagus bulb width, ExP- excretory pore position, Vu- vulva position, ELA- average length of four eggs, EWA- average width of four eggs, spines- no. of spines of tail, LA- lateral alae, caudial trunk: widest point (CT1) and narrowest point (CT2), third papillae width: 3pl- tip, 3p2- middle, 3p3- insertion point, 3PL- third papillae length. Abbreviations for locations are as follows: Wel- Wellington, Cant- Canterbury, Mac- Macraes Flat, GB- Great Barrier Island. Full host names can be found in Table 2.2 (chapter 2). All measurements are in micrometers. See Jorge et al. (2013) for measurement descriptions. (J) shows individuals which were juveniles.

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**Appendicies**

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**APPENDIX B: PHYLOGENETIC TREES**

Figure B.1: RAxML tree based on 18s data obtained from the parasitic nematodes infecting New Zealand native skinks and geckos. Numbers at nodes are bootstrap values (%). Codes represent nematode individuals. Information on each nematode can be found in chapter 2, Table 2.2.
Figure B.2: MrBayes inference tree based on 28s data obtained from the parasitic nematodes infecting New Zealand native skinks and geckos. Numbers at nodes are posterior probabilities. Codes represent nematode individuals. Information on each nematode can be found in chapter 2, Table 2.2.
Figure B.3 RAxML tree based on 28s data obtained from the parasitic nematodes infecting New Zealand native skinks and geckos. Numbers at nodes are bootstrap values (%). Codes represent nematode individuals. Information on each nematode can be found in chapter 2, Table 2.2.
Figure B.4 MrBayes inference tree based on 28s and COI data obtained from the parasitic nematodes infecting New Zealand native geckos. Data was partitioned by gene and position. This tree excludes the third position. Numbers at nodes are posterior probabilities (%). Codes represent nematode individuals. Information on each nematode can be found in chapter 2, Table 2.2.
Figure B.5: MrBayes inference tree based on 28s and COI data obtained from the parasitic nematodes infecting New Zealand native skinks. Data was partitioned by gene and position. This tree excludes the third position. Numbers at nodes are posterior probabilities. Codes represent nematode individuals. Information on each nematode can be found in chapter 2, Table 2.2.
Figure B.6: MrBayes inference tree based on the 28s data obtained from the parasitic nematodes infecting New Zealand native skinks and geckos. This data was subjected to Gblock analysis using the less stringent criteria (see chapter 2). Numbers at nodes are posterior probabilities. Codes represent nematode individuals. Information on each nematode can be found in chapter 2, Table 2.2.
Figure B. 7: MrBayes inference tree based on the 28s data obtained from the parasitic nematodes infecting New Zealand native skinks and geckos. This data was subjected to Gblock analysis using the more stringent criteria (see chapter 2). Numbers at nodes are posterior probabilities. Codes represent nematode individuals. Information on each nematode can be found in chapter 2, Table 2.2
Figure B.8: MrBayes inference tree based on the COI data obtained from the parasitic nematodes infecting New Zealand native skinks. Numbers at nodes are posterior probabilities. Codes represent nematode individuals. Information on each nematode can be found in chapter 2, Table 2.2.
Figure B.9: MrBayes inference tree based on the COI data obtained from the parasitic nematodes infecting New Zealand native geckos. Numbers at nodes are posterior probabilities. Codes represent nematode individuals. Information on each nematode can be found in chapter 2, Table 2.2
**Figure B.10**: RAXML inference tree based on the COI data obtained from the parasitic nematodes infecting New Zealand native skinks. Numbers at nodes are bootstrap values (%). Codes represent nematode individuals. Information on each nematode can be found in chapter 2, Table 2.2.
Figure B.11: RAxML inference tree based on the COI data obtained from the parasitic nematodes infecting New Zealand native geckos. Numbers at nodes are posterior probabilities. Codes represent nematode individuals. Information on each nematode can be found in chapter 2, Table 2.2
APPENDIX C: DATA USED IN THE CO-PHYLOGENETIC ANALYSIS

C.1 NEW ZEALAND GECKO-SPAULIGODON LINKS

Table C.1: Matrix showing the Gecko-Skrjabinodon links used in the PACo analysis

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C.2 NEW ZEALAND SKINK-SKRJABINODON LINKS

Table C.2: Matrix showing the Skink-Spauligodon links used in the PACo analysis

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</table>
C.3. R CODE FOR CO-PHYLOGENETIC RELATIONSHIP ANALYSIS

```r
library(ape)
library(vegan)

# Set file directory

### 1. DATA INPUT

# 1.1 Host and parasite phylogenetic data:
# 1.1.2 Aligned sequences
seqH <- read.dna("Host_Data.fasta", format="fasta")
seqP <- read.dna("Parasite_Data.fasta", format="fasta")

# Compute distance matrices from sequence data
host.D <- dist.dna(seqH, model = "F84", as.matrix=TRUE)
# Uses the Felsenstein (1984)
para.D <- dist.dna(seqP, model = "F84", as.matrix=TRUE)

# 1.2 Read HP: host-parasite association matrix
# Hosts in rows, parasites in columns. Taxa names are included in the file and should
# match those in tree, sequence or distance files.
HP <- as.matrix(read.table("H-P_linkG.txt", header=TRUE))
# sort host and parasite taxa in distance matrices to match the HP matrix
host.D <- host.D[rownames(HP), rownames(HP)]
para.D <- para.D[rownames(HP), colnames(HP)]

### 2. PACo FUNCTION: adjusted prior to Procrustes analysis

PACo <- function (H.dist, P.dist, HP.bin)
{
  HP.bin <- which(HP.bin > 0, arr.in=TRUE)
  H.PCo <- pcoa(H.dist, correction="cailliez")$vectors
  # Performs PCo of Host distances
  P.PCo <- pcoa(P.dist, correction="cailliez")$vectors
  # Performs PCo of Parasite distances
  H.PCo <- H.PCo[HP.bin[,1],] # adjust Host PCo vectors
  P.PCo <- P.PCo[HP.bin[,2],]  # adjust Parasite PCo vectors
  list (H.PCo = H.PCo, P.PCo = P.PCo)
}

### 3. APPLY PACo FUNCTION

PACo.fit <- PACo(host.D, para.D, HP)
HP.proc <- procrustes(PACo.fit$H.PCo, PACo.fit$P.PCo)# Procrustes Ordination
NLinks = sum(HP) # Number of H-P links; needed for further computations

## Superimposition plot:
```
HostX <- HP.proc$X  # host ordination matrix
ParY <- HP.proc$Yrot  # parasite ordination matrix, scaled and rotated to fit host
plot(HostX, asp=1, pch=46)  # plotting host and parasite ordinations
points(ParY, pch=1)
arrows(ParY[,1], ParY[,2], HostX[,1], HostX[,2],
    length=0.12, angle=15,
    xpd=FALSE)
HostX <- unique(HP.proc$X)
ParY <- unique(HP.proc$Yrot)  # unique () removes duplicated points - convenient for labelling of points bellow

## identify the user can label the data points interactively by clicking near each data point on the plot. First label the parasite locations (DOTS) and then those of the hosts
identify(ParY[,1], ParY[,2], rownames(ParY), offset=0.3,
    xpd=FALSE, cex=0.8)# press "Esc" to finish!!
identify(HostX[,1], HostX[,2], rownames(HostX), offset=0.3,
    xpd=TRUE, cex=0.8)# press "Esc" to finish!!

##3.2 Goodness-of-fit test:
m2.obs <- HP.proc$ss  # observed sum of squares
N.perm = 10000  # set number of permutations for testing - 100 000 is best; for most situations < or = 10 000 would be enough for hypothesis testing

P.value = 0
seed <- .Random.seed[trunc(runif(1,1,626))]
set.seed(seed)
# set.seed(5) ### use this option to obtain reproducible randomizations
for (n in c(1:N.perm)) {
    if (NLinks <= nrow(HP) | NLinks <= ncol(HP))  # control statement to avoid all parasites being associated to a single host
    { flag2 <- TRUE
        while (flag2 == TRUE){
            HP.perm <- t(apply(HP,1,sample))
            if(any(colSums(HP.perm) == NLinks)) flag2 <- TRUE
        }
    }
    else flag2 <- FALSE
}
else { HP.perm <- t(apply(HP,1,sample))}  # permutes each HP row independently
PACo.perm <- PACo(host.D, para.D, HP.perm)
m2.perm <- procrustes(PACo.perm$H.PCo, PACo.perm$P.PCo)$ss  # randomized sum of squares
write (m2.perm, file = "m2_perm.txt", sep = "\t",
append = TRUE)  # option to save m2 from each permutation
if (m2.perm <= m2.obs)
\{P.value = P.value + 1\}

P.value <- P.value/N.perm
cat(" The observed m2 is ", m2.obs, "\n", "P-value = ", P.value, " based on ", N.perm," permutations.")

#3.3 Contribution of individual links
HP.ones <- which(HP > 0, arr.in=TRUE)
SQres.jackn <- matrix(rep(NA, NLinks**2), NLinks)# empty matrix of jackknifed squared residuals
colnames (SQres.jackn) <- paste(rownames(HP.proc$X),rownames(HP.proc$Yrot), sep="-")
#colnames identify the H-P link
t.critical = qt(0.975, NLinks-1) #Needed to compute 95% confidence intervals.
for (i in c(1:NLinks)) #PACo setting the ith link = 0
{HP.ind <- HP
 HP.ind[HP.ones[i,1],HP.ones[i,2]]=0
 PACo.ind <- PACo(host.D, para.D, HP.ind)
 Proc.ind <- procrustes(PACo.ind$H.PCo, PACo.ind$P.PCo)
 res.Proc.ind <- c(residuals(Proc.ind))
 res.Proc.ind <- append (res.Proc.ind, NA, after= i-1)
 SQres.jackn [i, ] <- res.Proc.ind #Append residuals to matrix
}

SQres.jackn <- SQres.jackn**2 #Jackknifed residuals are squared
SQres <- (residuals (HP.proc))**2 # Vector of original square residuals

#jackknife calculations:
SQres.jackn <- SQres.jackn*(-(NLinks-1))
SQres <- SQres*NLinks
SQres.jackn <- t(apply(SQres.jackn, 1, "+", SQres)) #apply jackknife function to matrix

phi.mean <- apply(SQres.jackn, 2, mean, na.rm = TRUE)
#mean jackknife estimate per link
phi.UCI <- apply(SQres.jackn, 2, sd, na.rm = TRUE)
#standard deviation of estimates
phi.UCI <- phi.mean + t.critical * phi.UCI/sqrt(NLinks)
#upper 95% confidence interval

#barplot of squared jackknifed residuals
pat.bar <- barplot(phi.mean, names.arg = " ", space = 0.25, col="white", xlab= "Host-parasite links", ylab= "Squared residuals", ylim=c(0, max(phi.UCI)), cex.lab=1.2)
text(pat.bar, par("usr")[3] - 0.001, srt = 330, adj = 0, labels = colnames(SQres.jackn), xpd = TRUE, font = 2, cex=0.6) arrows(pat.bar, phi.mean, pat.bar, phi.UCI, length= 0.05, angle=90)
\texttt{abline(}a=\texttt{median(}phi.mean\texttt{)}, \ b=0, \ lty=2, \ xpd=\texttt{FALSE}) \ #\text{draws a line across the median residual value}

\#
REFERENCES


References


References


References


References


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