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Ultraconserved Elements Based Phylogeny of the New Zealand Mite Harvester Genus Rakaia

Zade Alafranji
Macalester College, zadealafranji01@gmail.com

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Zade Alafranji

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Advisor: Sarah Boyer, Department of Biology
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Abstract

New Zealand is home to a remarkable number of endemic taxa, some of which existed on the archipelago before the breakup of Gondwana. The mite harvesters (suborder Cyphophthalmi), tiny arachnids which dwell in forest leaf litter and caves, are one such group. The mite harvester family Pettalidae exhibits a classic Gondwanan distribution, informative for describing ancient patterns of diversification. Within New Zealand, there are three genera of pettalids; our research focuses on the phylogeny of the most widespread and diverse of these: *Rakaia.* Through phylogenetic analysis, we provide a window into patterns of ancient diversification and infer historical biogeographic trends.

The culminating phylogeny, based on ultraconserved elements retrieved using an Arachnid designed probe-set, resulted in a total of 68 taxa. The taxa were of the following breakdown: 54 *Rakaia* specimens, six of the sister genus *Aoraki*, and eight part of Opiliones outgroups. The final 50% and 75% taxon-occupancy matrix retrieved 729 and 355 loci, a large improvement from historic attempts. The phylogeny retrieved was fully resolved, and time-calibration using Bayesian Analysis yielded divergence dates across the genus. The study validated that the origin of *Rakaia* predates that of Gondwana, and that divergence within the genus may have been spurred by geologic events such as the Oligocene Drowning and the Last Glacial Maximum. The results need further validation through repeated attempts at time-calibration using different models, as well as estimation of evolutionary rates using Bayesian Analysis of Macroevolutionary Mixtures (BAMM). But, this study provides a novel high resolution depiction of the genus *Rakaia* with accompanying deep-time divergences.

Table of Contents

Preface

Prior to starting this research project, I had no idea what the process of research was. I had written some papers, done some projects, but never had I embarked on a project this comprehensive nor lengthy. Here are a couple of insights I have gained since starting this project. 1) research is a marathon, not a race, 2) research can at times be the most exciting thing you have in your life, but also at times be frustrating and demoralizing, 3) research is (usually) not a linear process. Most often it's two steps forward, then one step back, and seeing immediate results is not realistic, and finally, 4) results of research are most often not what you anticipate. Through writing this thesis, I have come to better understand what it means to do research, and the endurance you need to see a project through.

I also understand that the research on *Rakaia* will not end with my contributions, and that the fraction I have contributed will hopefully be built upon in the future. I am looking forward to adding on to my research on *Rakaia* in the summer, but I am also excited to hear about the next steps beyond my contributions as well. There are still lots of questions to be asked about this genus, and I can not wait to hear about how advances in methods contribute to a clearer picture of how *Rakaia* has evolved over time.

I would also like to preface this research with a note on the history of settler colonialism that has influenced New Zealand. Long before European contact, the Māori people had inhabited New Zealand Aotearoa for over 700 years. With European contact came new cropping techniques, new plants, new animals, and new diseases. Aside from land use changes, the addition of invasive species brought to New Zealand from afar has had a drastic impact on the native flora and fauna. Additionally, the arrival of Euorpeans led to a sharp decline in the Māori population at the time due to disease and guns. This history is important to acknowledge and mention as it has relied on the ability for other researchers to transport themselves to New Zealand to make collections and retrieve genetic data from animals on the islands, often in more remote and undisturbed areas. While the process of going into these areas to collect specimens is with the intention to catalog and provide a history of what has existed on the islands and for how long, it is important to understand the underlying historical significance of the land which the science is based on.

Acknowledgements

I would like to thank Sarah Boyer for her guidance and knowledge in making this study possible, as well as for being an amazing mentor with so much patience. This project has certainly had its trying moments, but I have always felt supported throughout the process. Additionally, Sarah has been critical for my path towards going to graduate school, and I am immensely grateful that she took me into her lab and gave me such a comprehensive research experience. Prior to meeting Sarah, I was unsure what I wanted in life post-graduation, but through working in her lab I now have a much clearer vision of what I see myself doing in the future. And aside from my future aspirations, I am glad I got to know her as she not only is a brilliant scientist, but also a fantastic person.

I would also like to thank my labmates in the Boyer Lab for livening up the lab space when I am spending hours at the computer, as well as for letting me hog the lab computer when I needed to. Our lab meetings always provided some respite, especially when the research was not going how I wanted it to, and I always felt that everyone helped me to see different perspectives from a research point of view. Former lab member Haley also deserves special thanks, as she walked me through the step-by-step process of using phyluce last summer, which was crucial to this project. I would also like to extend my thanks to Shahan Derkarabetian, whose expertise in the realms of phylogeny building and software know-how was critical to this research going forward. Without him, I likely would have spent twice as long trying to figure out the many bumps in the road, and for that I am grateful.

Lastly, I would like to thank my family and friends who have been so supportive of me throughout the process of this research project. I spent many hours in the lab working alone on this project, so always having my mom and dad available for a phone call was instrumental. I'd also like to thank my roommates Ollie and Max for always bringing me back to life when I would come home after hours of editing in Geneious. And Avery, for cheering me on and encouraging me when I was stuck with the research and didn't know where to go. You were all immensely important to me during this project, and for that I am thankful.

Introduction

Earth's ancient history leaves much to be known, and traces of what once was have captivated the scientific community for centuries. Gaps in knowledge regarding the speciation of organisms have puzzled researchers since questions began to be asked, with answers proving difficult to find with strong supporting evidence. Answers are often complicated; the field of biogeography works to tease apart these complicated results in a manner which is corroborated with an interdisciplinary approach incorporating facets of biology, geology, and history. The main concern of biogeography is the distribution of animals, plants, and other organisms on earth. A way of framing biogeography comes in the example of a puzzle, writes George Gibbs, a prominent scholar in the field. Analyzing biogeographical trends is a lot like assembling pieces of a puzzle, without having a picture of what the puzzle should look like. Researchers use genetic information and knowledge of geologic history to piece together an understanding of complicated questions and answers. This approach is especially pertinent when trying to understand an archipelago like New Zealand (Aotearoa), which has a rich geologic history full of change, resulting in a treasure trove of questions to be answered regarding its flora and fauna.

The mystery of the research, and the long history of life evokes storytelling; this storytelling is not embellished or falsified. It relies on science which helps put the pieces of the puzzle together. It focuses on the "why" of organisms–why they came to be where they are now (Gibbs, 2006). In the case of New Zealand, its ancient geologic-ties to the supercontinent Gondwana open questions regarding the origins of the species seen today.

Work on the molecular level is critical for biogeographers in inferring stories. Using molecular data, trends from the past can be illuminated. In the case of New Zealand, the use of the molecular clock has been instrumental in distinguishing vicariance vs dispersal. While the ancient origins of many New Zealand organisms had been disputed up until the 2000s, studies utilizing the molecular clock and fossils to date back approximate divergence dates of orders, sub-orders, families, and genera has allowed concrete assertions of origins to be made. By lining up approximate divergence dates generated from these studies with known geologic time frames or events, the long history of many organisms in New Zealand can be traced back to the time when New Zealand was a part of a much larger landmass (Goldberg et al. 2008).

At the level of species, New Zealand is home to high proportions of endemics – typical of island-like, isolated environments. Rates of endemism rival those seen in Hawaii and the Galapagos Islands. Broken down into groups of endemic species in New Zealand, there are 2000 endemic higher plants and 18,000 endemic insects, among many endemic species. Contrary to this stark endemism is the lack of presence of organisms one might expect to reside in New Zealand, such as: monotremes, snakes, crocodiles and land turtles. When analyzed through the lens of its turbulent geologic history, New Zealand is a fascinating place of study (Gibbs, 2006).

Another facet of New Zealand's unique biota are the remnants of the past which continue to inhabit the island nation. These organisms have persisted and adapted for millions of years with a shared history: a Gondwanan distribution. Millions of years ago, when Gondwana broke apart into fragments, the inhabitants of Gondwana remained on those pieces as they spread apart through time. Examples of taxa which experienced

vicariance are the chironomid midges and the harvestman family, Pettalidae. While many remnants of Gondwana dispersed elsewhere, and some current inhabitants like the Southern Beech came to reside in New Zealand due to dispersal, current techniques in systematics and molecular phylogenetics have allowed for a level of certainty when identifying a Gondwanan vicariant taxa (Boyer & Giribet, 2007).

The mite harvester (Fig. 1) family Pettalidae (Arachnida, Opiliones, Cyphophthalmi) typifies a Gondwanan distribution; except for Antarctica, all major remnants of Gondwana, including Chile, South Africa, Madagascar, the Indian subcontinent, Australia, and New Zealand are represented in their distribution. They are an excellent study system for vicariance, as they experience little to no dispersal during their lifetimes and are not known to inhabit any Darwinian or mid-ocean islands, suggesting they are incapable of trans-oceanic dispersal. Commonly known as mite harvesters, Cyphophthalmi have ancient origins. The oldest Cyphophthalmi fossil recovered is approximately 100 million years old, found in Burmese amber (Poinar, 2008). Further, non-cyphophthalmid Opiliones fossils have been found and dated back to approximately 411 million years ago, during the early Devonian (Dunlop, Anderson, Kerp, & Hass, 2004). Furthermore, Cyphophtalmi are sister to all living Opiliones, which suggested that they must be as old as the oldest dated Opiliones fossil recovered (Garwood, Sharma, Dunlop, & Giribet, 2014; Sharma and Giribet, 2014). Dated phylogenies of Opiliones have indicated that Cyphophthalmi diversified during the Permian or Carboniferous (359.2-252.2 Ma). With this deeply rooted history, the family Pettalidae and more broadly Cyphophthalmi, are an optimal study system for evaluating biogeographical trends (Baker et al. 2020).

Threats to New Zealand Flora and Fauna

While understanding biogeographical trends is important for informing our understanding of how the past has influenced the present distribution of organisms, it is crucial to understanding the future as well. It is well documented that the world is undergoing an accelerated rate of climatic change due to human induced pressures. Climate change presents itself in many forms, with increases in severe weather events as well as average annual temperatures both proven to be a product of increased greenhouse gas emissions from anthropogenic sources. Increases in human populations are also leading to decreases in historical ranges of animals, and the driving demand for increases in food production lead to land use changes at the detriment of local flora and fauna. Additionally, rates of pressure on species are steadily increasing to the detriment of biodiversity (Butchart et al. 2010). Models made to predict impacts on global biodiversity going forward indicate damning results: 20% of vertebrate species are at risk of extinction in the wild, with 11% of birds and 17% of threatened mammals having moved closer to extinction (Pereira et al. 2010).

Climate change within New Zealand is predicted to increase rapidly within the coming decades. By 2090, predictions from the Intergovernmental Panel on Climate Change (IPCC) indicate that air temperatures in New Zealand will increase by an average of 2.1 degrees celsius by 2090 for mid-range scenarios. Patterns of rainfall on New Zealand are also predicted to change, with more rainfall predicted in the western parts of the islands and reduced rainfall on the easter parts of the islands (Lundquist et al. 2011). This change will be accompanied by more intense rainfall events, and extreme heat waves. To combat these changes in temperature, species are predicted to shift their ranges

up in elevation or southward. Areas which experience decreased precipitation will be at an enhanced risk for wildfires, and areas with prolonged drought will only be at more risk (Lundquist et al. 2011). For organisms like mite harvesters, these pressures are damning. Unlike more motile invertebrates, mite harvesters are unable to uproot themselves from their given ranges due to their dispersal limitations and lifecycles. All families of Cyphophthalmi are located on continental landmasses or continental islands, which indicate a stark lack of dispersal ability given the lengthy evolutionary history of mite harvesters (Giribet and Kury, 2008). While this makes them prime targets for biogeographical studies, it makes them vulnerable to climate induced pressures.

Globally, arthropods seem to be in danger. Land use changes have been identified as a major contributor to the decline of arthropod species, and changes to forest and grassland composition have also been a key cause of declining populations. While monitoring arthropod populations across time is challenging on a large scale, a study taken on by Seibold et al. which took place from 2008 to 2017 in grasslands and forests of Germany found that arthropod species declined significantly. They found that biomass, abundance, and number of species declined by 67%, 78%, and 34% respectively (Seibold et al. 2019). This trend of arthropod decline was also found closer to the equator in the rainforest environment of Puerto Rico. This study looked at changes in arthropod biomass sampled from 1976 to 2012, and the results showed that there was a four to eight times decline in arthropod biomass (Lister and Garcia, 2018). It should be noted that there was a 2 degree celsius increase in average temperature recorded from the start of the study, which is approximately the same degree of change New Zealand is expected to see in the next 60 years.

While these biomes are far different from the southern reaches of New Zealand, changes to climate will impact the native flora and fauna with certainty. Already, there are examples of animals in decline as a result of climate change such as the Hihi bird, which is declining due to loss of habitat and ecosystem change (Hare et al. 2019). Despite New Zealand's historical knack for enacted laws bent on conserving biodiversity such as the NZ Biodiversity Strategy, which calls on a moral obligation for its citizens to protect nature and reduce rates of biodiversity decline, many species are still at risk of extinction within the next century. Other examples include the Grand and Otago Skinks, and the forest ringlet butterfly, among many others. Even with attempts at conservation, the animals are all in decline due to a variety of factors which all wind back to human introduced pressures (Hare et al. 2019).

While reporting many of these facts is despairing, a main motivation behind studies such as this is to uncover and understand the current biodiversity in order to inform and catalog what exists in the world. With the ever changing climate and the rapid rates of decline species are undergoing, using genetic tools to identify diversity, which is not always easy to see, is becoming more and more paramount.

Geology of New Zealand

Spanning back approximately 180 million years ago to the separation of Gondwana, New Zealand was a tiny part of the one of the largest land masses earth has seen. Plate tectonics led to the breakup of the continent into fragments spread around the globe, which now represent pieces of the past. Modern day New Zealand broke off from Gondwana as a subsidiary of a much larger land mass named Zealandia. Zealandia was a large descendent of Gondwana, which approximately 82 million years ago broke apart

into Australia, Antarctica, South America, and New Zealand (Trewick et al. 2007; Wallace & Trewick, 2009). A major feature of Zealandia is its submergence, or partial submergence, underwater as a result of the continent thinning. While it is still debated how much of Zealandia was submerged, the impact of the flooding must have drastically impacted terrestrial life. The last major flooding event in the Early Miocene (23-53 Ma) into the Pliocene (5.4-2.4 Ma) marked the end of the series of floods which forever changed the landscape (Wallace & Trewick, 2009).

Perhaps the defining geologic activity for the formation of New Zealand was activity on the Alpine Fault 25-23 Ma. This fault lies where the Pacific and Indo-Australian plates collide, and subsequent tectonic action caused the land to rise up and form the modern day New Zealand. This upward movement has persisted today, averaging 40 millimeters of movement per year since the Late Miocene (10.4-5 Ma). The Southern Alps, a result of compression along the Alpine Fault for the past 8-10 Ma, is a main feature of the South Island. The Southern Alps are one of the fastest rising mountain ranges in the world, experiencing acceleration of uplift around 6 Ma. The Southern Alps created a climatic gradient not seen before in New Zealand with its alpine addition, and the range sees uplift rates of 2-11 millimeters per year (Wallace & Trewick, 2009). The varying gradients resulted in environments beneficial to many organisms, resulting in multiple radiations from tiger beetles, aquatic beetles, multiple cicada lineages, and the moas (Marske and Boyer, 2022).

Another impactful event was the series of glaciation events which occurred during the Pleistocene (2.58 Ma-11,700 years ago). Up to 30% of the South Island of New Zealand was covered in glaciers, while many areas such as Marlbourough, central/eastern

Otago, and the North Island were untouched. Further, the glaciated areas were not continuous. Montane areas left fragmented valleys untouched by glaciation. The glaciation of New Zealand notably resulted in the connection of the the North, South, and Stewart Islands due to reduced sea levels, but also had varying results on taxa due to its lack of continuity (Wallace & Trewick, 2009, Marske and Boyer, 2022). This connection between the North and South Islands, likely due to low sea levels during the height of the last glacial maximum approximately 20,000 years ago, connected the North and South Islands with shrubs, grassland, and patches of forest (Boyer and Giribet 2009).

A common disjunction of species seen in New Zealand is the presence of closely related species in Nelson/Marlborough, the North Island, and Stewart Island–the most southern New Zealand landmass. This disjunction is seen in species of *Rakaia*, with species from the North Island, Nelson/Marlborough, and Stewart Island forming a clade sister to all the other species (Boyer and Giribet 2009). This clade is highly supported by phylogenetic analysis, and also supported by morphology. The two competing hypotheses for this disjunction are: 1) the disjunction forms around the Alpine Fault, and 2) the disjunction is a feature of a previously much larger distribution of species prior to the Last Glacial Maximum (LGM). Once the glacial maximum reached its peak, any Cyphophthalmi present in places of ice extent likely went extinct (Boyer and Giribet 2009). This is reflected in current distributions of *Rakaia* around the North, South, and Stewart islands of New Zealand (Fig. 2).

Molecular Phylogenetics of Pettalidae and *Rakaia*

The first attempts at developing phylogenies of the family Pettallidae utilized five nuclear and mitochondrial genes as well as morphology (18S rRNA, 28S rRNA, 16S

rRNA, cytochrome c oxidase subunit I and histone H3) in 2007 by Boyer and Giribet. With this early attempt at a phylogeny, Pettalidae arriving in New Zealand via dispersal was put up for debate. There are three distinct, monophyletic clades representing the three endemic genera that inhabit New Zealand: *Neopurcellia* (Forster, 1948), *Rakaia* (1925), and *Aoraki* (Boyer and Giribet, 2007). These endemic genera are not each other's closest relatives when looking at a phylogeny of the family Pettalidae. This suggested the three lineages must have evolved independently of each other prior to New Zealand breaking apart from other Gondwanan remnants. While this paper provided evidence for Gondwanan origins of Pettalidae, further resolution was lacking at the time to date the phylogeny with more certainty (Boyer and Giribet, 2009).

A more resolved phylogeny of Pettalidae came in 2018, with Oberski et al. using the molecular clock to explain the presence of Pettalidae in Australia. It uncovered that the diversification of Pettalidae coincided with the window of time when Gondwana rifted apart, meaning Australian and New Zealand pettalids had vicariant origins dating back to Gondwanan times (Oberski et al. 2018). In 2020, Baker et al. tried establishing the Gondwanan origins of the family Pettalidae, this time with transcriptomic data. This study corroborated the past studies, finding that the timing of diversification for Pettalidae coincided with the breakup of Gondwana. The study also revealed that the order in which cladogenesis occurred aligned with the order in which Gondwana broke into pieces, cementing Pettalidae as a classic example of a Gondwanan distribution (Baker et al. 2020). While transcriptomic data results in well resolved topologies for phylogenies at the level of family, it requires high-quality RNA inputs, which is not always possible. In contrast, the next development in phylogenomics for non-model

systems has become ultraconserved elements (UCEs) which can be used with lower quality DNA inputs (Starrett et al. 2016).

Ultraconserved Elements: History and Utility

A new era of phylogenomics has arrived within non-model systems. The word phylogenomics was coined in the late 1990s by Eisen, with its meaning translating to how the function of genes impacts genome-scale datasets and their resulting phylogeny. This term was preceded by the advent of Sanger Sequencing and PCR, which through multi loci sampling approaches allowed for a broadening of the field of phylogenetics. Then came next-generation sequencing (NGS) in the early 2000s, which allowed for the simultaneous sequencing of millions of nucleotides in parallel, significantly changing the field and uprooting prior methodologies. The utility for resolving relationships in non-model systems quickly became apparent with NGS, and since then, many specially designed workflows for working with these systems have been designed for even greater accuracy (Young and Gillung, 2020).

Traditional Sanger sequencing techniques resulted in difficulties when applied to the genus *Rakaia*. The phylogeny of the genus was not fully resolved by the dataset, and taxonomically it was not comprehensive. A further challenge was Sanger sequencing is not reliably effective in old, less well preserved samples. To overcome those obstacles, a new methodology was adopted in using ultraconserved elements (UCEs) to create much larger datasets with well resolved and supported phylogenies, while also being able to include samples which were previously unable to be sampled due to their condition. This method utilizes NGS to massively parallel sequence thousands of loci instead of the previous limitation of one target locus for Sanger sequencing (Faircloth et al. 2012).

With the case of Cyphophthalmi, many museum samples are old and degraded making genetic analysis difficult. They are often collections which have been stored for years in conditions which may not be optimal for genetic analysis. Samples of *Rakaia* have been stored in museums for almost 100 years now, with the earliest samples coming from Hirst in 1926 (Hirst, 1926). This long history of collection thus makes methods which can utilize this old, degraded data important. Sanger sequencing can use degraded samples, however it is difficult to use degraded samples as PCR relies on primers instead of baits, which UCE's use. A further challenge of Sanger sequencing is the chance of PCR contamination, which can slow down analysis. Transcriptome sequencing needs high quality RNA, which is challenging for cyphophthalamid based studies. While UCE's also run the risk of PCR contamination, they work with DNA of low quantity/quality, an advantage over Transcriptomes, and they can in parallel sequence thousands of loci, an advantage over Sanger Sequencing, all while still producing high resolution results (Faircloth et al. 2012). Additionally, the dawn of NGS came with emerging methodologies for combatting the issues that came with traditional sequencing methods.

A benefit, but challenge of NGS is the massive amount of data output, which compared to Sanger sequencing, produces millions more sequences. This can be misleading, though, as there are still careful considerations for using NGS. With Sanger sequencing, each base is typically evaluated by a human and colored peaks from chromatograms, which reflect the strength of nucleotide signals, are intuitive to interpret. With the case of NGS datasets, this level of quality control is not attainable due to the millions of sequences. Instead, NGS datasets are evaluated based on NGS quality scores which are integers representing bases called by the sequencing platform. This makes

coverage, or the number of reads which support a specific base call, of NGS datasets crucial. Proper coverage makes sure that all of the DNA within the data pool is sampled, and also ensures sequencing error can be detected (McCormack et al. 2013). The use of NGS combined with UCEs allows for a massive dataset to be analyzed all while retaining a level of resolution previously unattainable.

UCEs are highly conserved DNA regions of the genome shared by divergent taxa. While their function in vertebrates is still unclear, although may be linked to regulation or enhancement of gene expression, in arthropods UCEs are primarily exonic in nature (Hedin et al. 2019). They are easy to identify due to how conserved they are, which makes them optimal for aligning across multiple divergent genomes. Another characteristic of UCEs is their consistent variability in flanking regions, which suggests that they may be "molecular fossils," or have retained a molecular signal of evolutionary history (Fig. 3). This characteristic variability around a highly conserved core region is what makes them particularly useful from the lens of phylogenetics at a shallow level, as sequencing out from these regions allows for variable DNA sequences to be captured from hundreds of loci. At deeper levels, the exonic nature of core UCE regions is what is useful for phylogenetics. As Faircloth et al. define them, UCEs have a sequence similarity of $\geq$80% identity over $\geq$100 bp across divergent taxa (Faircloth et al. 2012). UCE datasets are generated through the use of a probe-set specifically tailored to UCEs from a certain target domain; that is, there are multiple probe sets in existence for different target organisms, with the one in this study targeting arachnids.

While the scope of what is sequenced can span the whole genome, smaller sections of the genome can be actively targeted for sequencing in what is called target

enrichment or target capture–as such, UCEs can be used for target enrichment with promising results (Morisawa 2020). This allows for much of the genome to be ignored in favor of the parts which are of interest, which is important due to the scope of data which is evaluated with NGS. Furthermore, this method is helpful for analyzing non-model organisms which lack in sampling quantity or sample quality (Faircloth et al. 2014). Target enrichment relies on a pre-designed probe set, which is built upon genomic information from related organisms. Target enrichment works via the following process: 1) DNA probes mix with fragmented gDNA, 2) hybridize to form an array or solution, 3) non-target DNA is washed away, and target DNA is eluted, 4) target DNA is sequenced with NGS (Fig. 4). This method of target enrichment is a non-random way of reducing the size of the genome, thus making it more manageable (McCormack et al. 2013).

The arachnid probe set, designed by Faircloth in 2016, works by targeting the known conserved areas of arachnid genomes to enrich loci. This works by having probes match target sequences with the following specifications: 1) if UCE sequences are >180 bp long, than 120 bp probes were tiled at that region at two-times density (thus probes overlapped target regions by 60 bp), 2) if UCEs were <180 bp, a single probe is selected from the center of the UCE, 3) sequences shorter than 120 bp had sequence added to them by selecting equal amounts of 5' and 3' flanking sequence from an arachnid genome assembly (Faircloth et al. 2012, Faircloth 2016, Starret et al. 2016).

UCEs have become prevalent within the study of non-model systems for the previously stated reasons. They are highly conserved across a wide array of taxa, reveal divergences at shallow and deep timescales evolutionarily, and have rapid generation at low costs (Smith et al. 2014). Studies looking at Australasian Scirtinae, or marsh beetles,

(Bradford et al. 2022), Sulawesi squirrels, (Hawkins et al. 2016), and Galliformes, or landfowl, (Chen et al. 2021) have been successful in their generation of well supported topologies, previously unattainable for various reasons. As previously stated, most UCE regions within arachnids are exonic. Recent studies have unveiled the arachnid specific probe set targets different parts of the same coding genes, which may be detrimental to returning a resolved phylogeny. However, at shallower levels the arachnid probe set shows high utility for population level analyses due to flanking UCE regions (Kulkarni et al. 2020). Within Pettalidae, they have proven to be extremely successful in deriving highly resolved phylogenetic trees across the level of genera, with much better resolution at the species-level in each respective genera. This has been shown in recent studies by Morisawa 2020 with the genus *Rakaia* in New Zealand, delimitation of cryptic species in *Aoraki* (Heine et al. 2024), and phylogenetic placement of the newly discovered pettalid genus Archaeopurcellia eureka in Southern Australia (Giribet et al. 2022).

Already, new studies show that order-specific probe sets demonstrate strong returns in resolution as well as number of loci returned. Through a combination of genomes, transcriptomes, and the most useful probes from the Arachnida probe set, Derkarabetian et al. (2023) was able to design an Opiliones specific probe set which demonstrates a high level of resolution in shallow population level divergences, even when using highly degraded data which even the Arachnida probe set struggled to resolve. This probe set targets twice the number of UCEs that the Arachnida probe set does, and likewise returns a larger number of loci (Derkarabetian et al. 2023). As the emergence of these probe sets continues to grow and UCEs gain further traction within

the phylogenomic community, it is likely that the results will continue to return a clearer picture of many ancient evolutionary lineages.

Pettalidae Systematics

The mite harvester sub-order, Cyphophthalmi, is a part of the order Opiliones. Known for the aforementioned global distribution and low vagility, Cyphophthalmi members are Neogoveidae, Stylocellidae, Sironidae, Ogoveidae, Troglosironidae, and Pettalidae. The divergence of the sub-order Cyphophthalmi is dated back to the Jurassic period (~199-145 ma), followed by a period of diversification during the Cretaceous (~145-66 ma) by Fernandez et al. 2017. However, more recent estimates have attributed the diversification of Pettalidae to the Jurassic period, and the diversification of Cyphophthalmi to the Carboniferous (~359-299 ma), which is consistent with the breakup of Gondwana (Oberski et al. 2018, Fernandez et al. 2017).

The family Pettalidae can be split into 11 genera, each of which has a different geographical distribution following the split up of Gondwana. The 11 genera are split globally as follows: *Chileogovea* is found in southern Chile, *Austropurcellia, Archaeopurcellia,* and *Karripurcelia* in Australia, *Parapurcellia* and *Purcellia* in South Africa, *Manangotria* in Madagascar, *Pettalus* in Sri Lanka, and *Chilegovea* in Chile, and *Aoraki*, *Rakaia,* and *Neopurcellia* in New Zealand (Giribet et al. 2022).

The endemic Pettalidae of New Zealand each have specific distributions across the North, South, and Stewart Islands. The genus *Neopurcellia* is found throughout the west coast of the South Island and *Aoraki* is found in the North and South Islands. The genus *Rakaia* is widespread, and is found in the North, South, and Stewart Islands. *Neopurcellia* and *Rakaia* do not overlap in their distribution, but *Aoraki* and *Rakaia*

overlap in the Nelson/Marlborough region of the South Island and the North Island. A feature of this overlap is that areas of high *Rakaia* diversity, particularly in the south and east coasts of the South Island, have no *Aoraki* presence (Boyer and Giribet 2007).

The genus *Rakaia* is the most speciose of the three endemic genera in New Zealand. As of 2020, there have been 18 named species of *Rakaia* (Giribet 2020), with four new, unnamed species identified by Morisawa (2020). On the South Island of New Zealand, the species distribution of *Rakaia* are small and distinct. This is likely due to the environment of patchy, discontinuous forest that is separated by the Southern Alps. Three species of *Rakaia* are located on the south coast of the South Island, with no distribution overlap and adjacent ranges. On the drier east side of the Southern Alps, there are four species with adjacent, non-overlapping distributions. In contrast to the species of *Aoraki* in the north and west of the island, which coexist in the continuous *Nothofagus* and podocarp forests, *Rakaia* species south of Nelson and Marlborough do not inhabit overlapping ranges (Boyer and Giribet 2009). In fact, across the thousands of cyphophthalmi collections there are only 34 recorded cases of two or more species being collected from the same locality (within 1-2 km, Heine et al. 2024).

The most northern tip of the South Island, referred to as Nelson/Marlborough, and the North Island, are home to many coexisting species of *Aoraki* and *Rakaia*. Examples of sympatrically living species include: *A. granulosa* and *R. media, A. inerma* and *R. media, A. denticulata* and *R. magna australis, A. denticulata* and *R. florensis*, and *A. denticulata* and *R. minutissima*.

The Molecular Clock

The molecular clock is a powerful tool for deducing biogeographic trends within flora and fauna. In practice, the molecular clock relies on several factors: suitable fossil calibrations, widely sampled taxa with well resolved topology, and suitable applications of molecular clock methodology. Results from the analysis are deep-time dates which can inform diversification histories for the organisms of interest, and overturn paradigms of biogeographic history. For example, a major paradigm shift attributed to molecular clock dating is the belief that placental animals and Neornithes birds diversified prior to the Cretaceous to Paleogene Extinction, instead of after (Chen et al. 2021). Important information about the history of organisms can be inferred from the use of the molecular clock, as has been demonstrated with Cyphophthalmi.

The main software this study relies on to create the time calibrated phylogeny is BEAST2, which stands for Bayesian Evolutionary Analysis by Sampling Trees (second generation; Bouckaert et al. 2014). Bayesian statistics rose to prominence within the phylogenetic community in the early 2000s with the creation of MrBayes, a similar tool for phylogenetic reconstruction which, like BEAST2, uses the Bayesian Markov chain Monte Carlo (MCMC) algorithm. This method of combining statistics with biological data helped to better inform parameter estimates based on coalescent-based inferences from genetic data and was much faster than previous optimization models due to the use of maximum likelihood criterion. Like other comparable phylogenetic reconstruction programs, BEAST2 works by creating a posterior set of possible evolutionary parameters (Fig. 5) based on sequence data (Suchard et al. 2018).

BEAST2 also allows for a relaxed molecular clock to be used, which unlike other models, does not assume all lineage of the phylogeny evolve at the same rate. This relaxed clock model was used for this study to allow for each branch of the resulting tree to have its own uncorrelated clock, evolving independently of other branches, and allowing for the increase and decrease of evolutionary rates through time (Drummond and Rambaut, 2007). This approach is especially useful, as this study is interested in understanding how rates of evolution may change over time in conjunction with geologic events which drastically changed landscapes. While BEAST2 can be used to track pathogenic spread and monitor fast-evolving viruses, it also has high utility for reconstructing ancient lineages (Bouckaert et al. 2014). The passages below highlight this utility, and provide context for the use of BEAST2 within cyphphthalmid dating.

Within the context of Patellidae, divergence dating seeks to answer questions related to the breakup of Gondwana into Western and Eastern Gondwana starting approximately 180-170 Ma, then into its smaller parts approximately 80 Ma (Wallace & Trewick, 2009). It is well documented that the main driving force of divergence for many dispersal-limited organisms, such as Cyphophthalmi, is vicariance (Giribet et al. 2022; Boyer & Giribet, 2007; Boyer & Giribet, 2009). Many studies of other dispersal-limited organisms have corroborated diversification dates with the break-up of Gondwana. Further, in contrast to vagile generalists, organisms which are dispersal-limited and restricted to particular environments are intrinsically more connected to the geology of the localities they inhabit, making their divergence interesting when evaluating biogeographical trends (Derkarabetian et al. 2020).

With transcriptomic data, the age of Pettalidae is estimated to be approximately 212 Ma, with the East Gondwanan taxa (*Rakaia, Pettalus, Austropurcellia, Neopurcellia, Karripurcellia* and *Aoraki*) diverging from the West Gondwanan taxa (*Parapurcellia, Purcellia* and *Chileogovea*) 151 Ma (Baker et al. 2020). Western Gondwanan taxa diverged the earliest, with *Chileogovea* of Chile diverging from the South African taxa. Eastern Gondwana had its first rifting of taxa ~151 Ma, when *Rakaia* diverged from the rest of taxa. This was followed by *Neopurcellia* diverging from *Aoraki* and *Karripurcelia* ~114 Ma, and *Purcellia* and *Austropurcellia* diverging ~130 Ma (Baker et al. 2020).

Some of the divergences between pettalid genera occurred prior to the rifting of Gondwana. *Parapurcellia* diverged from the rest of the so-called "Eastern Gondwanan taxa" prior to Gondwana splitting apart. Additionally, it is important to note that the New Zealand genera diverged from each other prior to New Zealand rifting from the landmass which constituted modern day Antarctica, Australia, and New Zealand (~80 Ma). The genera *Rakaia* and *Aoraki* even have been dated back to New Zealand, when it was still a constituent of Gondwana. This suggests that, while the rifting of Gondwana played a role in the wide diversity of these genera today, there was likely cladogenesis while the landmasses were still connected (Baker et al. 2020).

Within the genus *Rakaia*, there are still questions to be answered regarding internal topology, and the timing of diversification. It is still unclear whether the closely related *Rakaia* species which inhabit the North, South, and Stewart Islands are remnants of a more widely distributed lineage, which diverged with the disappearance of land bridges following the LGM. Using the molecular clock to provide approximate diversification dates will seek to remedy this lack of information. Further, dating the tree

will be informative for parsing apart hypothesized extinction events following the Last Glacial Maximum, as well as tell a story about possible radiations following the rising of the Southern Alps.

Combining high-resolution UCE based topologies with molecular dating has resulted in studies which effectively categorize taxa and allowed for a better understanding of dynamics between clades, genera, and species. This has been shown in the case of Australian Scirtinae, Sulawesi squirrels, and Galliformes (Chen et al. 2021, Bradford et al. 2022, Hawkins et al. 2016). The molecular clock will be applied to the genus *Rakaia* with the goals of better understanding the dynamics between species in the genus and how geologic events have impacted its history of speciation.

Evolutionary Rate Shifts

When evaluating a phylogenetic tree, one can ascertain the relationship between sister lineages or perhaps the ages of nodes on the tree. What can also be gathered through the use of Bayesian Analysis of Macroevolutionary Mixtures (BAMM) is the rate at which branches on a tree have evolved over time. This process, like BEAST2 , relies on Markov chain Monte Carlo algorithms with the idea that there is no fixed number of distinct processes that occur in a phylogenetic tree, but that instead there is a Poisson distribution of processes that can occur. BAMM also operates on the premise that individual branches do not evolve at the same rate, the number of evolutionary instances are randomly estimated by the data, and that through Bayesian analysis, a greater number of models are able to be explored through incremental approaches. With that said, BAMM also provides for each branch in a phylogeny speciation and extinction distributions (Rabosky et al. 2014).

The implementation of BAMM within phylogenetic datasets has recently been used to evaluate the rates of ray-finned fish evolution, and showed the resulting differences in evolutionary rates across ray-finned fish (Rabosky et al. 2013). Some groups of fish experienced high-rate evolution, while others experienced low-rate evolution, and this relationship was prevalent throughout the phylogeny. The paper identified a median number of rate shifts from the posterior of 65, which was reflective of the size and contents of the dataset and number of terminals in the phylogeny. This finding implied to the authors that disparity in evolutionary rates not only applied to ray-finned fish, but also to nearly half of vertebrate diversity (Rabosky et al. 2013). Another example of BAMM in action was the analysis of extant bat radiations by Shi and Rabosky in 2015, which identified one evolutionary rate shift and indications of decreasing speciation rates within the taxa sampled (Shi & Rabosky 2015).

Utilizing BAMM in this study will be critical for understanding how evolutionary rate shifts have occurred throughout the evolutionary history of *Rakaia*. Part of the goal of this study is to understand how geological events have impacted rates of speciation within the genus. Events of the past approximately 100 million years in New Zealand have included events ranging from major weathering of mountains to spreading of ocean floor and inundation of landmass. Using BAMM to elucidate potential changes in evolutionary rates across these lineages will be important for informing the relative importance of each geological event with respect to the speciation of *Rakaia*.

Methods

The following methods were used and adapted from Heine et al. 2024 and Derkarabetian et al. 2021.

Specimen Collection

Data used in analysis was collected from 68 specimens. Of the 68, 54 were *Rakaia,* and six were *Aoraki*, a closely related outgroup. The remaining eight specimens represented taxa from Opiliones groups which have dated fossil records used to calibrate the molecular clock. The genus *Aoraki,* as sister to *Rakaia,* was used as the outgroup within Pettalidae. Nested within Pettalidae, *Rakaia* is sister to nine other genera in the family, with a recent study by Giribet et al. elucidating the challenging placement of the genera within the family (Giribet et al. 2022). The other outgroup sequences were acquired through a prior analysis conducted by Derkarabetien et al. 2021, and consisted of the following specimens all within the Opiliones order: *Dibunis* sp., *Holoscotolemon unicolor*, *Peltonychia leprieurii*, *Pseudobiantes japonicus, Leiobunum calcar, Sabacon cavicolens, Sclerobunus robustus,* and *Siro boyerae* (Derkarabetien et al. 2021).

The specimens within *Rakaia* sampled were gathered and loaned from a variety of institutions: the Harvard University Museum of Comparative Zoology (MCZ), the New Zealand Arthropod Collection (NZAC), the Museum of New Zealand Te Papa Tongarewa (MONZ), and the Field Museum in Chicago, IL. The MCZ specimens were generally collected with the following protocol: 1) sifting leaf litter followed by in situ sorting, 2) resulting specimens preserved in 95% ethanol, and 3) corresponding locality data point marked with GPS. Historic specimens more than 20 years old came from the MONZ, NZAC, and Field Museum.

Ultraconserved Elements Collection and Processing

Specimens which had been preserved in 95% ethanol had genomic DNA extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). This was done using either a set of appendages or the whole body, following the manufacturer protocol with modification. Older specimens, preserved in 70% ethanol, as well as the small and degraded specimens were processed using separate protocol, as described in (Derkarabetien et al. 2019). Following extraction, DNA was prepared for sequencing; DNA was sheared to 500bp with a Covaris S220 Focused-ultrasonicator. The UCE libraries used in the analysis were prepared with a KAPA Hyper Prep Kit (Roche Sequencing and Life Science, Indianapolis, IN), and ligation cleanup done with AMPure beads. Further, a KAPA Library Amplification Kit protocol, with some modification, was used to amplify UCE libraries. Target enrichment of ultraconserved elements was done using MYbaits Arachnida 1.1K version 1 kit (Arbor Biosciences, Ann Arbor, MI), and DNA was quantified using PicoGreen at the University of Minnesota or with a Qubit fluorometer.

The Phyluce pipeline (v1.7.3, Faircloth, 2023) was used to process raw data reads, and reads were cleaned and adapters removed using the Illumiprocessor wrapper (Faircloth, 2013). ABySS (Simpson et al. 2009) and Trinity (Grabherr et al. 2011) were used to assemble contigs and matched with probes using minimum coverage and minimum identity values of 65. UCE loci were aligned with MAFFT (Katoh, 2013) and trimAI (Capella-Gutierrez et al. 2009) was used to trim alignments. Loci with 50% taxon coverage, meaning loci were present in 50% or more of taxa, were imported into Geneious (Kearst et al. 2013), and then sequences were manually edited. Sequences that

were determined to be non-homologous were trimmed, and specimens with poor sequence quality removed. RAxML (v8.2.12, Stamakis, 2014) with 200 bootstrap replicates and a GTRGAMMA model was used to construct a phylogeny.

Molecular Clock Analysis

The molecular clock analysis was performed using divergence dating in BEAST 2.6.6 (Bouckart et al. 2014). The calibrations used were adapted from Derkarabetian et al. 2021, and included the use of three fossil calibrated priors. All calibrated priors were set to a uniform distribution with the minimum age corresponding to the age of the fossil, and the maximum age of 514 Ma, the age of the oldest chelicerate fossil. The root calibration was set to 405 Ma, which reflects the age of the oldest Opilliones fossil. The second calibration was assigned to the order Palpatores using two fossils, *Leiobunum* and *Sabacon*, dating to 305 Ma. The third calibration was assigned to the order European Cladonychiidae, using *Peltonychia leprieurii* and *Holoscotolemon unicolor*, dating to 44 Ma. A *Siro* specimen was used as an outgroup, and the as well as a *Sclerobunus* specimen used to provide additional branching leading to the shallower calibration (Derkarabetian et al. 2021).

Using a relaxed log normal clock, a fossilized birth-death model was used with 600 million generations, logging every 1000 steps. An additional analysis (totaling two) was used to validate prior results. The birth-death model was used as it is appropriate when sample taxa and fossils included in the analysis are a part of the same diversification process. The distribution choice for the three priors was uniform. The upper bounds of each prior were set to 514 Ma, which reflects the age of the oldest known chelicerate fossil. The lower bounds of the distributions reflect the ages of the

fossils, with the Opiliones lower bound being 405 Ma which corresponds to the age of *Eophalangium sheari*, the oldest known Opiliones fossil (Dunlop et al., 2003). The lower bound of the Palpatores prior was set to 305 Ma, and the lower bound for European Cladonychiidae was set to 44 Ma. The results of the analysis included a ucldStdev mean of 0.37 (95% highest posterior density [HPD] of 0.28-0.39) and a coefficient of variation mean of 0.34 (95% HPD of 0.29-0.4). The program TRACER (Rambaut et al., 2018) was used to examine convergence with ESS values >200 (Rambaut et al. 2018). The results were then displayed in TREEANNOTATOR to create a maximum clade credibility tree.

BAMM Analysis

The BAMM analysis including all taxa sampled was run using BAMM version 2.5 (Bayesian Analysis of Macroevolutionary Mixtures: Rabosky et al. 2013; Rabosky 2014) for one billion generations using a speciation-extinction model, with a sampling frequency of every 1000 events from the posterior. The prior block attained using the R package BAMMtools (v.2.5) were the following: expected number of shifts = 1.0, prior on initial lambda = 25.99, prior on the lambda shift parameter = 0.0025, and prior on the extinction rate = 25.99. Convergence was checked for by plotting the number of generation against the likelihood score, as well as exceeding effective sample sizes of 200. Furthermore, 20% of burnin was then discarded (Robosky et al. 2014)

Results

Phylogenetic Analysis

A total of 1,119 UCE loci were sampled, with a total length of 132,509 bp and a mean locus length of 118 bp. The 50% taxon-occupancy matrix resulted in 729 loci being retrieved, and the 75% taxon-occupancy matrix retrieved 355 loci. Compared to previous attempts at resolving the *Rakaia* phylogeny by Morisawa 2020, which resulted in a 50% taxon-occupancy matrix of 719 loci and a 75% taxon-occupancy matrix of 93 loci, this is a significant improvement. The numbers of loci retrieved by Heine et al. 2024 in her study on *Aoraki,* while higher, validates this phylogeny as an advancement in the the resolution of this genus as numbers are within a similar range (Morisawa, 2020, Heine et al., 2024).

The resulting RAxML tree (Fig. 6) retrieved *Rakaia* as a monophyletic clade with >95% bootstrap support, sister to the *Aoraki* outgroup. Within *Rakaia*, almost all nodes were supported with >95% bootstrap support, save for seven nodes. Additionally, major lineages inferred in the past by previous studies within *Rakaia* were recovered (Boyer and Giribet, 2009). These clades (A,B,C, and D) reflect geographic history, understanding of climatic events, and knowledge of the system (Fig. 7, Fig. 8). Clade A consists of the most widespread distribution of species, located on the southern part of the North Island, the north and south of the South Island, and Stewart Island. Clade B is made up of species from the southern part of the South Island. Clade C is made up of species from primarily the east coast of the South Island. Finally, Clade D consists of species from the north and south of the North Island, and north of the South Island. Each

clade is supported with >95% bootstrap support, and generally there is high resolution within clades.

Looking at the BEAST phylogeny, all nodes had posterior probabilities >0.95, save for the node which represents the split of *Rakaia* from its outgroups (Fig. 9). This posterior probability of 0.64, while lower than desired, likely represents a lack certainty in the topology of the tree at that node; this is likely a product of the taxonomic coverage within outgroups as that node represents the divergence of cyphothalamid samples from outgroups. Like the RAxML phylogeny, the tree arranged itself into the typical four clades seen in *Rakaia*.

Diversification within *Rakaia*

Under the chosen birth-death model with incomplete sampling, the age of *Rakaia* was inferred to be 76 Ma (95% HPD [highest posterior density]: 57–95 Ma), which falls through the Cretaceous and Paleocene. Looking at the four clades within *Rakaia*, their inferred ages were as follows: Clade A was inferred to be 51 Ma (95% HPD: 37–66 Ma), Clade B was inferred to be 45 Ma (95% HPD: 33–59 Ma), Clade C was inferred to be 49 Ma (95% HPD: 37–61.1 Ma), and Clade D was inferred to be 49 Ma (95% HPD: 37.3–61 Ma). These age distributions fall within the Paleocene and Eocene (Fig. 10). Topology estimates also indicate the presence of new species which have not yet been named. There also may be the possibility that putative species previously synonymised into a single species may need to be re-evaluated as multiple species.

The analysis in BAMM revealed one distinct evolutionary rate shift across all sampled taxa and two macroevolutionary cohorts (Fig. 12, Fig. 13). The first cohort contained the outgroup taxa, while the second group contained the pettalid taxa. Within

the taxa sampled a single shift occurs with a prior probability (pp) = 77%, two shifts occur with a pp = 18%, three shifts occur with a pp = 4%, and four shifts occur with a pp = 0.6%. The frequency of one shift with highest posterior probability is 99.15% (Fig. 14, Fig 15, Fig. 16)

Discussion

Fully Resolved *Rakaia* Phylogeny

The RAxML phylogeny retrieved provides a fully resolved UCE based tree of *Rakaia*, which is a novel advance within the family Pettalidae. Unlike previous attempts at estimating the *Rakaia* phylogeny with methods such as Sanger Sequencing, transcriptomics, and UCEs, this attempt provided a level of support previously unattainable. Previous attempts struggled with degraded samples and low scalability potential, leading to lower resolution for nodes at deeper and shallower levels of divergence. UCEs have all but eliminated these issues, allowing for massive parallel sampling of samples which are not of the highest quality, an important quality for non-model organisms. Furthermore, this study shows the utility of UCE's when combined with organism-specific probe sets for resolving relationships within a genus and family which formerly proved to be intractable.

This high resolution result, likely due to the use of the Arachnida designed probe-set, will only increase in support with future attempts as UCE probe sets become more and more specialized in the future. Already, an Opiliones-specific probe set, designed by Derkarabetien et al. 2023 could likely be applied to this system with great success in improving further upon the results of this analysis. While the Arachnida

probe-set has proven effective for the dataset used in this study, other datasets may show improved results from a more specialized probe-set such as the that (Derkarabetien et al. 2023).

Rakaia and Gondwana

With the age of diversification of *Rakaia* inferred to be 76 Ma, this analysis corroborates that its origin post-dates New Zealand's split from Gondwana. Zealandia, the continent which formerly consisted of Australia, Antarctica, South America, and New Zealand, rifted from Gondwana approximately 82 Ma. This implies that the origin of *Rakaia* is Gondwanan, as *Rakaia* split from its sister group ~111 Ma. With the current BEAST analysis resulting in such a large distribution of estimated ages, it is plausible that *Rakaia* diverged from sister groups while Gondwana and Zealandia were still part of the same landmass, or, *Rakaia* diverged following the rifting of Zealandia from Gondwana. Either way, the divergence of *Rakaia* prior to New Zealand rising out of the submerged Zealandia is the most likely explanation from this study.

Major Lineages of *Rakaia* Divided by Geography

This study confirms the allopatric nature of major lineages of *Rakaia* (i.e., they were separated by major geographical features which prevented gene flow between each clade). Like Morisawa (2020) was able to do with her thesis work on *Rakaia*, the distinct monophyly of the four lineages or clades was recovered, with convincing spatial distributions based on knowledge of the system. Clade A, consisting of *R. florensis, R. minutissima, R. lindsayi, R. stewartiensis,* and an unnamed species from Stewart Island, represents the most widespread clade with members being located on the south of the

North Island, the north of the South Island, and Stewart Island. Possible reasons for this widespread distribution are the possibility of a land bridge which existed during the LGM which connected the islands of New Zealand (Newnham et al. 2013). The competing hypothesis, which follows the idea that the rise of the alpine fault resulted in the disjunction of this clade, is also possible based on the current distributions, which place divergences from approximately 25 Ma onwards. This roughly puts the divergence of *Rakaia* post Oligocene Drowning and around the time of New Zealand starting to form out of Zealandia with activity occurring on the Alpine Fault around 25-23 Ma. These hypotheses need to be further explored as a possibility with additional attempts of a time-calibrated tree in order to validate estimated divergence dates.

Clade B, consisting of *R. sorenseni sorenseni, R. sorenseni digitata,* and two unnamed species of *Rakaia,* are all restricted to the southern tip of the South Island. The South Island, where the *Rakaia* species from Clade B dwell, is characterized by patchy, discontinuous forest. The species ranges of the included Clade B specimens are each restricted to their own individual forest which are disjunct from each other. These ranges do not overlap, and this is likely the cause for the distinct distribution of Clade B in the southern part of the South Island (Boyer and Giribet, 2009). Furthermore, there appears to be a steady rate of divergence occurring from ~50 Ma until antiquity. That is, from ~50 Ma, there seems to be speciation occurring every "x" number of years within the clade until the present (Fig. 11). This pattern appears in Clade B and C, while Clades A and D seem to display different patterns of speciation.

Clade C, consisting of *R. antipodiana, R. collaris, R. pauli, R. macra,* and four other unnamed species reflects a range which is continuous, a pattern which Morisawa

(2020) also derived. This clade, as stated above, seems to have undergone a steady rate of divergence within the last ~50 Ma. The ranges of species are discontinuous, which reflects the patchy forests in the central part of the South Island eastern coast.

The final clade recovered was Clade D, consisting of *R. dorothea, R. media, R. solitaria, R. magna australis, R. uniloca,* and three other unnamed species. The widespread distribution could, again, be a result of the loss of the land which existed during the LGM. It is possible that a common ancestor lived on this land bridge connecting the North and South Islands, and that the resulting loss of land caused the diversification of the species to occur. However, looking at the estimated ages, its seems that the divergence times for the species living on the most southern tip of the North Island (*R. solitaria* and *R. dorothea*) and the species on the most northern tip of the South Island (*R. uniloca*) both were retrieved to be approximately 25-15 Ma, which is not consistent with the timeframe of the LGM, which occurred approximately 20 thousand years ago. This could suggest that a stronger driver of divergence for all species was the Oligocene Drowning, however more research needs to be done to validate this hypothesis.

Finally, further analysis must be done with BAMM to investigate rates of evolutionary change within the genus. While preliminary analysis of all taxa sampled revealed one rate shift, with increases in rates occurring between cyphophthalamid and non-cyphophthalamid taxa, no rate shifts were revealed within the genus *Rakaia* alone. This could be a result of sampling, as all samples were included in the analysis and multiple samples from the same reported species included. This over-weighting of certain species may have resulted in some branches not being represented accurately. Further

attempts at using BAMM will include just the use of pettalid taxa as well as just the genus *Rakaia*, which may reveal rate-shifts consistent with geological events of the past ~80 million years.

Conclusion

This study validates the Gondwanan origins of the mite harvester genus *Rakaia*, placing its origin prior to the rifting of New Zealand from Gondwana and Zealandia. It also demonstrates the high utility of UCE based target enrichment methods for capturing a high resolution phylogeny in a non-model organism. The high level of support provided by the phylogeny is novel and only possible due to the high volume of data able to be utilized with this UCE and NGS approach. Further, it is a step in the direction towards resolving the pettalid family phylogeny. The intergeneric relationships recovered from this analysis are the same as that recovered by Baker et al. 2020 (Fig. 17). While not the primary goal of this study, returning this result is important for validating the species level relationships we hope to uncover.

The results of the study indicate that likely causes for diversification within *Rakaia* occurred within the last 15-30 Ma, and that hypotheses related to the last LGM may not be responsible for divergence of species. However, this timeline does highlight the possibility that the Oligocene Drowning had a significant impact on speciation. Due to the current 95% confidence intervals on the posteriors of the BEAST generated tree, *Rakaia's* pre-Gondwanan origins can be confidently assumed. Continental crust thinning and sea floor spreading ~85-80 Ma occurred, leading to *Rakaia* diversifying on the new landmass of Zealandia and persisting. The next ~40 million years saw *Rakaia* diversify,

even with the marine transgression occurring and the Oligocene Drowning reaching its peak ~35-30 Ma. This seems to disqualify the Oligocene Drowning as a major driver of speciation for all species across *Rakaia*. However, looking at individual clades of the tree it seems like clades A and D experience radiation-like events following the Oligocene Drowning, whereas clades B and C seem to steadily diversify steadily during this time. Furthermore, this study seems to rule out the hypothesis that these radiations occurred as a result of the LGM leaving behind a disjunct forested landscape as the tree does not seem to reveal any patterns of diversification as pertains to the last 20,000 years. So overall, it seems like the Oligocene Drowning may have had impacts on individual clades within the tree. But, this must be further analyzed through replications of the BEAST workflow as well as recovery of diversification rates within the genus using BAMM across pettalid taxa.

Figures

Figure 1: Pictures of Rakaia stewartiensis, Museum of Comparative Zoology, Harvard, photo taken by Gonzalo Giribet

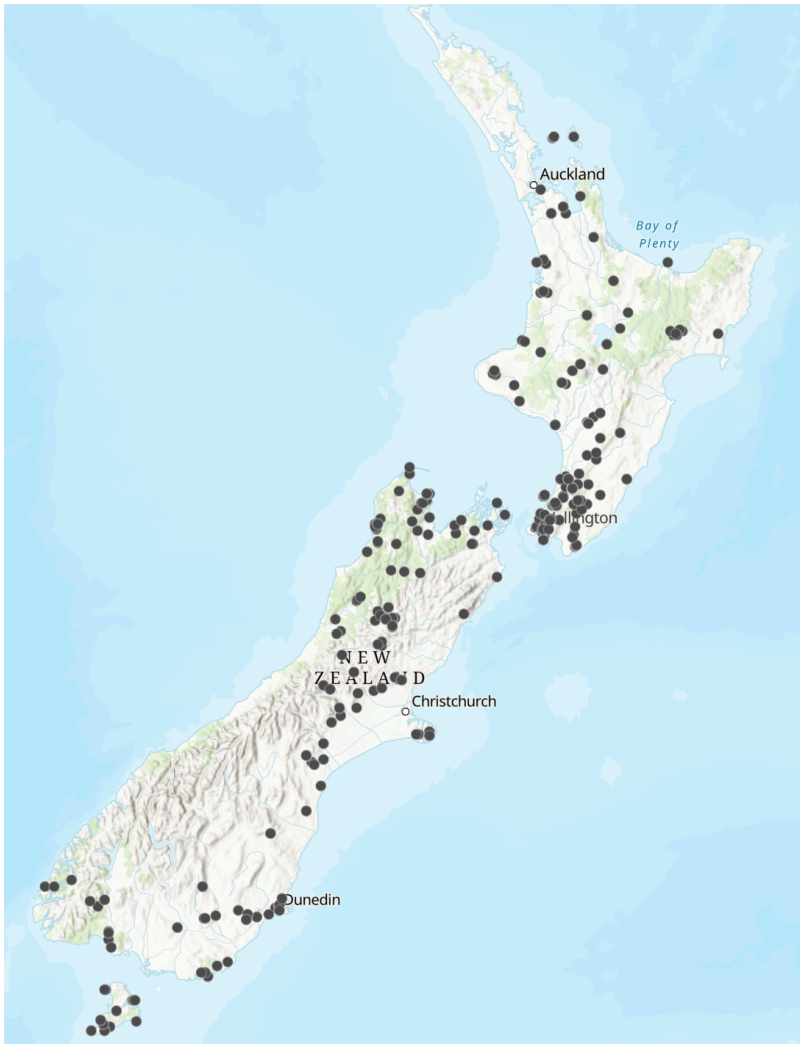

Figure 2: Distribution of *Rakaia* plotting known collections of the genus across the North, South, and Stewart Islands of New Zealand.

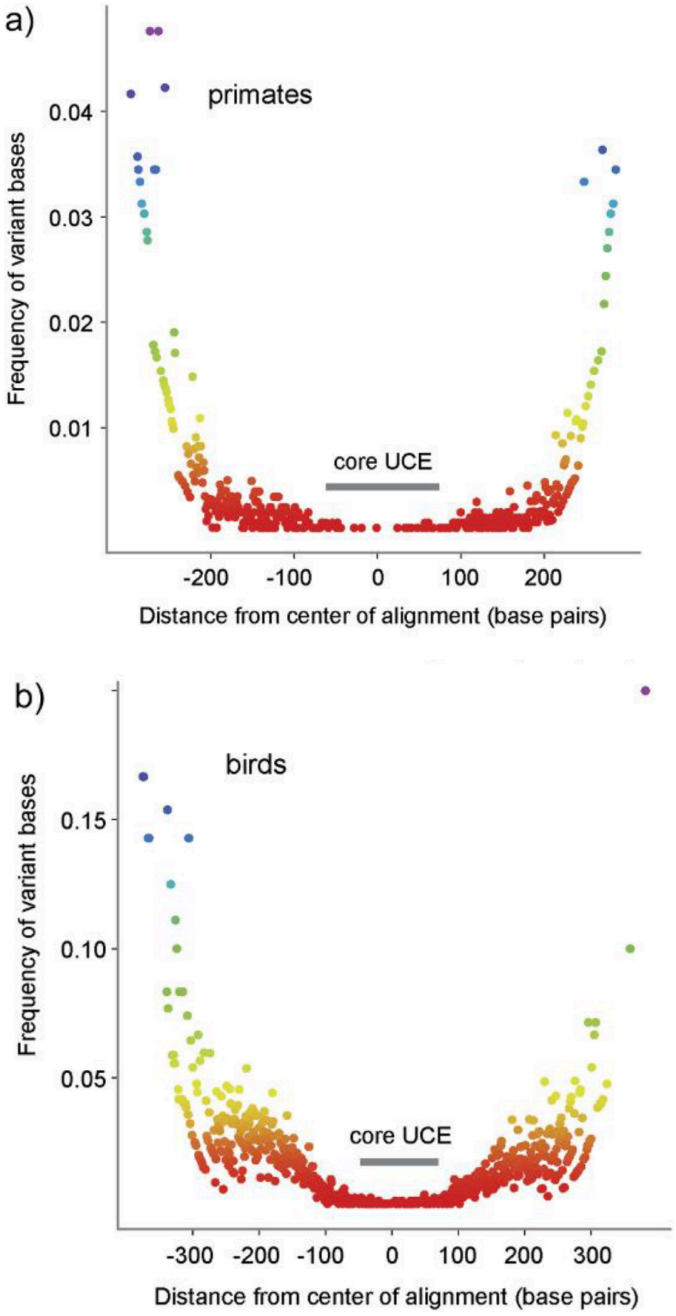

Figure 3: Faircloth et al. 2011, demonstrating in (a) primates and (b) birds that variability around core UCE-loci increases as distance from core increases.

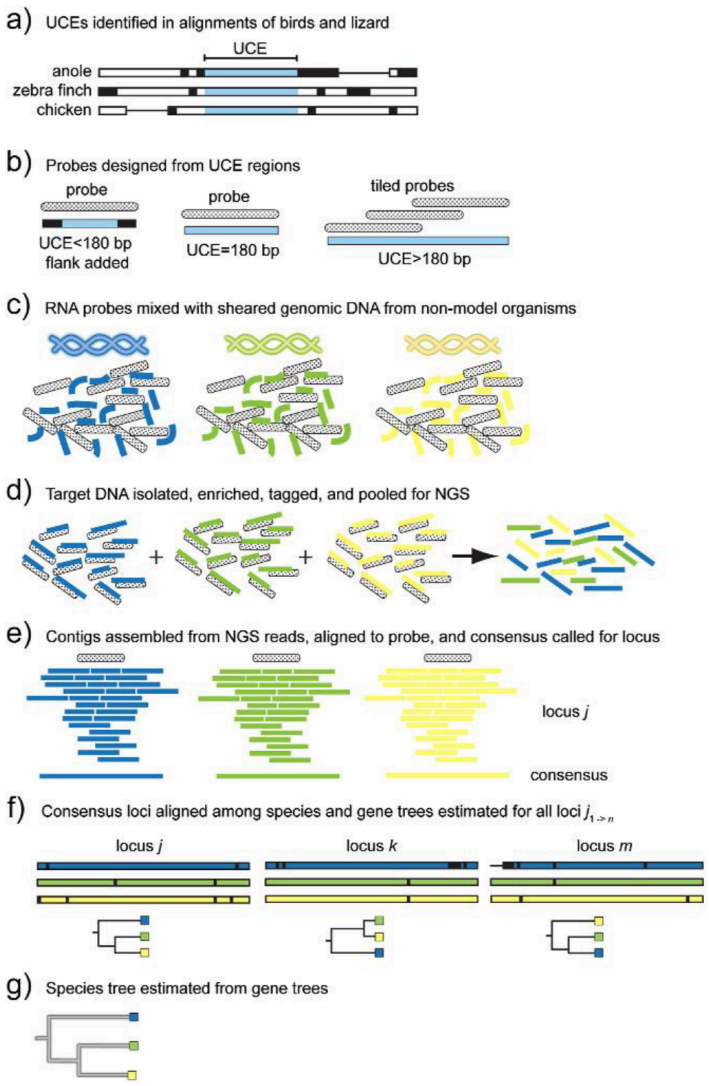

Figure 4: Faircloth et al. 2011, illustrating the work-flow of target enrichment in conjunction with UCE-loci.

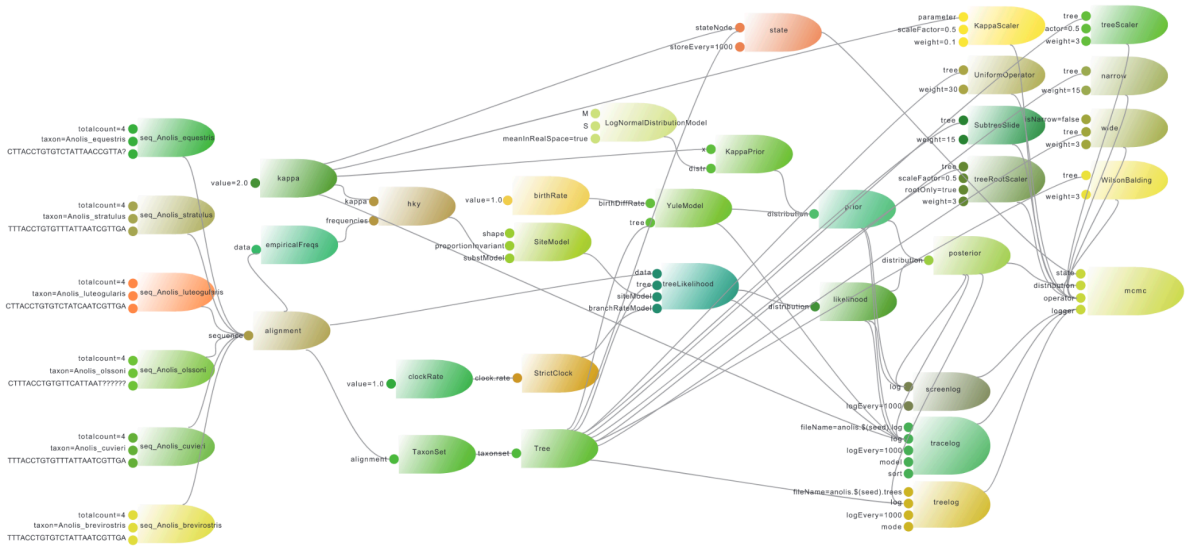

Figure 5: Example of complete model with 6 samples in BEAST2, displaying tree priors, choice of model, operators, and resulting output logs (Bouckaert et al. 2014). Workflow proceeds from left to right, with three loggers shown on bottom right as “screenlog”, “tracelog”, and “treelog”.

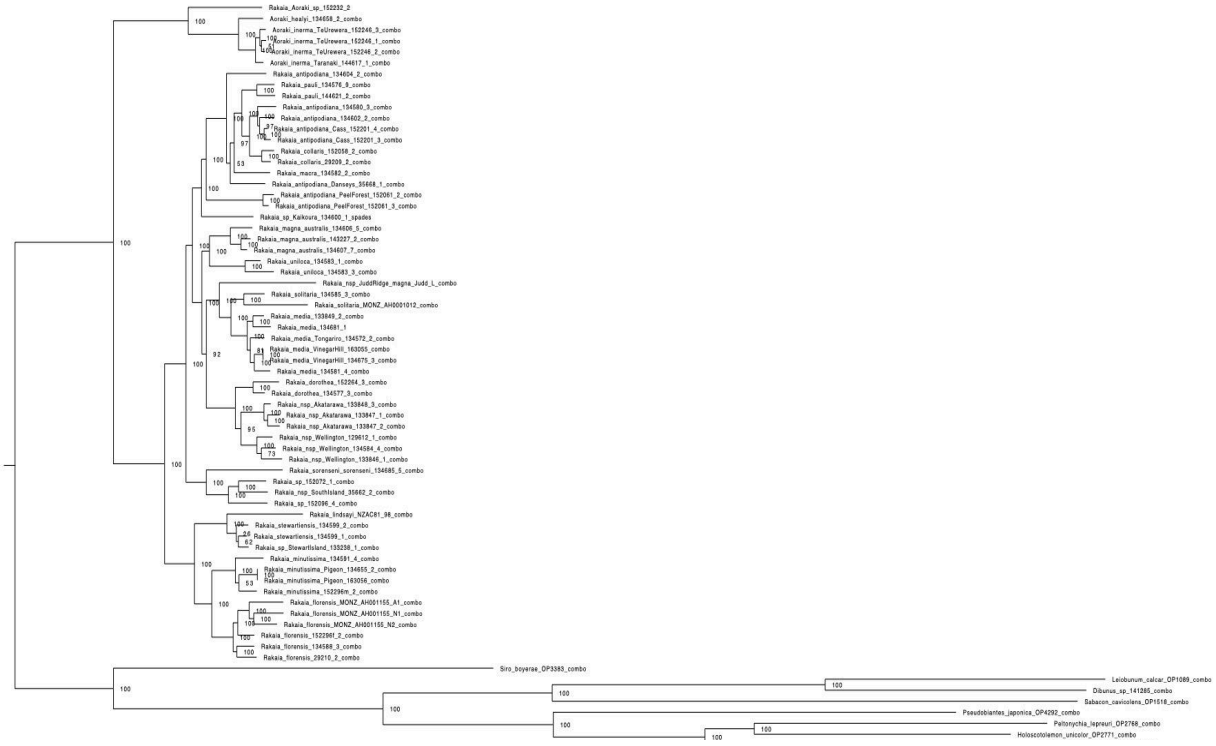

Figure 6: RAxML tree of *Rakaia* including 68 total specimens using rapid bootstrap with 500 replicates. Of the 68 specimens, 54 are *Rakaia,* six are *Aoraki,* and eight are other outgroups in the family Opiliones. Bootstrap values are displayed at each node.

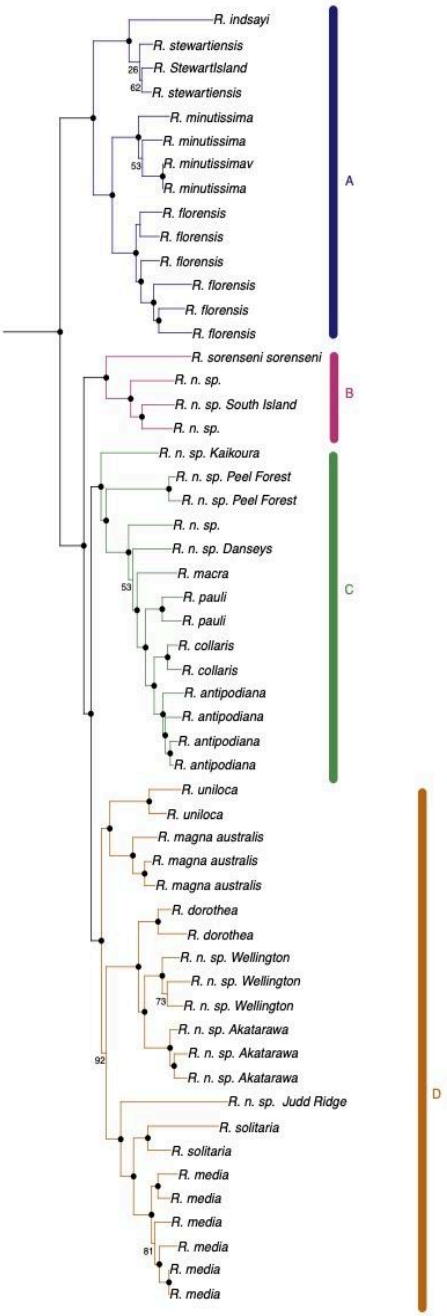

Figure 7: RAxML tree of the genus *Rakaia* using rapid bootstrap with 500 replicates. Parts of the tree are color coded by clade (A,B, C, and D), which is based on understanding of geography and the system. Black dots at each node represent bootstrap values >95.

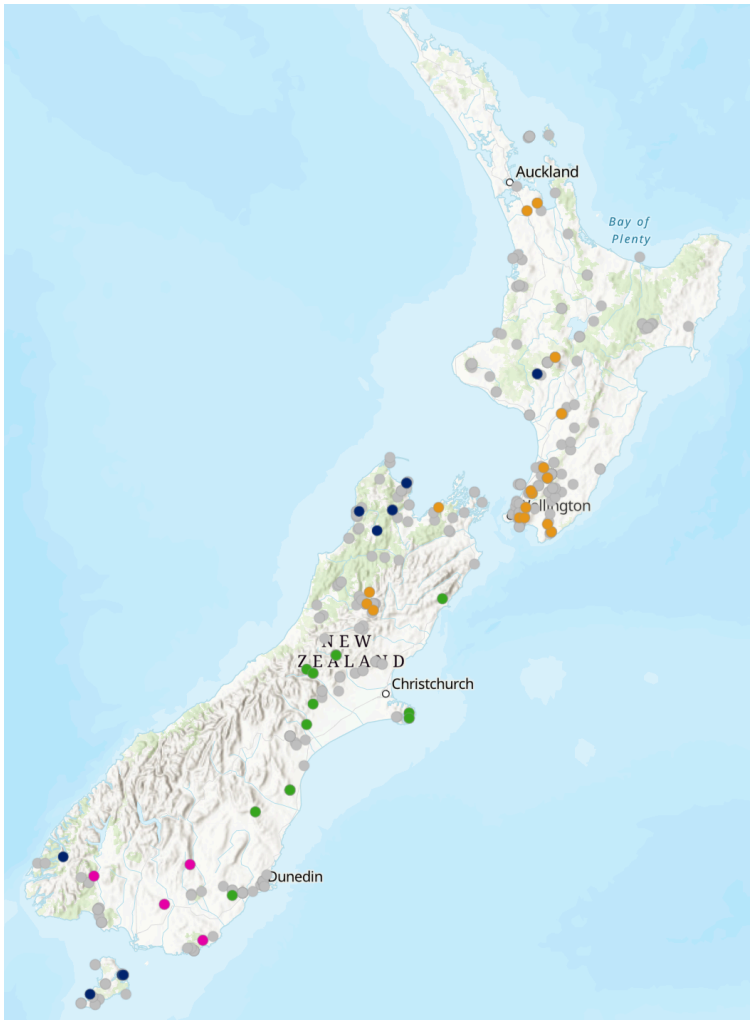

Figure 8: Distribution of *Rakaia* with colored points indicating clade inferred by knowledge of the system (Clade A = Blue, Clade B = Red, Clade C = Green, Clade D = Orange). Grey points indicate collections of *Rakaia* not included in phylogenetic analysis.

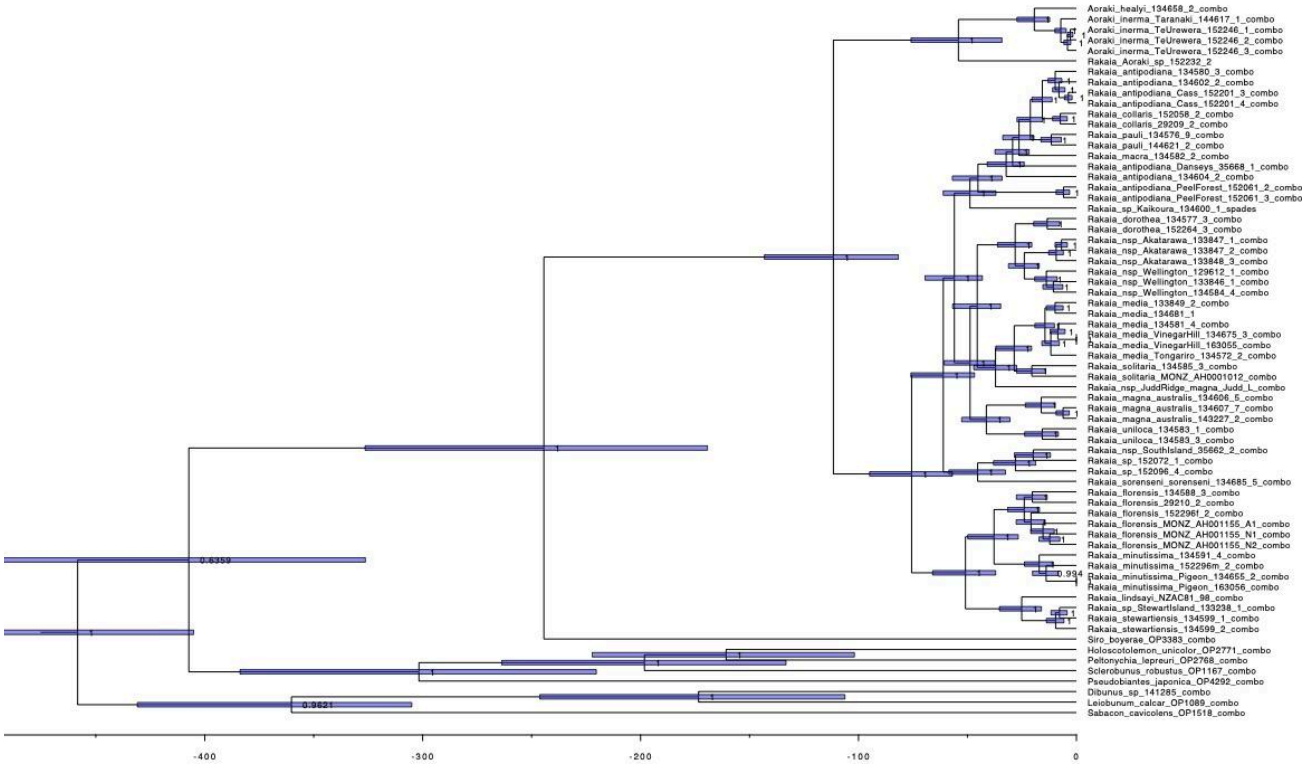

Figure 9: Time-calibrated phylogeny using Bayesian Evolutionary Analysis Sampling Trees 2 (BEAST2) showing posterior probabilities and 95% Highest Posterior Density (HPD). Numbers at each node represent posterior probability in the posterior set of trees after 20% burnin was discarded. Blue scale bars represent the 95% HPD for the age of each node in the tree. All 68 specimens are included, and the x-axis represents time in hundreds of millions of years.

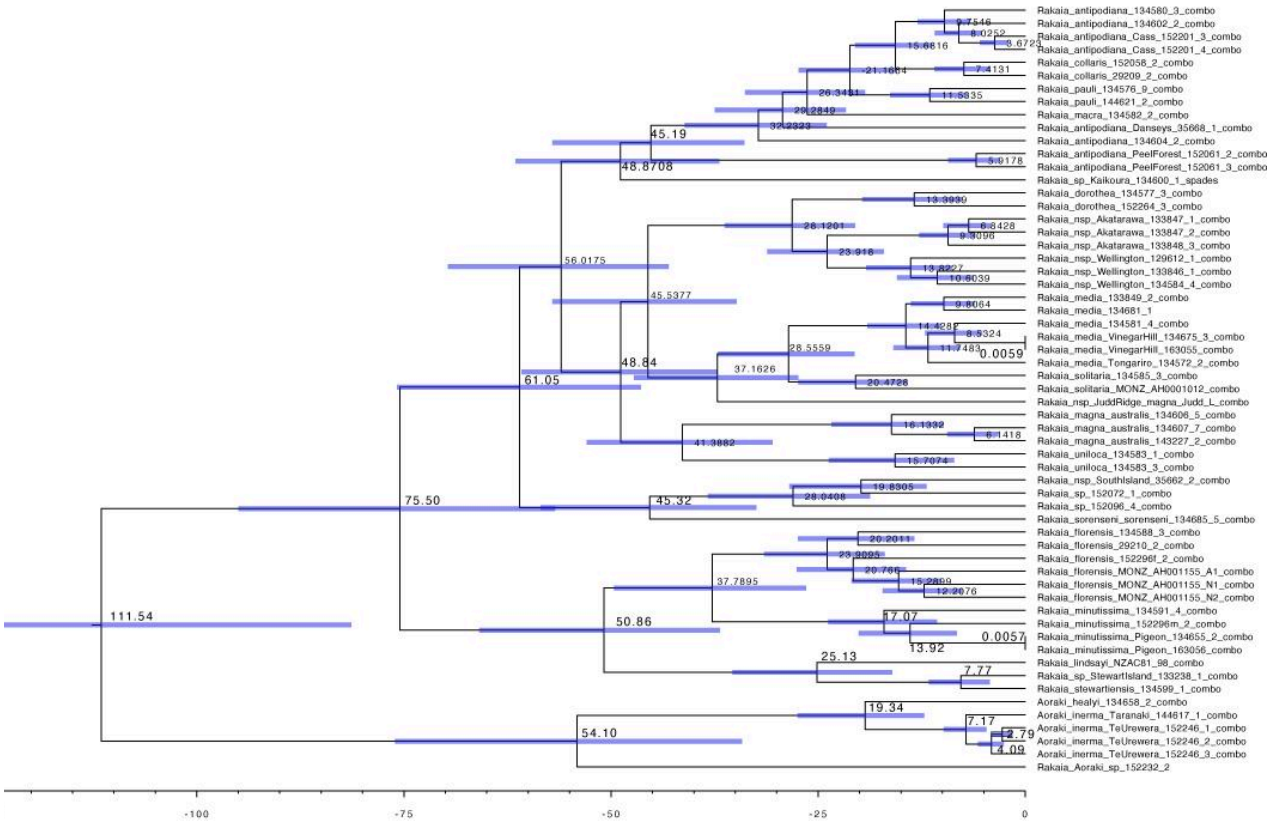

Figure 10: Time-calibrated phylogeny using Bayesian Evolutionary Analysis Sampling Trees 2 (BEAST2) showing age estimates at each node and 95% Highest Posterior Density (HPD). Only *Rakaia* and *Aoraki* specimens were included, and the x-axis represents time in hundreds of millions of years.

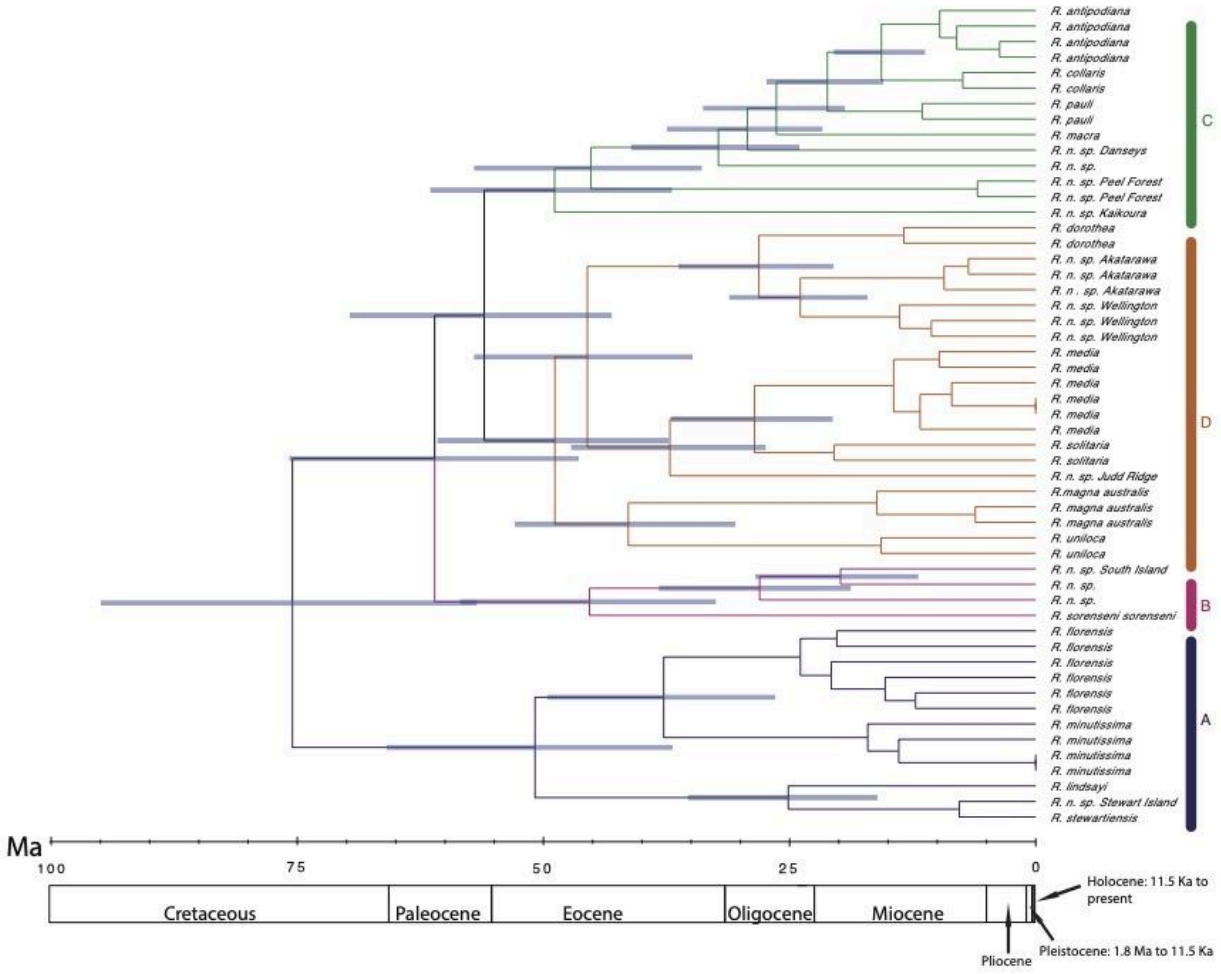

Figure 11: Time-calibrated phylogeny using Bayesian Evolutionary Analysis Sampling Trees 2 (BEAST2) showing 95% Highest Posterior Density (HPD) and color coded by clade. Only *Rakaia* is included. The x-axis represents time in hundreds of millions of years, as well as the geological time scale.

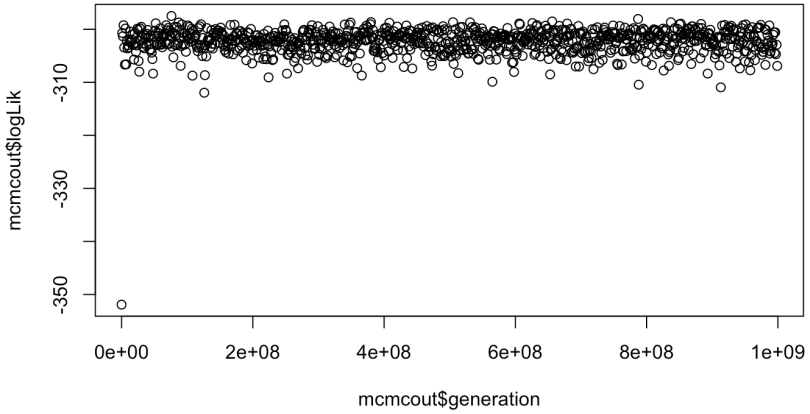

Figure 12: Convergence of MCMC algorithm by plotting the number of generations (one billion) against the log likelihood scores in BAMM 2.5. This plot represents the BAMM analysis of all taxa sampled.

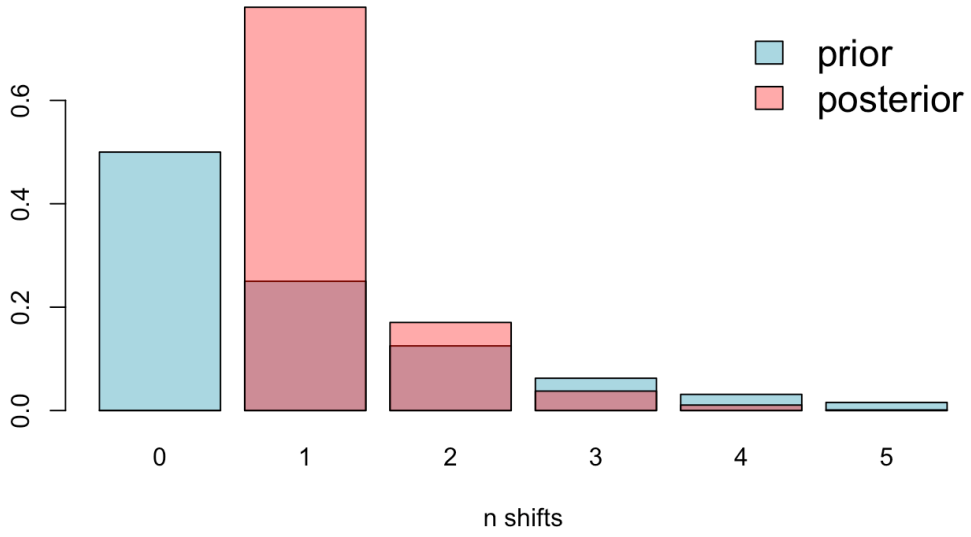

Figure 13: Probability of Core Evolutionary Rate Shifts across all taxa sampled, analyzed using BAMM 2.5. The plot indicates one core rate shift (Rabosky et al. 2014).

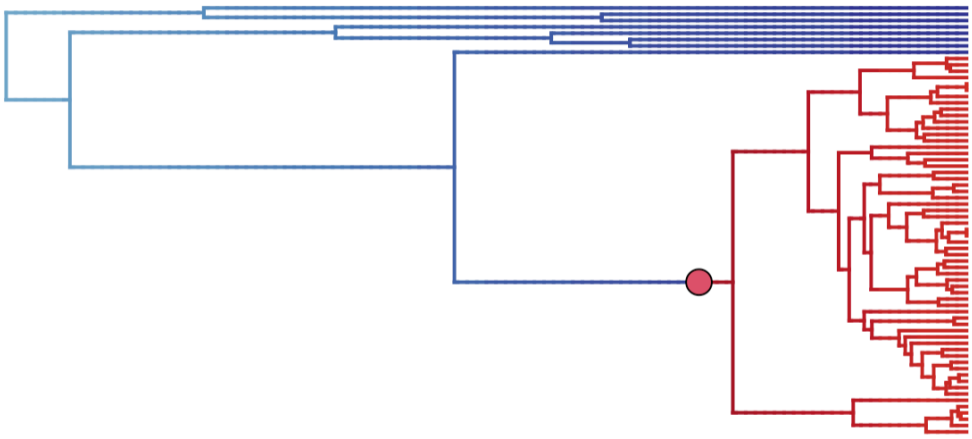

Figure 14: Maximum Shift Credibility Tree generated from BAMM 2.5.

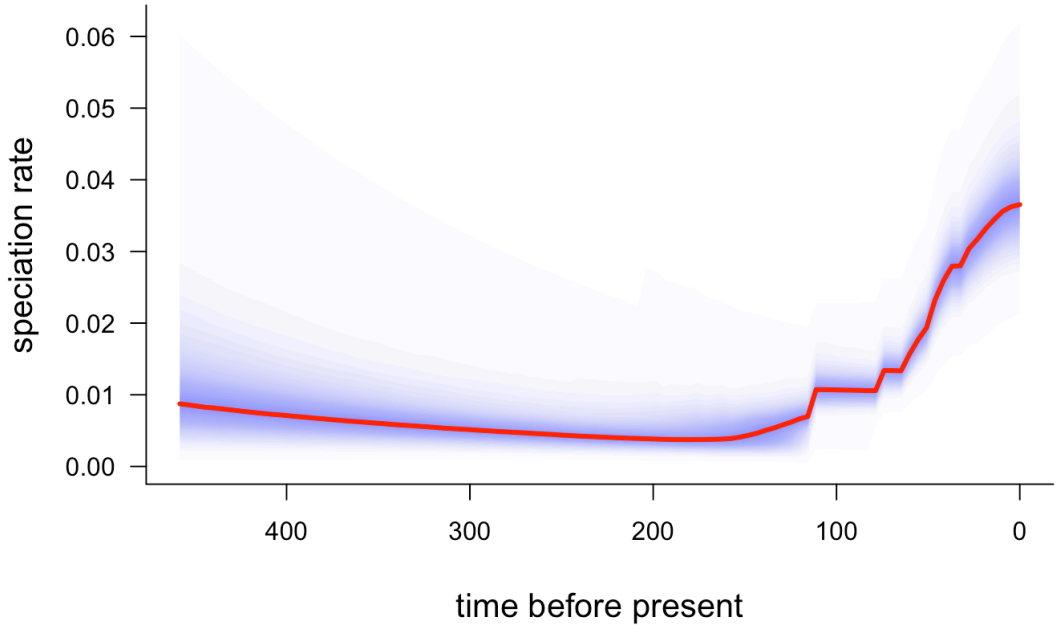

Figure 15: Through Time analysis of Evolutionary Rates generated in BAMM 2.5, indicating increase in diversification during approximate time of New Zealand rifting from Gondwana.

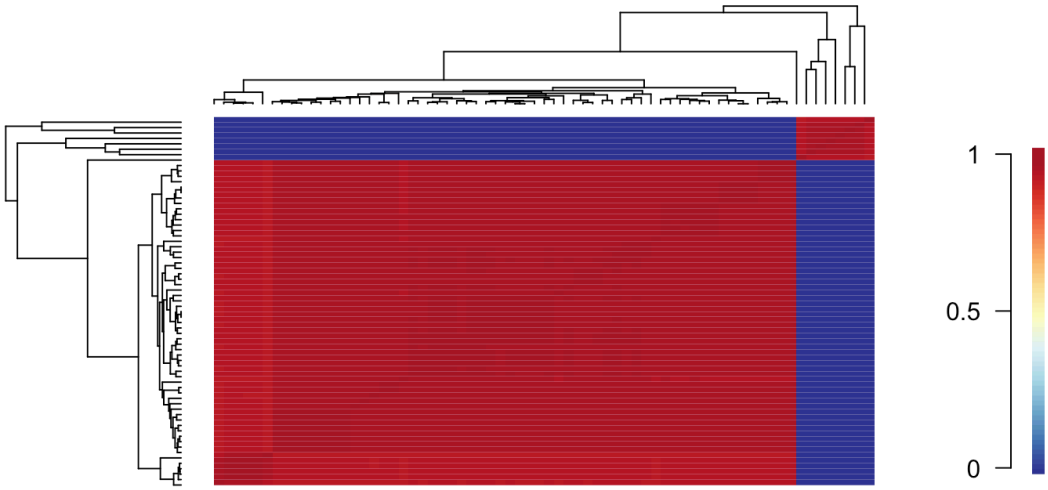

Figure 16: Macroevolutionary cohorts established in BAMM 2.5, indicating that there are two distinct cohorts of taxa which share different macroevolutionary parameters (Rabosky et al. 2014)

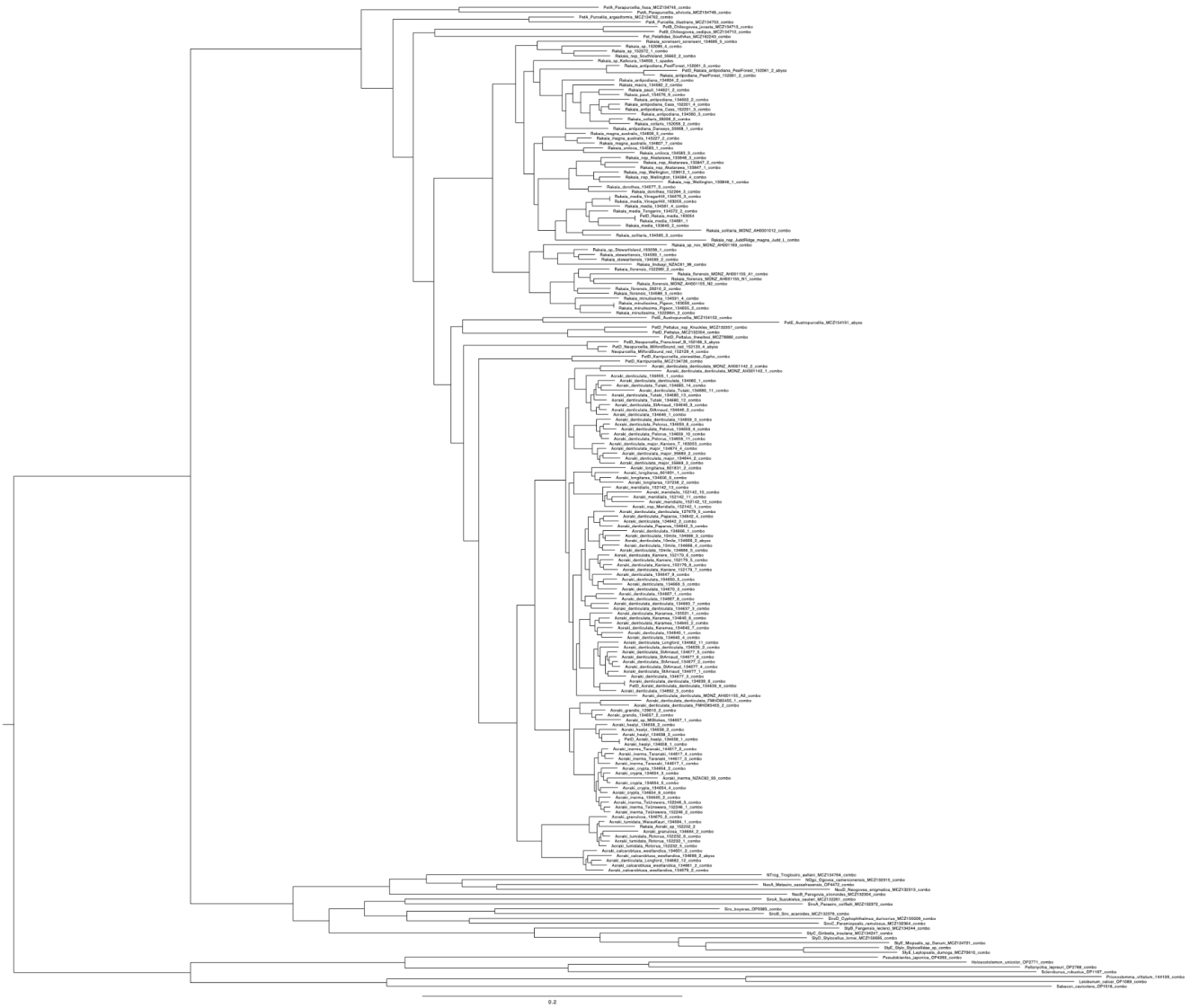

Figure 17: RAxML phylogeny with larger sampling within Pettalidae and outgroups (n = 203).

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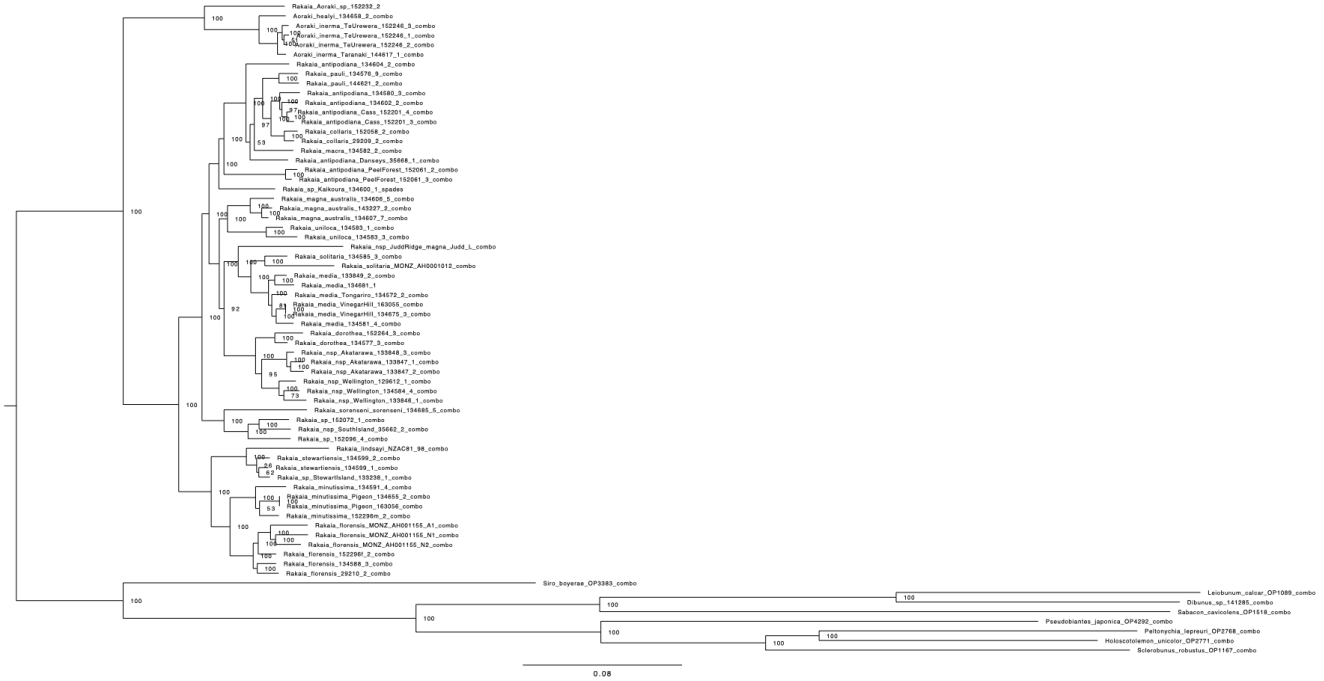
Appendix

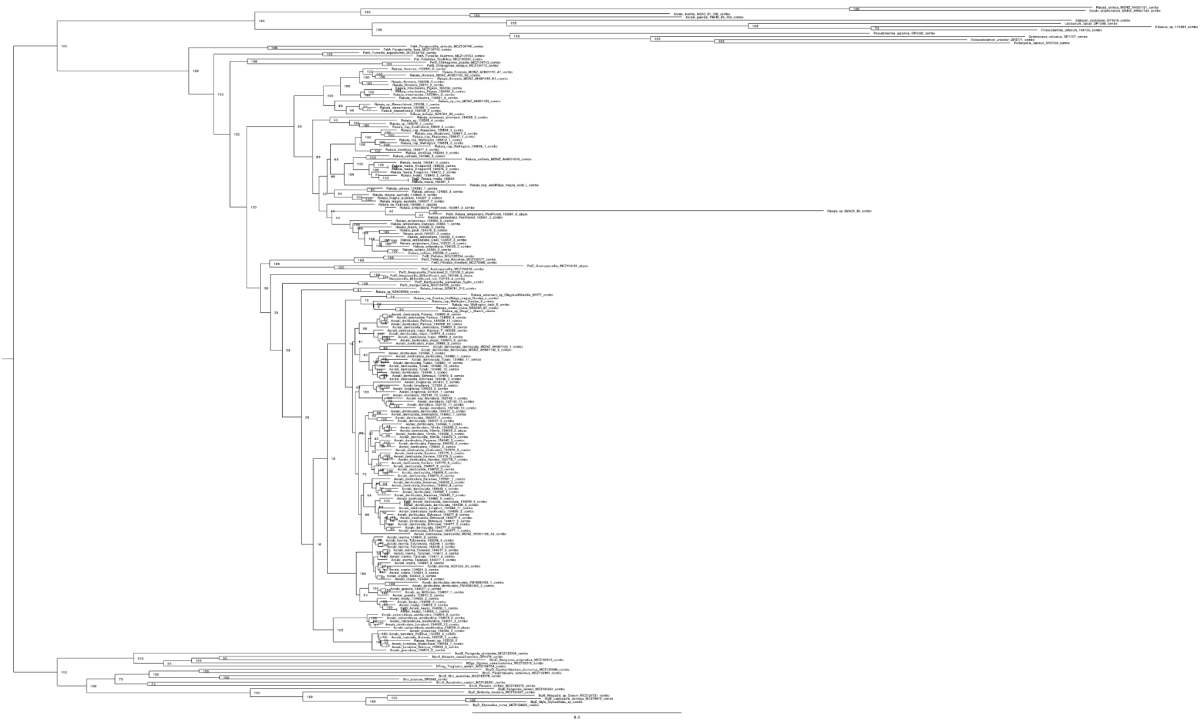
Species	Specimen Voucher	Sample Number	Latitude	Longitude	Locality	Collectors
Rakaia antipodiana	MCZ IZ-134580	3	-43.2514532000	171.3676180000	Mt. Algidus, Rakaia Gorge	Sarah L. Boyer, Cyrille D'Haese, Gonzalo Giribet
Rakaia antipodiana	MCZ IZ-134602	2	-43.1996333300	171.2514170000	Mt. Thomas Forest Cons. Area, Glentui Bush Rd., Waimakariri District	A. Schomann, Jan Pedersen
Rakaia antipodiana	MCZ IZ-134604	2	-43.6372222000	171.3750000000	Alford Forest Conservation Area, Ashburton District	A. Schomann, Jan Pedersen
Rakaia antipodiana	MCZ IZ-152201	3	-43.0345000000	171.7646167000	Cass Field Station, near creek	Caitlin M. Baker, Sarah L. Boyer, Rina Morisawa, Eliza Pessereau, Pietro Tardelli Canedo
		4				
Rakaia antipodiana	MCZ IZ-35668	1	-44.9662333300	170.3735	Dansey's Pass	Richard Leschen, J. Nunn, M. Gimmel
Rakaia antipodiana	MCZ IZ-152061	2	-43.8900000000	171.2586167000	Fern walk to Blandswood, Peel Forest	Caitlin M. Baker, Sarah L. Boyer, Rina Morisawa, Eliza Pessereau, Pietro Tardelli Canedo
		3				
Rakaia collaris	MCZ IZ-152058	2	-43.7500833333	173.0157000000	Otepatotu Reserve Trail	Caitlin M. Baker, Sarah L. Boyer, Rina Morisawa, Eliza Pessereau, Pietro Tardelli Canedo
Rakaia collaris	MCZ IZ-29209	2	-43.80876199	173.021372	Banks Peninsula, Hinewai Private Reserve, Big beech	Stephanne Boyer, Rosa Fernández, Gonzalo Giribet, Kim Youngnam
Rakaia dorothea	MCZ IZ-134577	3	-41.2816360400	174.9096040000	Williams Park, Days Bay	Sarah L. Boyer, Cyrille D' Haese, Gonzalo Giribet
Rakaia dorothea	MCZ IZ-152264	3	-41.281792	174.9993	East Harbour Regional Park, Kereru Road Track, Days Bay	Caitlin M. Baker, Benjamin Snawder
Rakaia florensis	MCZ IZ-134588	3	-40.83257901	172.9689690000	Pigeon Saddle, Abel Tasman National Park	Sarah L. Boyer, Gonzalo Giribet
Rakaia florensis	MCZ IZ-152296	2	-41.20660996	172.1571500000	Fenian Track, Oparara	Caitlin M. Baker, Benjamin Snawder
Rakaia florensis	MCZ IZ-29210	2	-41.1824959800	172.7318800000	Kahurangi National Park, Flora Hut	Rosa Fernández, Gonzalo Giribet
Rakaia florensis	MONZ AH001155	A1	-41.4590000000	172.4640000000	Denniston Plateau	BioBlitz 2012
		N1				
		N2				

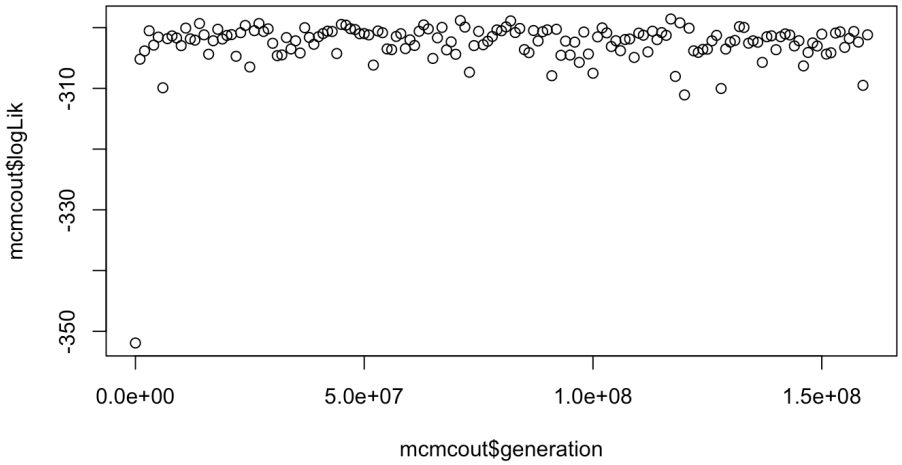
Rakaia lindsayi	NZAC 81	212				
	NZAC 81/198	1			Codfish Island, Summit	B.A. Holloway
Rakaia macra	MCZ IZ-134582	2	-45.9674400000	169.9818920000	Waipori Falls	Sarah L. Boyer, Greg D. Edgecombe, Gonzalo Giribet
Rakaia magna australis	MCZ IZ-134606	5	-42.2450000000	172.3333330000	Warbeck Scen. Res., nr. Maruia Saddle, Nelson Lakes NP, Tasman District	A. Schomann, Jan Pedersen
Rakaia magna australis	MCZ IZ-134607	7	-42.3824444000	172.2889444	between Rough Ck, and Jackson Ck.	A. Schomann, Jan Pedersen
Rakaia magna australis	MCZ IZ-143227	2	-42.47007	172.3985300000	Lewis Pass, Palmer Lodge	Gonzalo Giribet, Miquel A. Arnedo, Caitlin M. Baker, Rosa Fernández, Fernando Álvarez Padilla, Gustavo Hormiga, Robert J. Kallal
Rakaia media	MCZ IZ-133849	2	-37.1245833300	175.2214583000	Hunua Ranges Reg Park, Workman Trek	A. Schomann, Jan Pedersen
Rakaia media	MCZ IZ-134581	4	-39.9347809609	175.6404610164	Vinegar Hill Campground	Sarah L. Boyer, Gonzalo Giribet
Rakaia media	MCZ IZ-134681	1	-37.2204060000	175.0295500000	Mt. William Scenic Reserve	Robert S. Anderson
Rakaia media	MCZ IZ-134572	2	-39.1834259900	175.5248530000	Tongariro National Park, Whakapapa Village, Whakapapanui Walk	Sarah L. Boyer, Gonzalo Giribet
Rakaia media	MCZ IZ-134675	3	-39.9347809600	175.6404610164	Vinegar Hill Campground	Sarah L. Boyer, Gonzalo Giribet
Rakaia media	MCZ IZ-163055	1	-39.93478	175.640461	Vinegar Hill Campground	Sarah L. Boyer, Gonzalo Giribet
Rakaia media insula	NZAC 84.61	1			Little Barrier Island	
Rakaia minutissima	MCZ IZ-134591	4	-39.4164139800	175.2185880000	Raetihi, Tongariro National Park	Sarah L. Boyer, Gonzalo Giribet
Rakaia minutissima	MCZ IZ-152296m	2	-41.20660996	172.1571500000	Fenian Track, Oparara	Caitlin M. Baker, Benjamin Snawder
Rakaia minutissima	MCZ IZ-134655	2	-40.8325790148	172.9689690191	Pigeon Saddle	Sarah L. Boyer, Gonzalo Giribet
Rakaia minutissima	MCZ IZ-163056	1	-40.832579	172.968969	Pigeon Saddle	Sarah L. Boyer; Gonzalo Giribet
Rakaia n. sp. Akatarawa	MCZ IZ-133847	1	-40.9759549890	175.1174600143	Akatarawa Divide	Sarah L. Boyer, Gonzalo Giribet
		2				

Rakaia n. sp. Akatarawa	MCZ IZ-133848	3	-40.9458333333	175.1100000000	Akatarawa Divide	Sarah L. Boyer, Gonzalo Giribet
Rakaia n. sp. Dundas Hut Ridge	NZAC sifted litter 84/83				Dundas Hut Ridge, Tararua	B.G. Bennet & T.K. Crosby
Rakaia n. sp. Judd Ridge	NZAC litter 80/101				Judd Ridge, Otaki Forks, 8	C.F. Butcher
Rakaia n. sp. Mt. Bengar	MCZ IZ-35662	2	-45.5895000000	169.25755	Mt. Bengar near summit, Mt. Bengar Rd.	Richard Leschen, J. Nunn, M. Gimmel
Rakaia n. sp. Wellington	MCZ IZ-129612	1	-40.6388299819	175.3223660309	Waiopehu Reserve, Wellington Conservation	Pierre Paquin, Sebastián Vélez
Rakaia n. sp. Wellington	MCZ IZ-133846	1	-40.766667	175.383333	Tararua Forest Park, Holdsworth loop trail	Gustavo Hormiga
Rakaia n. sp. Wellington	MCZ IZ-134584	4	-41.1575699600	175.0216870000	Wi Toko Scenic Reserve	Sarah L. Boyer, Cyrille D'Haese, Gonzalo Giribet
Rakaia n. sp. Wellington Dundas	NZAC sifted litter 84/83				Dundas Hut Ridge, Tararua	B.G. Bennet & T.K. Crosby
Rakaia n. sp. Wellington Judd	NZAC litter 80/101				Judd Ridge, Otaki Forks, 8	C.F. Butcher
Rakaia pauli	MCZ IZ-134576	9	-44.7007340379	170.9655789938	Kelcey's bush track, near Waimate	Sarah L. Boyer, Cyrille D'Haese, Greg D. Edgecombe, Gonzalo Giribet
Rakaia pauli	MCZ IZ-144621	2	-44.70055	170.96715	Waimate, Kelcey's Bush	Fernando Álvarez Padilla; Gonzalo Giribet; Miquel A. Arnedo; Caitlin M. Baker; Rosa Fernández; Gustavo Hormiga; Robert J. Kallal
Rakaia solitaria	MCZ IZ-134585	3	-41.4685297000	175.4488610000	Opouawe Gully	Sarah L. Boyer, Gonzalo Giribet
Rakaia solitaria	MONZ AH0001012	1	-41.36764926	175.3931513	Huarangi Road	B.A. Marshall
Rakaia sorenseni sorenseni	MCZ IZ-134685	5	-45.7358300000	167.6194000000	West slopes of Mt. Burns, west of Monowai	Alexey Solodovnikov, Lars Vilhelmsen
Rakaia sorenseni digitata	MCZ IZ-152072	1	-46.5000000000	169.4877167000	Matai Falls Walk	Caitlin M. Baker, Sarah L. Boyer, Rina Morisawa, Eliza Pessereau, Pietro Tardelli Canedo
Rakaia n. sp. Croydon	MCZ IZ-152096	4	-46.060866	168.82685	Croydon Bush Scenic Reserve	Caitlin M. Baker; Sarah L. Boyer; Rina Morisawa; Eliza Pessereau; Pietro Tardelli Canedo
Rakaia n. sp. Kaikoura	MCZ IZ-134600	1	-42.323813	173.593247	Mt. Fyffe Cons. Area (at hut), Kaikoura District	A. Schomann; Jan Pedersen
Rakaia n. sp.	NZAC litter rep 1.5.82	1			Breaksea Sound	C.F. Butcher
Rakaia n. sp. Otago	Otago SPM 001604, 16	1			Leith Saddle	R.R. Forster

Rakaia n. sp. Stewart Island	MCZ IZ-133238	1	-46.89276	168.1233	Stewart Island, Rakiura NP, Fern Gully Track, Halfmoon Bay	Fernando Álvarez Padilla, Miquel A. Arnedo, Caitlin M. Baker, Rosa Fernández, Gonzalo Giribet, Gustavo Hormiga, Robert J. Kallal
Rakaia n. sp. Fiordland	MONZ AH001169	1	-45.506167	167.074	Wilmot Pass, Fiordland	J. Tweed
Rakaia stewartiensis	MCZ IZ-134599	1	-46.8932720200	168.103976	Fem Gully Track, Stewart Island, Halfmoon Bay (Giribet Loc. 187)	Sarah L. Boyer, Cyrille D' Haese, Greg D. Edgecombe, Gonzalo Giribet
		2				
Rakaia uniloca	MCZ IZ-134583	1	-41.1588900000	173.5284610000	Whangamoa Saddle	Sarah L. Boyer, Jessica M. Baker
		3				
Rakaia uniloca	MONZ AH001101					
Aoraki healyi	MCZ IZ-134658	2	-41.0908330000	174.1016670000	Mt. Stokes	Sarah L. Boyer
Aoraki inerma	MCZ IZ-144617	1	-39.2737600000	174.9365000000		
Aoraki inerma	MCZ IZ-152246	1	-38.7427719900	177.1639590000	Waikareiti Track, Te Urewera	Caitlin M. Baker, Benjamin Snawder
		2				
		3				
Aoraki tumidata	MCZ IZ-152232	2	-38.1184333333	176.2006000000	Mt. Ngongotaha Scenic Reserve	Caitlin M. Baker, Benjamin Snawder



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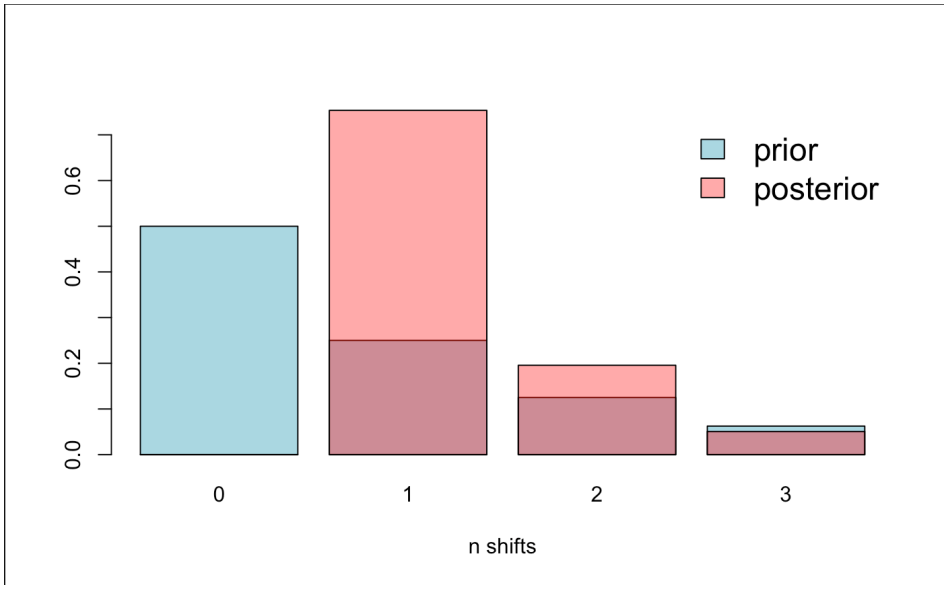
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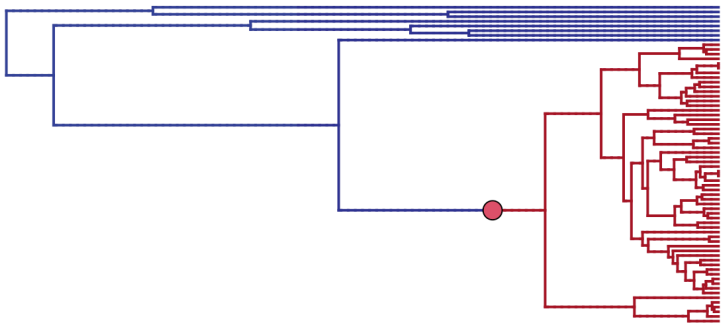

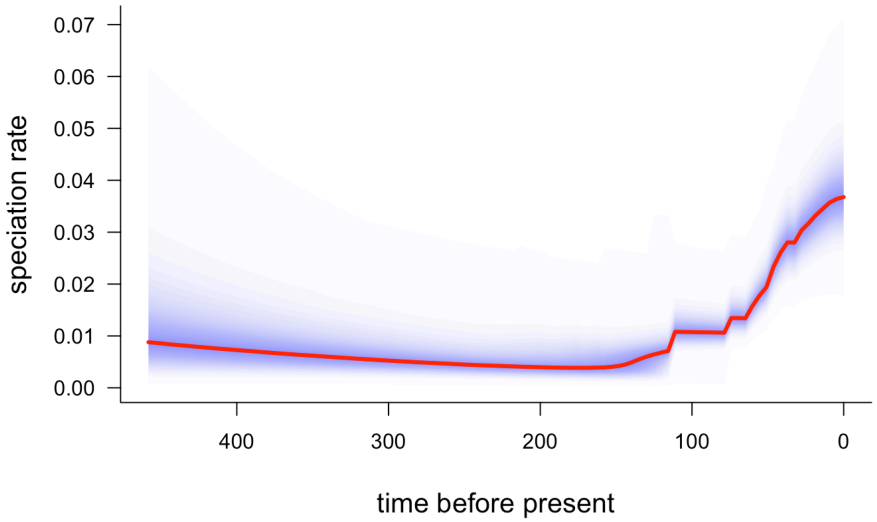
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