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Dating the emergence of the divaricate habit in the New Zealand flora

A thesis

submitted in fulfilment

of the requirements for the degree

of

PhD in Biological Sciences

at

The University of Waikato

by

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THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

2021

Abstract

The New Zealand divaricates are a collection of shrubs, short trees and tree juveniles whose crowns are made of tough interlaced twigs branching at wide angles and bearing small leaves. These species represent c. 13% of the native woody flora, a proportion not seen in any other region of the world. Since the late 19th century, ecologists and botanists have sought to understand the of drivers this unique case of convergent evolution. Debate has been dominated by two main competing hypotheses invoking (1) the effect of browsing by now-extinct avian herbivores (moa) and (2) a response to frosty and droughty Plio-Pleistocene climates. Observational and experimental evidence, as well as theoretical discussions, have not clearly favoured one over the other. More recently, a synthetic hypothesis involving both climate and browsing has been proposed, but has not been specifically tested yet: the divaricate habit did not become advantageous as an anti-browsing defence until Plio-Pleistocene climatic constraints prevented young trees and shrubs from growing quickly out of reach of ground-dwelling herbivores.

These hypotheses imply different expected divergence periods between divaricate species and their non-divaricate relatives. The focus of this PhD project is to produce a dated phylogeny including as many divaricates as possible, with the aim of bringing new evidence to help tease apart the various hypotheses about their evolution.

This PhD thesis is built from a previously published paper and draft manuscripts intended for submission to scientific journals. The first two papers report phylogenies of small genera (*Pennantia* J.R.Forst. & G.Forst. and *Corokia* A.Cunn.) that include divaricate species. These studies helped develop lab methods and offered the opportunity to test diverse phylogenetic methods on a smaller scale in preparation for the third paper, which is the core dating work of this PhD.

The phylogenies of *Pennantia* and *Corokia* provided in this thesis (Chapter 2 and 3, respectively) are the first published dated phylogenies of all the species of each genus. Building the phylogeny of *Corokia* was also the occasion to discuss two theories trying to explain the distribution of extant species on landmasses formerly part of Gondwana: vicariance and long-distance dispersal—*Corokia* appears to be one of an increasing number of cases where long-distance dispersal is indicated. The dated phylogeny of most New Zealand divaricate species presented in Chapter 4 reveals that, in the great majority of genera with divaricate representatives, the divaricate habit appeared in New Zealand within the last 5 My, i.e. since the beginning of the Pliocene.

On one hand, this research constitutes a valuable methodological addition to the field of molecular phylogenetics by (1) disseminating a method for retrieving extra genetic markers, at no marginal cost, from Next Generation Sequencing shotgun sequencing data of DNA samples enriched for a specific set of markers, and (2) developing a guide to using a piece of phylogenetic reconstruction software (treePL), which was missing from the literature and needed by users. On the other hand, dating the emergence of the divaricate habit brought new and crucial evidence to the debate over what promoted the evolution of the divaricate habit in New Zealand: the findings are clearly consistent with a major effect of Plio-Pleistocene climates—and given evidence and discussion from past studies of these plants and similar plants around the world, the effect of browsing by moa was also probably involved.

Acknowledgements

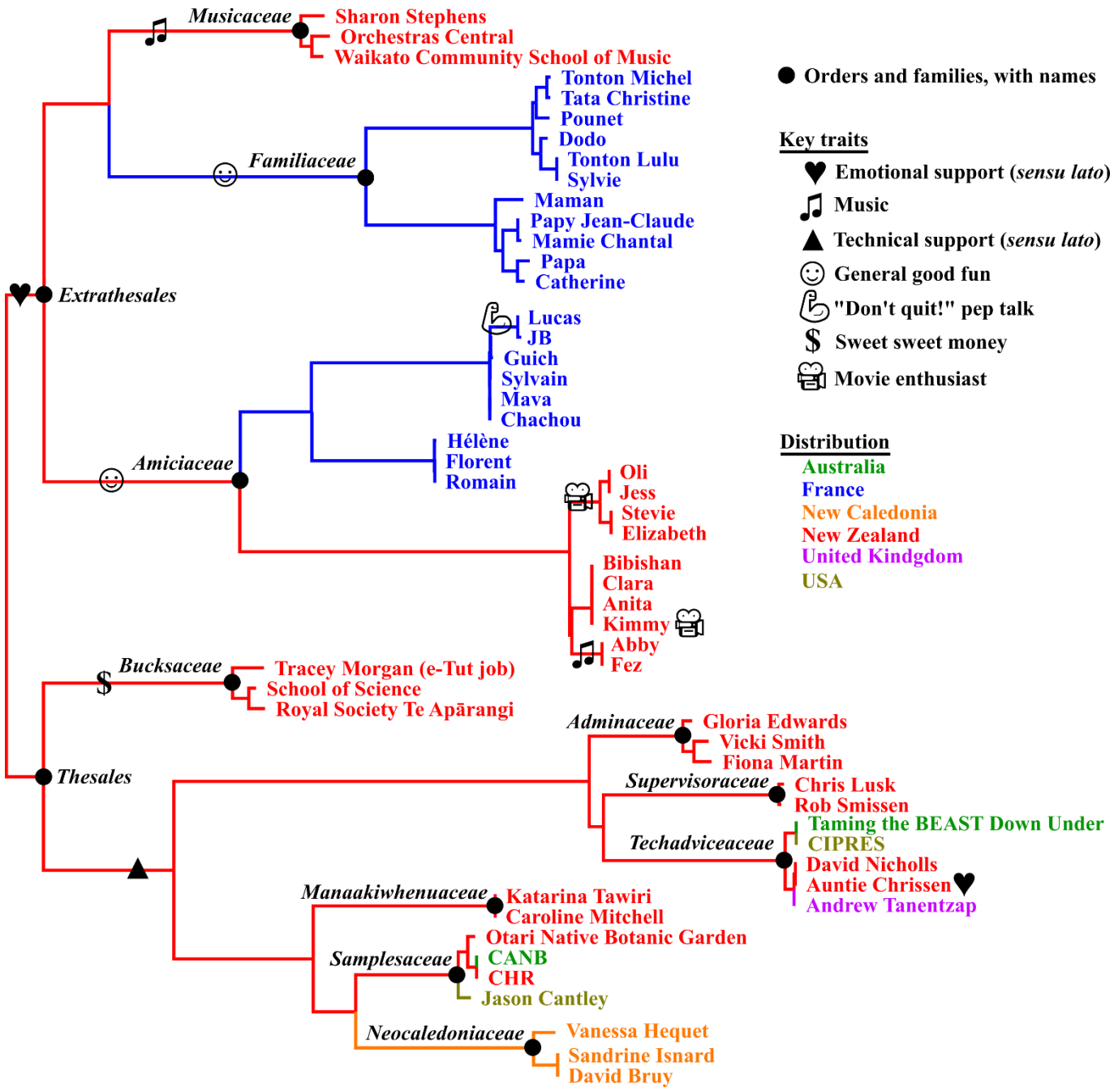

Figure 0. *Molecular phylogeny of the 55 entities who I consider brought the most significant support (of any kind) during the three years of my PhD. DNA samples were collected in obscure ways (how could I get DNA from non-living entities otherwise?), then prepared for sequencing and sequenced using good old alchemy recipes. The phylogeny was built with the usual black magic tricks. The names of the orders and families are indicated at their respective common ancestors. Symbols indicate key character traits displayed by the corresponding clade. All nodes have ≥ 95% support. My apologies to entities who think they rightfully deserve a branch in this phylogeny—55 was not a randomly chosen number, and there are only so many that can be included.*

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Chapter 1 Thesis introduction

1.1 Literature review

Preliminary note

This literature review was accepted for publication in *New Zealand Natural Sciences* in January 2021 under the following reference:

Maurin, K. J. L., & Lusk, C. H. (2021). 120 years of untangling the divaricate habit: A review. *New Zealand Natural Sciences, 46*. https://doi.org/10092/101662

It was published under a CC-BY 4.0 International licence (https://creativecommons.org/licenses/by/4.0/). The content of this section is therefore an exact copy of the published article, except that (1) the heading, the ORCID numbers of the authors and the lists of keywords and of references were omitted, (2) footnotes were added to add elements pointed out by the examiners without having to modify the published text, and (3) for consistency with the rest of the thesis, the citations were reformatted and the numbering of figures and tables adjusted.

Abstract

The evolution of divaricate plants in New Zealand has been the subject of long-running debate among botanists and ecologists. Hypotheses about this remarkable case of convergent evolution have focused mainly on two different types of selective pressures: the Plio-Pleistocene advent of cool, dry climates, or browsing by now-extinct moa. Here, we review the scientific literature relating to New Zealand divaricates, and present a list of 81 taxa whose architectures fall on the divaricate habit spectrum. We recommend a series of standardised terms to facilitate clear communication about these species. We identify potentially informative areas of research yet to be explored, such as the genetics underlying the establishment and control of this habit. We also review work about similar plants overseas, proposing a list of 53 such species as a first step towards more comprehensive inventories; these may motivate further studies of the ecology, morphology and evolutionary history of these overseas plants which could help shed light on the evolution of their New Zealand counterparts. Finally, we compile published

divergence dates between divaricate species and their non-divaricate relatives, which suggest that the divaricate habit is fairly recent (< 10 My) in most cases.

Introduction

The earliest mention we have found of what we call today "divaricating plants" or "divaricates" was made in 1896 by German botanist Ludwig Diels. He described them as "systematically distant descendants of the New Zealand forest flora that converged towards a xerophytic structure" (Diels, 1896, pp. 246-247, translated from German). He expressed surprise at seeing apparently drought-adapted species in climates that are generally more humid than in his native Central Europe, where plants do not show similar architectures. These plants are nowadays recognised as a collection of shrubs and early growth stages of heteroblastic trees bearing small leaves on tangled branches diverging at wide angles.

Such a case of convergent evolution naturally attracted much attention from local and overseas botanists and ecologists. The centre of this attention was to identify putative selective forces that may have driven this evolution. Diels (1896) initially proposed drought as the main selective factor, and McGlone & Webb (1981) considered that frost and wind might also have been important. Diels' climatic hypothesis remained largely unchallenged until Greenwood & Atkinson (1977) developed the moa-browsing hypothesis that several authors had previously hinted at (e.g. Carlquist, 1974; Denny, 1964; Taylor, 1975), igniting a passionate debate that is still ongoing today. Concurrently, a non-selective evolution process was proposed by Went (1971): the horizontal transfer of "divaricate" genes; it however was strongly criticised on theoretical grounds (Greenwood & Atkinson, 1977; Tucker, 1974) and has not been empirically investigated so far.

Rationale for and content of this review

Although about 120 years have passed since the first publications on the topic, the real debate around the evolution of divaricates only started in the late 1970s. Yet, no recent literature review (e.g. Wilson & Lee, 2012) offers an exhaustive account of all the scientific material published about these plants. The aim of this review is to provide a comprehensive resource for anyone with an interest in divaricate plants.

First, we review past attempts at defining the divaricate habit and describing its variability in New Zealand. We propose a series of terms to try to standardise the

vocabulary to be used when discussing these species (in bold in the text). We also report and discuss observations of divaricate-like species overseas, compiling a list of such occurrences.

We then review the published hypotheses that have been formulated to explain how such a diversity of architectures was selected in the New Zealand flora, and comment on the weight of evidence for or against each hypothesis. Finally, we examine the handful of studies that, rather than focusing on the evolution of these species, have looked at developmental aspects of these peculiar architectures. We conclude our review by pointing out new areas of research that might enhance our understanding of divaricate plants.

Characterising the diversity of divaricating habits: variations on a New Zealand theme

Past attempts at defining the divaricate habit in New Zealand

"Divaricate" comes from a Latin root meaning "stretched apart", which in botany refers to the usually wide angle at which branches of these species grow from the stem on which they originate. Indeed, the branching angle of divaricating species is on average more than 70°, sometimes over 90° (Bulmer, 1958; Greenwood & Atkinson, 1977), whereas their broadleaved relatives branch on average at < 55° (Kelly, 1994). However, simplifying the definition of a divaricate species by its branching angle is misleading: Pott & McLoughlin (2014) and (Pott et al., 2015) discussed the evolutionary adaptations of shrub or low-growing tree species of the extinct gymnosperm family Williamsoniaceae by making a parallel between them and New Zealand divaricates, claiming that they share similar architectures. Although the species they described undeniably branched at wide angles, they did not look anything like what New Zealand researchers call "divaricates": they bore much larger leaves (4-25 cm long, cf. < 2 cm in most New Zealand divaricates), and their branches were not interlaced. Likewise, many examples of extant species can be cited as having wide branching angles while not satisfying the definition of a divaricate, e.g. *Araucaria heterophylla* (Salisb.) Franco or *Piper excelsum* (G.Forst.). Indeed, the divaricate habit in New Zealand is also defined by a collection of other traits, including: small leaves (leptophyll and nanophyll classes of Raunkiær, 1934); interlaced and abundant branching; relatively long internodes compared to the size of their leaves (Kelly, 1994 and references therein; Maurin & Lusk, 2020)—although some species show "short-shoot development" (Tomlinson, 1978), i.e. stubby shoots with densely crowded nodes

and leaves. The exact set of features used to define the habit however varies between authors (Grierson, 2014; Kelly, 1994; see Table 1 in Supplementary Material for a list of traits used by past authors). Finally, New Zealand divaricates are notably lacking in spines, except for *Discaria toumatou* Raoul which has spinescent congeners in Australia and South America. Some divaricating species, such as *Melicytus alpinus* (Kirk) Garn.-Jones and *Aristotelia fruticosa* Hook.f., have been considered spinescent by some authors (e.g. Burns, 2016; Greenwood & Atkinson, 1977), but we argue that their pointed branchlets are not sharp enough to pierce the skin and therefore probably did not have the same adaptive value as actual wounding spines or thorns.

Because the divaricate habit has evolved independently multiple times in the New Zealand flora, it appears under different structural forms that were tentatively grouped by various authors to form classifications. Bell (2008) recognised four branching pattern types in divaricate species: branching at wide angles (e.g. *Aristotelia fruticosa*), zig-zagging by sympodial (e.g. juvenile form of *Elaeocarpus hookerianus* Raoul) or monopodial (e.g. *Muehlenbeckia astonii* Petrie) growth, and "fastigiate". The use of "fastigiate" (meaning narrow branching angles) to categorise divaricate plants may seem paradoxical, but Bell's (2008) example, *Melicytus alpinus*, sometimes does show a fastigiate habit in shaded habitats. In our experience, however, in sunny environments *M. alpinus* has wider branching angles and is compactly interlaced. Tomlinson (1978) tried to assign divaricate species to Hallé et al.'s (1978) architectural models, without success. Halloy (1990) defined five groups based on branching patterns and assigned one species per group as examples, but his proposal has been largely ignored.

These variations around the features which characterise the divaricate habit led Wardle & McGlone (1988) to propose the word "filiramulate" to describe lianes and shrubs with reduced apical buds that have some (but not all) of the traits usually regarded as integral to the divaricate habit. These reduced buds exert a weakened apical dominance (Wardle & McGlone 1988), and thus do not prevent the outgrowth of lateral branches. This first definition of the term "filiramulate" emphasised the wiry branches that may be flexuose to truly divaricating, and divaricate plants were therefore considered a type of filiramulate species. However, this definition of "filiramulate" has not been widely adopted by the scientific community.

The lack of a consensus word-based definition of the divaricate habit led to two attempts to find a mathematical quantification of divaricateness. Atkinson (1992) focused on branch density (number of lateral branches subtended per cm of main branch) and branching angle; Kelly (1994) also focused on branching angle, and included leaf size

and density (the relative width of the leaves to the size of the internodes that bear them). Although these two indices emphasise different features of the divaricate habit, they correlate well for New Zealand species (Grierson, 2014; Kelly, 1994). In spite of these indices, which are rarely used in the literature, consensus definitions of the divaricate habit and its variations are still lacking.

Heteroblastic divaricate species

Although most of the species showing the divaricate habit keep it their whole life, some heteroblastic species produce a divaricating form early in life, then later switch to a non-divaricating form (Cockayne, 1958). Very few quantitative data exist regarding the age before the non-divaricating form appears (Table 1.1), which may depend on the degree of exposure to sunlight in many cases (Cockayne, 1958), or even on latitude at least in *Sophora microphylla* Aiton (E. J. Godley, 1979). We propose referring to them as **heteroblastic divaricate** species; the term "habit-heteroblastic" used by Philipson (1963) for such species is inadequate as it does not mention "divaricate", and the juvenile and adults forms of some heteroblastic divaricate species do not only differ in architecture but also in leaf shape (e.g. *Pennantia corymbosa* J.R.Forst. & G.Forst.). Both forms often coexist on the same individual at least for some time, and the transition can be abrupt ("metamorphic" species (Ray, 1990), such as in *Pennantia corymbosa*; see Figure 1 in Supplementary Material), or gradual with transitional forms between the divaricating bottom and the non-divaricating top of the plant ("allomorphic" species (Ray, 1990), such as in *Hoheria sexstylosa* Colenso). Day et al. (1997), studying the transition of the heteroblastic divaricate *Elaeocarpus hookerianus* from its juvenile form to its adult form, described a distinctive transitional form characterised by a less plastic growth pattern than the juvenile form, while not showing the morphological attributes that identify the adult form.

Table 1.1. *Published quantitative measurements and estimations of the age reached by heteroblastic divaricate species before their adult form appears.*

Species	Duration of the juvenile form	Reference
Elaeocarpus hookerianus Raoul	At least 60 years, depends on light conditions (source not specified by the author)	Cockayne (1958)
Prumnopitys taxifolia (D.Don) de Laub.	(1) Up to 60 years (source not specified by the author) (2) At least 47 years (based on ring counts)	(1) Dawson & Lucas (2012) (2) Lusk (1989)
Sophora microphylla Aiton	(1) ca. 15 years (source not specified by the author) (2) variable according to location: from absence of juvenile form in some parts of the North Island, to ca. 3.5 years in the Auckland region and at least 23 years in the south-east of the South Island (based on field observations and a common garden experiment)	(1) Cockayne (1958) (2) Godley (1979)

The ubiquitous use in the literature of the adjectives "juvenile" and "adult" (sometimes "mature") to name, respectively, the early divaricating form and the ultimate non-divaricating form of heteroblastic divaricate species, is potentially misleading. Jones (1999) criticised the use of "juvenile" to describe early forms of heteroblastic species because it better characterises a phase of plant development that is incapable of sexual reproduction. She therefore suggested that "juvenile" should be restricted to non-flowering stages of heteroblastic species. Yet, it was observed in New Zealand that the early form of some heteroblastic divaricate species are capable of flowering, such as those of *Pennantia corymbosa* (Beddie, 1958; Cockayne, 1958) or *Plagianthus regius* (Poit.) Hochr. subsp. *regius* (Cockayne, 1958): they should therefore not be termed "juvenile". However, alternative terms such as "young" and "old" carry ambiguities of their own, so it is not obvious to us how to improve upon "juvenile" and "adult", which have become deeply anchored in the literature. We however recommend the use of **juvenile/ adult form** instead of the more commonly used juvenile/adult "stage" or "phase" to avoid the confusion between growth habit and reproductive state that Jones (1999) pointed out.

Two hypotheses have been proposed to try to explain the origin of heteroblastic divaricate species:

1. Hybridisation between a divaricate species and a non-divaricate relative

It is well known that some divaricate species hybridise with broadleaved congeners (e.g. Dansereau, 1964; see lists of known (and potential) hybrids compiled by Cockayne, 1923; Cockayne & Allan, 1934; Greenwood & Atkinson, 1977). These hybridisation events were hence proposed as a source for the origin of heteroblastic divaricate species

(Godley, 1979; 1985). Carrodus (2009) addressed the question of whether *Pittosporum turneri* Petrie, a heteroblastic divaricate small tree, is a hybrid between *Pittosporum divaricatum* Cockayne, a divaricating shrub, and *Pittosporum colensoi* Hook.f., a broadleaved tree. The study used plastid and nuclear DNA markers as well as a morphological analysis and found evidence supportive of such an event, e.g. that *P. tuneri* shows an ISSR band and morphological traits (for example in leaves, flowers and fruits) that combine those of the putative parents. They however suggested more investigation: their cross-pollination experiments between *P. divaricatum* and *P. colensoi* did not produce progeny, and given the limitations of the ISSR technique they recommend using more nuclear markers in more individuals. Shepherd et al. (2017) and Heenan et al. (2018) used chloroplast DNA and microsatellite markers respectively to study hybridisation and introgression events in New Zealand *Sophora* L.: even though their findings showed that these species hybridise readily, they reported little support for the hypothesis that the heteroblastic divaricate species *Sophora microphylla* arose through hybridisation between divaricate species *Sophora prostrata* Buchanan and the non-divaricate species *Sophora tetraptera* J.F.Mill.

However, as Godley (1985) makes explicit, his hypothesis allows for multiple generations after an initial hybridisation and for selection of the heteroblastic divaricate form from a variable population of hybrid derivatives (such as a hybrid swarm). Therefore, genetic signal of a hybrid origin might be weak and difficult to detect in studies employing only modest numbers of genetic markers.

2. Neotenous loss of a putative adult non-divaricate form

A mirror image of the previous hypothesis, this hypothesis states that divaricate species arose from heteroblastic divaricate ancestors which later lost their forest-adapted adult form in response to new selective pressures in more open environments. It was first suggested by Cockayne (1911, p. 25–26; 1958, p. 141) and further developed by Day (1998c). It is difficult to see how to test such a hypothesis, which may explain why it has not been the subject of published research so far.

The divaricate habit in New Zealand and overseas

Variations of the divaricate habit are found in ca. 81 taxa in New Zealand (Appendix 1.1), including heteroblastic divaricate taxa. 80 are Eudicots, one is a Gymnosperm, and they represent 20 families. According to statistics about the New Zealand vascular flora produced by de Lange et al. (2006), this number represents almost 13% of indigenous

woody spermatophytes. We refer to all these species as **divaricates**, a term that encompasses architectures that fall on a spectrum with two extremes. On one end, there are the **true divaricates** (or **truly divaricating** species), i.e. species with the most characteristic traits of the habit (such as tightly interlaced tough branches with relatively long internodes compared to leaf size, and leaves < 2 cm in length); typically shrubs that are common in open environments such as forest margins. To characterise the other end of the spectrum, we propose to use the term **semi-divaricate** as used by Greenwood & Atkinson (1977); these are species with traits that are not as typical as the traits of the true divaricates, such as slender branches in a more open architecture, and larger leaves—sometimes species that appear clearly divaricate in open areas tend towards a semi-divaricate habit when growing in the shade (Christian et al., 2006; Philipson, 1963; pers. obs.). Furthermore, we use the term **divaricate habit** to refer to the habit as a phenomenon, which manifests itself through a variety of architectures that we refer to as **divaricating habits**.

Although divaricates are present in a wide range of environments throughout New Zealand, several environmental patterns in their abundance have been noted. They can be found in most forest types and successional shrublands (Wardle, 1991), from the coast to alpine environments (Greenwood & Atkinson, 1977). Divaricates have been reported as especially common in open environments such as forest margins (McGlone & Webb, 1981), though relevant quantitative data are lacking. The percentage of divaricate species in woody assemblages increases from north to south (McGlone et al., 2010). Quantitative analyses have shown strong associations with frosty (and to some extent, droughty) climates such as are typical of the eastern South Island (Garrity & Lusk, 2017; Lusk et al., 2016) where notably divaricate species often comprise the majority of arborescent assemblages (Lusk et al., 2016). It has been stated that divaricates are commonest on fertile young soils, such as those derived from recent alluviums or volcanic ashes (Greenwood & Atkinson, 1977; McGlone et al., 2004). Consistent with this proposal, the largest known concentrations of divaricate species occur on alluvial terraces derived from mudstone in the Rangitikei and Gisborne areas (Clarkson & Clarkson, 1994). However, an analysis of > 1,000 plots by Lusk et al. (2016) did not detect a significant association with terraces, or with any other topographic position.

Even though broadly similar plants occur in many other regions of the world, few of them show the full range of traits that are typical of New Zealand divaricates. Species showing aspects of the divaricate habit have been reported from Madagascar (Bond & Silander, 2007; Grubb, 2003), Patagonia (McQueen, 2000; Wardle & McGlone, 1988) or

South America in general (Böcher, 1977), mainland Australia and Tasmania (Bulmer, 1958; A. Mitchell et al., 2009; Stajsic et al., 2015; Thompson, 2010), Arizona and California in the USA (Carlquist, 1974; Tucker, 1974) and New Guinea (Lloyd, 1985). The reported species and their close relatives indeed show branching patterns similar to what is seen in New Zealand divaricates, but they often present rather large leaves. This is for example the case with the North American *Quercus dunnii* Kellogg ex Curran, reported by Tucker (1974), and the South African shrub species with dense, cage-like architectures studied by Charles-Dominique et al. (2017). Most overseas divaricate-like plants also differ notably from all but one New Zealand divaricates by the presence of wounding spines. A striking example is the African boxthorn (*Lycium ferocissimum* Miers; see Figure 2 in Supplementary Material), a South African species naturalised in New Zealand, which has tough interlaced branches similar to those of some New Zealand divaricates but bears sharp spines. However, this spinescence can sometimes be rather weak, for example in Australian species of *Melicytus* J.R.Forst. & G.Forst. (Stajsic et al., 2015). There are however some overseas divaricate look-alikes that show the same traits as New Zealand divaricates, for example *Tetracoccus hallii* Brandegee (Picrodendraceae), a non-spiny shrub with seemingly tough, interlaced branches, branching at wide angles and bearing small leaves (descriptions and pictures from SEINet Portal Network 2020 and Calflora 2020) from south-west USA (distribution data from GBIF 2020 and Calscape 2020).

We propose a list of the species that the studies cited above claim as "divaricate" and that we agree do resemble the architectural models we see in New Zealand divaricates (Appendix 1.2). We suggest the name **divaricate-like** to describe these species in order to emphasise their resemblance with New Zealand divaricates, yet stressing the fact that they often present distinguishing features (discussed above) and that they evolved in environmental conditions that were somewhat different from those experienced by the ancestors of New Zealand divaricates (reviewed below).

A review of theories about the evolution of New Zealand divaricates

The climatic hypothesis

Since its Upper Cretaceous separation from Gondwana (Wallis & Trewick, 2009), New Zealand has undergone wide-ranging climatic changes. There is some debate as to the climate of the Upper Cretaceous: some argue this period was probably warmer than today (e.g. Fleming, 1975), others that it was similar to present-day climates (e.g.

Kennedy, 2003; Mildenhall, 1980). Hornibrook's (1992) review of marine fossil evidence indicates mostly subtropical climates during the Paleogene, although a sudden cooling event may have occurred around the Eocene-Oligocene boundary; temperatures then warmed to a local peak around 16 Mya, during the Miocene; the climate remained subtropical until a Late Miocene cooling, with further cooling from the Pliocene. The combined effects of this global cooling and of the rapid uplift of the Southern Alps during the Kaikoura Orogeny (Batt et al., 2000) created local frosty and droughty environments, especially in the eastern South Island. These new climates are likely to have reduced plant growth on many sites (Lusk et al., 2016), as shown by comparisons of juvenile annual height growth rates of the small broadleaved tree *Aristotelia serrata* J.R.Forst. & G.Forst. on modern sites that differ in growing season length (Anton et al., 2015; Bussell, 1968).

Besides these climatic variations, a progressive submergence greatly reduced the extent of the New Zealand landmass from the Upper Cretaceous to the Early Miocene (85–22 Mya; Landis et al., 2008). It reaching a peak around 25–23 Mya known as the Oligocene marine transgression (Cooper, 1989), at which point the surface of the New Zealand mainland was about 18% of its present-day surface area (Cooper & Cooper, 1995). Landis et al. (2008) argued that, at that time, New Zealand was probably completely submerged, but this idea is now clearly refuted. Geological and paleobiological evidence show that New Zealand was not completely submerged during the Late Oligocene (reviewed by Mildenhall et al., 2014), particularly the 23 Myo Foulden Maar deposit (near Middlemarch, Otago), which notably contains fossils of diverse land plants (e.g. Lee et al., 2016). Moreover, recent molecular dating of the age of New Zealand lineages strongly suggest that some extant terrestrial plant and animal groups most probably originated from a Gondwanan vicariance (Heenan & McGlone, 2019; Wallis & Jorge, 2018).

Diels (1896) was the first to hypothesise an important role of Pleistocene climate in shaping the modern New Zealand flora, and as far as we are aware his work is the first attempt to explain the evolution of the divaricate habit. He proposed that, by reducing transpiration, the divaricate habit helped plants cope with droughty climates created in the eastern South Island by the uplift of the Southern Alps. Cockayne (1911) proposed that the divaricate habit was a response to past windy and droughty Pleistocene steppe climates, especially in the South Island. Similarly, Rattenbury (1962) hypothesised that the divaricate habit was an adaptation to dry or cool Pleistocene climates, and suggested an effect of the cage-like architecture as a windbreak, reducing transpiration. Wardle

(1963) suggested that the divaricate habit continues to be adaptive in the present-day drier forest and shrub environments of eastern New Zealand.

McGlone & Webb (1981) further developed the climatic hypothesis, joining the debate started by Greenwood and Atkinson with the moa-browsing hypothesis (Greenwood & Atkinson, 1977; see next section). They suggested that the divaricate habit represents the response of the "largely subtropical" Tertiary flora of the isolated New Zealand archipelago to the near-treeless glacial periods of the Pleistocene; this habit may have protected growing points and leaves from wind abrasion, desiccation and frost damage, which occurred unpredictably in the weakly seasonal New Zealand climates of the Quaternary. McGlone & Webb (1981) also argued that the cage-like architecture of the divaricate habit also provides a milder microclimate within the plant which promotes higher rates of photosynthesis. The transition from the juvenile form to the adult form in heteroblastic divaricate species occurs above the height of the most damaging frosts during temperature inversions on clear nights, and the absence of the habit on offshore and outlying islands can be explained by their more oceanic, hence milder and less frosty, climates. Burns & Dawson (2009) however noted that the heteroblastic divaricate species *Plagianthus regius* from the mainland has a heteroblastic divaricate subspecies (*P. regius* subsp. *chathamicus* (Cockayne) de Lange) on the historically avian-browser-free Chatham Islands: they propose that, because *P. regius* is a recent immigrant on the Chatham Islands, its juvenile form has not been counter-selected yet.

The climatic factors suggested as selective forces are certainly not peculiar to New Zealand, whereas divaricate-like forms are much less common in other regions with similar climates (Dawson, 1963). McGlone & Webb (1981) argued that what made New Zealand unique in the evolution of its subtropical flora in response to the cold, dry and windswept environments that appeared during the Quaternary was its isolation from sources of steppe-adapted floras, apparently believing that such floras might have provided plants with more conventional physio-morphological responses to cold, dry climates. This argument appears to overlook the fact that divaricate shrubs are also common in the Patagonian steppe, although those species are invariably spinescent (McQueen, 2000). Furthermore, if wind was one of the drivers of the evolution of the divaricate habit, it is strange that few divaricate species are found in some very windy parts of New Zealand (Greenwood & Atkinson, 1977): although they are often prominent in the vegetation of windswept areas such as Cook Strait (Wardle, 1985), they present a low species richness there (Gillham, 1960).

The photoprotection variant of the climatic hypothesis

Howell et al. (2002; see also Howell, 1999), proposed that the shading of inner leaves by the cage-like divaricate architecture protects them from high irradiance on cold mornings after frosts, thus minimising photoinhibition and photodamage. It is a derivative of the climatic hypothesis that includes the effect of solar radiation as a selective pressure under stressfully cold climatic conditions. Howell et al. (2002) tested this hypothesis with an experiment involving the pruning of the outer branches of three divaricate species, which resulted in a reduced photosynthetic capacity of the inner leaves of these shrubs for at least 3 months. This experiment was criticised by Lusk (2002), who pointed out that the failure to include non-divaricate species as a control undermined the authors' conclusions: without further research, we cannot know if non-divaricate plants would respond in a similar way to pruning of their outer branches.

Empirical appraisal of the climatic hypothesis

Experimental tests have produced little support for the climatic hypothesis. Although past climatic conditions cannot be reliably reproduced in a controlled experiment, it is possible to estimate the differential response of divaricate and non-divaricate species when they are subjected to present-day climatic conditions similar to those hypothesised to have selected the divaricate habit during the Pleistocene.

Kelly & Ogle (1990) were the first to publish a test of the response of divaricating habits to climatic conditions. They studied the effect of air temperature, humidity, frost and wind on internal and external leaves of a divaricate species and both juvenile and adult forms of a heteroblastic divaricate species. While they did not show a significant difference in leaf temperature and air humidity between the inside and the outside of divaricating habits, they did show that the habit provides some protection against frost.

Keey & Lind (1997) used four species showing various divaricating habits to test the effect of different branching architectures on the surrounding airflow patterns. Although they did not compare these species to non-divaricate species, they showed that dense branching patterns produce calmer zones, which may imply that they create a more favourable growing environment for leaves and other fragile organs by reducing wind damages.

Darrow and colleagues experimentally compared the frost resistance (2001) and water use efficiency (2002) of juvenile and adult forms of heteroblastic species, most of them divaricate at a juvenile stage. Darrow et al. (2002) found that most (though not all) divaricate juvenile forms had lower water use efficiency than the corresponding adult

forms, concluding their results were not consistent with the climatic interpretation of the divaricate form. Darrow et al. (2001) compared the frost tolerance of the leaf tissues of juvenile and adult forms of five heteroblastic divaricate species by chilling leafy twigs overnight in thermostatically controlled freezers. However, their findings are of limited relevance to the climate hypothesis, as this approach does not address the effect of leaf size on night-time chilling under a clear sky (cf. Lusk et al., 2018), nor any potential effect of stem vascular anatomy on freeze-thaw embolism. In a similar vein, Bannister et al. (1995) studied the development of frost tolerance of detached leaves of some divaricate and non-divaricate species of *Pittosporum* Banks & Sol. ex Gaertn. over the course of autumn and winter. As was the case for Darrow et al. (2001) this study of tissue-level responses to frost did not test the potential roles of any of the characteristic leaf or stem traits of divaricates in conferring frost resistance .

A test of the photoprotection hypothesis was provided by Christian et al. (2006), who compared carbon gain versus structural costs of three congeneric pairs of divaricate and non-divaricate species under different intensities of light exposure. They showed that the costs of divaricating habits may be too high to be compensated by the photoprotection it provides, although they did not subject their samples to especially stressfully cold temperatures. In parallel, Schneiderheinze (2006) studied photoinhibition in divaricate and non-divaricate species under high light loads and other stressful conditions, such as drought. She found plants of both habits showed similar levels of photoinhibition under high irradiance, whether the plants were water-stressed or not. Here again, the hypothesis as formulated by Howell et al. (2002; i.e. protection from photoinhibition under cold conditions) was not tested, but the study still provided a valuable insight into the absence of significant photoprotection in divaricate species compared to their non-divaricate relatives.

Recently, an observational approach was taken by Lusk et al. (2016), who examined the environmental correlates of the proportion of divaricate species in arborescent assemblages throughout the main islands of New Zealand. They concluded that divaricate species are generally more diverse and prominent at frosty and droughty sites. Garrity & Lusk (2017) also used an observational approach by correlating climatic data with the distribution of 12 congeneric pairs of divaricate and larger-leaved species of the main islands of New Zealand. They found that divaricate species were significantly favoured by colder mean annual temperatures, and especially by colder minimum July temperature, but there was little evidence of an association with droughtier environments. Their results also showed little support for the photoprotection hypothesis, as divaricate species tended

to predominate in cold environments irrespective of winter solar radiation levels. These two different observational approaches concur in showing that short frost-free periods and cold climates in general favour the abundance and diversity of divaricate species, but do not quite agree on the effect of drought. Given the limited number of species encompassed by Garrity & Lusk (2017), as well as evidence that the largest concentrations of divaricate species occur on middle North Island sites subject to significant water deficits (Clarkson & Clarkson, 1994), the balance of the evidence indicates that both frost and drought favour divaricate species.

Finally, a key component of the divaricate habit is small leaf size, which is known to be advantageous under harsh climates. A study by Lusk et al. (2018) compared leaf temperature during clear winter nights in relation to leaf size for 15 native New Zealand species, including four congeneric pairs of divaricate and non-divaricate species. They observed that small leaves chilled significantly less than large leaves. Their conclusions provide experimental support to leaf energy balance theory, which predicts that large leaves should be more vulnerable to frost because they cool below air temperatures on frosty nights whereas the smallest leaves stay close to air temperature (Parkhurst & Loucks, 1972; I. J. Wright et al., 2017). Although this effect does not explain the three-dimensional structure of the divaricate habit, it suggests that the characteristically small leaves of divaricates may have provided an adaptive value in open habitats with short annual frost-free periods (see also Lusk & Clearwater, 2015, a similar but less conclusive study on a smaller scale). Additionally, a study of the relationship between leaf dimensions and environmental variables in South African species of Proteaceae concluded that small leaves promotes convective heat dissipation under dry conditions and limited wind, enabling them to avoid overheating when water shortage forces stomatal closure (Yates et al., 2010). This effect was confirmed on Australian Proteaceae by Leigh et al. (2017). The small size of the leaves of most divaricates may therefore enable them to cope with drought better than large-leaved competitors.

The moa-browsing hypothesis

"Moa" is the Māori name for a group of now-extinct large (1-3 m and 10-250 kg; Atkinson & Greenwood, 1989; Worthy & Holdaway, 2002) flightless birds ("ratites") of the endemic order Dinornithiformes. Nine species are currently recognised, belonging to six genera and three families (Trevor Henry Worthy & Scofield, 2012). There are several hypotheses about how the ancestors of moa reached New Zealand (Allentoft & Rawlence, 2012): they may have inhabited the New Zealand landmass from the time it started to

separate from Gondwana about 80 Mya (the "Moa's Ark" of Brewster, 1987); alternatively their ancestors might have reached New Zealand either by walking before 60 Mya, when the New Zealand landmass was still connected to a disintegrating Gondwana, or by flying after the complete separation. This last possibility is consistent with recent molecular evidence that the closest living relatives of moa appear to be tinamous (Mitchell et al., 2014; Phillips et al., 2010), a group of volant birds. If the earliest ancestors of moa to inhabit Zealandia were volant, fossil evidence suggest that their descendants have been large flightless birds since at least 16-19 My ago (Tennyson et al., 2010). All moa species were extinct by about the mid-15th century CE (Perry et al., 2014), apparently because of hunting (Allentoft et al., 2014).

Moa subfossil remains are more common on the South Island than on the North Island (Anderson, 1989); moreover, they are more concentrated in the east of the South Island (Anderson, 1989). However, this does not necessarily mean that moa were more abundant in the eastern South Island than elsewhere in the country, since the subfossil record is probably influenced by preservation biases: natural moa bone deposits are mainly in alkaline swamps and limestone caves, which are near-ideal preservation environments (Atkinson & Greenwood, 1989) that happen to be more common in the eastern South Island than in most other parts of the country (Anderson, 1989). Furthermore, an estimation of population size and distribution of the different moa species based on mitochondrial DNA and fossil record of *Dinornis* spp. suggests, in contrast, that moa populations were more numerous on the North Island than on the South Island (Gemmell et al., 2004). Therefore, it seems difficult at present to draw clear conclusions about geographic variation in moa densities.

Although the potential influence of moa browsing on the evolution of the divaricate habit had been suggested by previous authors (e.g. Carlquist, 1974; Denny, 1964; Taylor, 1975), Greenwood & Atkinson (1977) were the first to fully develop and argue this idea. First postulating that moa fed by clamping and pulling vegetation in the same manner as present-day ratites, they hypothesised that the tough and highly tensile branches of many divaricate species are difficult to tear off, while the interlaced structure kept leaves and growing tips out of easy reach. Hence, browsing on these plants would be less energetically rewarding than browsing on broadleaved species. Greenwood & Atkinson (1977) did not completely exclude a cutting ability of moa beaks, later acknowledging that the feeding behaviour of moa could not be confidently inferred because fossil skulls do not retain all the relevant tissues (Atkinson & Greenwood, 1989). A recent study simulating the force of moa jaw muscles however concluded that different moa species

fed in various different ways, including cutting (Attard et al., 2016). This appears to confirm the findings of studies of moa gizzard contents, which concluded that that divaricate twigs consumed by moa had been sheared rather than broken off (Burrows, 1980, 1989; Burrows et al., 1981). These findings were later corroborated by a study of coprolites (Wood et al., 2008), yielding the same conclusion that divaricate species were by no means exempt from moa browsing (reviewed by Wood et al., 2020).

Moreover, Greenwood & Atkinson (1977) used evidence from the distribution of divaricate plants to support their hypothesis. One the one hand, they pointed out that divaricate plants often grow on lowland river terraces and swamps, which offer high nutrient levels and hence high plant productivity and nutrient content. They explained that divaricate species should be more subjected to moa browsing in such places, a sensible claim given that at least some studies show a positive correlation between herbivore abundance and soil fertility (e.g. Kanowski et al., 2001). Even if divaricate species have been reported from low fertility soils, such as the acidic soils of Stewart Island (McGlone & Clarkson, 1993), the largest known concentrations have been reported from fertile terraces derived from mudstone (Clarkson & Clarkson, 1994). On the other hand, Greenwood & Atkinson (1977) noted that divaricates are largely absent from areas where moa did not live, such as offshore islands, or where moa could not reach them, such as growing on cliffs or as epiphytes. Although *Myrsine divaricata* A.Cunn. is abundant on some of the subantarctic islands of New Zealand (McGlone & Clarkson, 1993; Meurk et al., 1994), which are unlikely to have harboured moa, Greenwood & Atkinson (1977) attributed such occurrences to recent colonisation from the mainland. Kavanagh (2015) lent support to this interpretation by comparing some traits used to describe the divaricate habit between related species of New Zealand mainland and Chatham Island (historically moa-free, with a flora largely derived from the mainland): he concluded that the absence of moa may have relaxed the selection for traits that deterred moa browsing on the main islands of New Zealand.

Greenwood & Atkinson (1977) also examined the bearing of the height of transition between the juvenile in adult forms in heteroblastic divaricate species on their hypothesis. They claimed that, in such species, the shift from the juvenile divaricate form to the adult non-divaricate form happens around 3-4 m high; this height corresponds to the approximate height of the tallest moa, implying that the adult form in these species only appears at heights where it is safe from browsing. Burns & Dawson (2006) brought support to this claim from New Caledonia: they mentioned that heteroblastic species there (which do not have a divaricating juvenile form) seem to shift form at about the estimated

height of the flightless birds which once lived there, although they called for quantitative support for this observation. There are however multiple counter-examples to Greenwood & Atkinson's (1977) claim. Field observations sometimes reveal that the shift can happen significantly lower; for example, Cockayne (1911) reported that the shift in *Sophora microphylla* can happen as low as 1.4 m, and we observed a shift in *Pennantia corymbosa* happening at about 2 m high (Figure 1 in Supplementary Material). Conversely, some homoblastic divaricate species can reach heights significantly above the size of the tallest moa without showing any relaxation of their divaricating habit; McGlone & Clarkson (1993) report such instances with individuals of *Coprosma crassifolia* Colenso, *Melicope simplex* A.Cunn. and *Myrsine divaricata* more than 5 m high; individuals of the latter species exceeding this height were also recorded by Veblen & Stewart (1980).

Finally, a crucial point of Greenwood & Atkinson's (1977) argument is the fact that the New Zealand flora is unique in having co-evolved with ratites but without browsing mammals. This phenomenon did not occur in areas where divaricate-like species co-evolved with ratites: in Madagascar, now-extinct elephant birds shared the island with giant tortoises and giant lemurs (Bond & Silander, 2007); in Patagonia, Darwin's rhea grazed side-by-side with diverse mammals, such as equiids, camelids and giant ground sloths (McQueen, 2000); in Australia, emus coexisted with many different herbivorous mammals, mostly marsupials (Roberts et al., 2001). Although these regions have all undergone megafaunal extinctions, they still host browsing mammals, and with the exception of Madagascar they have retained their ratites as well. No ratites or ratite fossils are known from North America; they are known only from former Gondwanan lands (Briggs, 2003).

Greenwood & Atkinson (1977) originally hypothesised that the divaricate habit evolved as a deterrent to moa browsing. Lowry (1980) instead suggested that the main effect of the divaricate habit is to help the plant survive browsing by spacing and multiplying palatable growing tips, with a side-effect of making the browsing less energetically rewarding. This idea that the divaricate habit enables plants to survive rather than to prevent browsing led Atkinson and Greenwood to reconsider their 1977 hypothesis by acknowledging Lowry's view (Atkinson & Greenwood, 1980). Consequently, this view raised the question of why the divaricate habit, if it is not a specialised moa-deterring adaptation, is much scarcer in other regions where non-ratite browsers existed (McGlone & Webb, 1981).

Indirect support for the moa-browsing hypothesis came from a fossil of a small-leaved woody species with wide-angle opposite branching that was discovered by

Campbell et al. (2000). It was estimated to date from 20-16 Mya, which corresponds to the Early Miocene, whereas the climatic conditions usually put forward as the drivers of the evolution of the divaricate habit did not occur before the Pliocene (i.e. not before 5.333 Mya, Cohen et al., 2013, updated). Despite the absence of information about the three-dimensional structure of the plant when alive, 12 out of 15 experts they consulted agreed it was most likely a divaricate species (potentially extinct), and had rather varied ideas about what genus it could belong to. They noted the presence of "small acute broken processes protrud[ing] from the branchlets at irregular intervals", which look like spines even though they are not opposite. Even though the processes might have been defensive spines that would be of little use against moa beaks, this discovery appears consistent with the moa-browsing hypothesis.

According to the moa-browsing hypothesis, the divaricate habit could be nowadays seen as an anachronism (Greenwood & Atkinson, 1977). As such, it was hypothesised that divaricate species may not be adapted to the current browsing pressure of introduced mammals because their costly ratite-resistant architecture was thought to be useless against mammals (Bond et al., 2004). Diamond (1990) imported the concept of "ghost" from overseas cases of anachronisms (later reviewed by Barlow, 2000) when defending the hypothesis that divaricates are adapted to a now-extinct fauna. However, the conclusions of Pollock et al. (2007) about the preferences of ungulates for New Zealand woody plants, as well as a study by Lusk (2014) on the regeneration of divaricate and non-divaricate species in a forest remnant that had been subject to ungulate browsing for decades, indicate that the divaricate habit may also be effective in deterring mammal browsing. Ungulates indeed tend to avoid some (though not all) divaricate species until more attractive foods are depleted (Forsyth et al., 2002; Lusk, 2014).

Experimental appraisal of the moa-browsing hypothesis

The moa-browsing hypothesis was first tested experimentally by Bond et al. (2004), who fed juvenile and adult form foliage of two heteroblastic divaricate species to present-day ratites (emus and ostriches). They found that the high tensile strength of divaricate branches reduces breakage, that the high branching angles make the twigs difficult to swallow because birds cannot use their tongue to properly orient the twigs, and that small and widely spaced leaves increase the time and the energy required to consume leaf biomass. These results brought support to the hypothesis that the divaricate habit represents an adaptation to deter moa browsing. However, whether the feeding behaviour

of the present-day ratites reliably reflect the feeding behaviour of extinct moa is a matter of debate (reviewed above).

A more elaborate cafeteria experiment was conducted a few years later by Pollock et al. (2007), comparing the offtake of deer, goats and ostriches from five divaricate species compared to five congeneric non-divaricate species. Their general finding is that features of the divaricate habit, such as small leaves and stem toughness, deter ungulates as well as ratites.

The moa-climate synthetic hypothesis

The idea that selection for the divaricate habit may have been driven by both past climatic conditions and the effect of moa browsing has been suggested several times since the debate started (Bond & Silander, 2007; A. Cooper et al., 1993; Wardle, 1985, 1991). Lusk et al. (2016) proposed a synthetic hypothesis with a specific mechanism integrating browsing and climatic factors. Although the ancestors of moa may have reached the New Zealand landmass as early as 80-60 Mya (reviewed by Allentoft & Rawlence, 2012), the divaricate habit may not have become advantageous as an anti-browsing defence until Plio-Pleistocene climatic constraints on plant growth resulted in juvenile trees being exposed for longer to ground-dwelling browsers. During this period the combination of global cooling (Hornibrook, 1992) and rapid uplift of the Southern Alps (Batt et al., 2000) created widespread frosty, droughty environments in the eastern South Island. The relatively fertile alluvial soils of these environments may have attracted high levels of browsing, but frost and drought would have reduced the ability of juvenile trees to grow rapidly out of the browsing zone, even in well-lit microenvironments such as treefall gaps. Evidence for a much earlier origin of divaricate plants, for example in the more benign climates of the Miocene or Oligocene, would refute both this hypothesis and the original climate hypothesis, and would point to moa browsing as the sole driver of divaricate evolution if no other factor can be identified.

The light trap hypothesis and its appraisal

The light trap hypothesis, formulated by Kelly (1994), relies on the conclusions of Horn (1971) that a multi-layered leaf distribution (i.e. leaves distantly scattered among multiple layers in the canopy) is more efficient at capturing a higher proportion of sunlight than mono-layered architectures (i.e. leaves distributed in a dense layer, the umbra of the outermost leaves completely obscuring the innermost leaves). Photosynthesis of most plants is indeed saturated well below full sunlight, the saturation point varying with, for

example, species' successional status (e.g. Bazzaz & Pickett, 1980). The scattered distribution of the leaves of divaricates over multiple branch layers therefore allows inner leaves to be in the penumbra of the outer leaves, thus better distributing light harvest throughout the canopy. The light trap hypothesis appears consistent with a modelling study of the impact of penumbral effects on shoot-level net carbon gain of conifers (Stenberg, 1995) which, like New Zealand divaricates, have small effective leaf diameters that result in short shadows; this modelling however does not explain the potential advantage of the architectural structure of divaricating habits. Moreover, even though penumbral effects are likely to result in higher carbon gain per unit area of foliage in small-leaved species growing in high light, Christian et al.'s (2006) data suggest that this advantage will be outweighed by the much higher (ca. threefold) leaf area ratio of congeneric broadleaved species, resulting in higher net carbon gain per unit of biomass in the latter. In divaricate species, this effect might be at least partially compensated by photosynthesis in stems, brought to light in one instance so far: the juvenile form of the heteroblastic divaricate *Prumnopitys taxifolia* (Banks & Sol. ex D. Don) de Laub. (Mitchell et al., 2019). More divaricate species will need to be investigated to determine how widespread stem photosynthesis is among divaricates. However, why would divaricating habits be scarce or absent in most other regions of the world if sunlight were the main driver of the evolution of these peculiar architectures in New Zealand, where solar irradiance levels are similar to those of other regions at comparable latitudes (Solargis, 2020)? The light trap hypothesis does not appear to offer a satisfying explanation of the evolution of the New Zealand divaricates.

Insights into the development of divaricate branching patterns

If the debate surrounding divaricate plants has mainly focused on how the divaricate habit has evolved, a handful of studies looked into describing the range of growth patterns that give rise to the spectrum of divaricating habits, and how such patterns translate into adaptations to local environments.

Tomlinson (1978) examined bifurcation ratios of 18 New Zealand divaricates, including two heteroblastic divaricate species. He concluded that the interlaced structure of most divaricates is a consequence of a sequential branching which may be supplemented by reiterative branching. Moreover, he suggested that this sequential branching is characterised by a lack of organisational control that translates into a dimorphism between orthotropic and plagiotropic branches. He recommended the study of the changes in the branching sequence of many divaricate species over their lifetime,

as he believed this could be the only way to understand how the diversity of divaricating habits was produced under a possibly single selective pressure, and to draw general conclusions about their development.

Subsequently, the development patterns of a few divaricates were studied in the 1990s. The species were: *Muehlenbeckia astonii* (Lovell et al., 1991); the juvenile form of *Elaeocarpus hookerianus* (Day, 1998c; Day et al., 1998; Day & Gould, 1997), *Carpodetus serratus* J.R.Forst. & G.Forst. (Day, 1998c, 1998b) and *Pennantia corymbosa* (Day, 1998a); *Sophora prostrata* and the juvenile form of *Sophora microphylla* (Carswell & Gould, 1998). Overall, these studies concluded that such a growth pattern, with many growing points scattered across the plant's crown, offers a plastic structure that can more easily accommodate changes in environmental conditions (e.g. forest canopy gap versus closed canopy or seasonal changes in environmental conditions). These case studies also agreed that the lack of apical dominance plays a key role in the establishment of the divaricating habits they observed.

In parallel to the study of developmental patterns, a handful of studies looked into the hormonal control of the divaricate habit. Horrell et al. (1990) showed that a gibberellic acid treatment on cuttings of the adult form of *Pennantia corymbosa* and *Carpodetus serratus* tends to revert them to their juvenile form. This phenomenon did not occur in *Elaeocarpus hookerianus*, a result later confirmed by Day et al. (1998) with treatments of adult cuttings with gibberellic acid and other growth factors, including a cytokinin. Day et al. (1998) also showed that the adult form is not precociously triggered in *E. hookerianus* seedlings by these treatments. In *Sophora*, a treatment with 6-benzylaminopurine (a cytokinin) reinforces the divaricateness of the juvenile form of *Sophora microphylla* (Carswell et al., 1996). Qualitative and quantitative measurements in *E. hookerianus* showed that the leaves of the divaricating juvenile form contain more active cytokinins than the non-divaricating adult form or transitional form leaves (Day et al., 1995, reviewed by Jameson & Clemens, 2015). A similar yet more questionable conclusion was drawn from a comparison of the ratio of active to storage forms of cytokinin between divaricate and non-divaricate forms in *Sophora* species (Carswell et al., 1996). In contrast with the heteroblastic divaricate species studied, the levels of cytokinins are relatively low in the divaricate species *Sophora prostrata*, suggesting that they might not play a role in the establishment of the divaricating habit itself (Carswell et al., 1996). There are however too few studies about these growth regulators to formulate general conclusions about their potential effects in controlling the expression of the divaricate habit.

Conclusions

The terms **divaricate** or **divaricating** have been variously applied to around 80 New Zealand species that we regard as occupying a spectrum from **truly divaricate** (small and widely-spaced leaves; wide-angle branching; tough, wiry, tightly interlaced stems) to **semi-divaricate** (plants that present some but not all of these traits). This spectrum of architectural forms, which we call **divaricating habits**, is the expression of a phenomenon called the **divaricate habit**. **Heteroblastic divaricate** species have a divaricate (or semi-divaricate) **juvenile form** and a non-divaricate **adult form**, in contrast to the generally smaller (< 8 m) homoblastic divaricates that retain the divaricate form throughout their entire lives. Finally, we coin the term **divaricate-like** to describe overseas instances of the divaricate habit phenomenon, which acknowledges their resemblances with New Zealand divaricates while stressing their peculiarities. We hope that adoption of these terms will help reduce ambiguities in future research and facilitate clear communication. Our recommendations nevertheless do not resolve the blurry boundary between true divaricates and semi-divaricates, like any categorisation involving a degree of subjectivity.

In spite of rather extensive experimental and observational evidence, no hypothesis about the evolution of divaricates in New Zealand has been decisively favoured over another. Among the most plausible hypotheses however, the moa-browsing hypothesis seems more supported than the climatic hypothesis, although neither are fully satisfying on their own. The synthetic moa-climate hypothesis has not been much discussed or tested so far, but given the evidence of both the moa-browsing hypothesis and the climate hypothesis individually, it appears to be a good candidate for a definitive answer to the divaricate question.

However, neo-ecological studies alone are unlikely to entirely resolve the origin of divaricate plants. One way still left to explore was suggested by Cooper et al. (1993): using molecular phylogenetics to date the divergences between divaricates and their closest non-divaricate relatives. Past studies estimating the age of New Zealand plant lineages (e.g. reviewed by Heenan & McGlone, 2019; Wallis & Jorge, 2018) have not focused on dating such divergences. Such studies, and studies on overseas groups that include New Zealand representative, can still offer isolated dates even though they might not have sampled the closest non-divaricate relative to the divaricate species they included (Appendix 1.3). The divergence dates between congeneric divaricate and non-divaricate species give us a first hint that the divaricate habit may have appeared less than

10 Mya in most cases. Table 1.2 provides the theoretical divergence dates one might expect from a study specifically dating splits between divaricate and non-divaricate species under the different hypotheses in play: the dates of the divergences in Appendix 1.3 hardly favour one hypothesis over the other, suggesting the need for a dating effort specifically targeting divaricate species and their closest non-divaricate relatives, as suggested by Cooper et al. (1993).

Table 1.2. *Theoretical divergence periods between New Zealand divaricates and their closest non-divaricate relatives under the different hypotheses that try to explain their emergence. 5.3 Mya represents the lower bound of the Pliocene, the period when the climatic factors that would have favoured the evolution of the divaricate habit appeared.*

Hypothesis	Implied theoretical divergence period
Climatic (including photoprotection)	Not older than ca. 5.3 Mya.
Moa-browsing	Much older than 5.3 Mya
Moa-climate synthesis	Not older than ca. 5.3 Mya.
Light trap	Unpredictable, as past sun radiation levels cannot be estimated (or with difficulty and questionable reliability).

There is still much to be done on developmental aspects of the divaricate form. First, our understanding of how the diversity of divaricating habits is produced needs more work despite having been the subject of numerous studies in the late 1990s. Second, the genes or gene networks that produce the diversity of divaricating forms have not been identified; such knowledge would help assessing Went's (1971) horizontal transfer hypothesis beyond theoretical arguments. These directions might even bring a new theory about the emergence of these species, or give birth to a new classification of the divaricating habits. However, we believe that such a new classification could only become consensual if it is based on quantitative measurements of the architectural features of all these species, that would be analysed by way of multivariate analyses. The main issue with such an endeavour is that each individual species will need to be measured in the wild, including several individuals in shaded and open habitats. Herbarium specimens cannot be used because the three-dimensional structure of the original individual is lost during pressing and, and only a small fraction of the architectural structure is usually represented. Such a classification may help significantly in clarifying the boundary between true divaricates and semi-divaricates, by identifying and discriminating architectural types within the spectrum of the divaricate habit.

Moreover, combined with the molecular phylogeny suggested by Cooper et al. (1993), it will be essential to try to answer the following pending questions:

1. Did similar architectures arise in closely related species? I.e. do different divaricating habits reflect different inherited pre-existing traits of the corresponding lineages (as suggested for example in Brown & Lawton, 1991)?

2. Did similar architectures arise in response to similar environmental selective pressures? I.e. what features of those architectures (e.g. branching angle, degree of interlacement, degree of branch toughness, etc.) were selected by climatic factors, moa browsing or another selective pressure yet to be identified? For example, do species typically found in open habitats present more interlaced and tougher branches than species of shaded environments, as field observations seem to suggest?

Finally, our understanding of the evolution of divaricate species in New Zealand might be aided by more extensive study of the ecology, morphology and evolutionary history of divaricate-like species in other regions of the world, which would lead to identifying the putative selective pressures under which they may have evolved. Generating a thorough inventory of divaricate-like species could be a useful first step that motivates further work on them.

Acknowledgements

We thank the Royal Society of New Zealand for support through Marsden contract 16- UOW-029. We thank Rob D. Smissen, Matt McGlone and two anonymous reviewers for insightful comments on the manuscript. KJLM thanks the researchers of the Institut de Recherche pour le Développement of Nouméa (New Caledonia), especially Sandrine Isnard and David Bruy, for fruitful conversations that provided new elements of thoughts about the divaricates.

Disclosure statement

No potential conflict of interest is reported by the authors.

Funding

This research is supported by the Royal Society of New Zealand – Te Apārangi under a Marsden fund (number 16-UOW-029); the Faculty of Science and Engineering of the

University of Waikato under the FSEN Student Trust Fund (number P102218 SoS/ PG Support).

Supplementary Material

The supplementary material file is available from the Zenodo repository: https://doi.org/10.5281/zenodo.4304900

Appendices

Appendix 1.1. *Complete list of 81 New Zealand taxa falling on the divaricate habit spectrum. This list is based on a compilation of published work amended by field observations. Names of families follow the nomenclature of the APG (Stevens, 2017a). H = heteroblastic species showing the divaricate habit during early life stages only; D = strongly divaricate; ± = semi-divaricate.*

Family	Taxon	Type of divaricate
Araliaceae	*Raukaua anomalus* (Hook.) A.D.Mitch., Frodin & Heads	D
Argophyllaceae	*Corokia cotoneaster* Raoul	D
Asteraceae	*Helichrysum lanceolatum* (Buchanan) Kirk	±
	Olearia bullata H.D.Wilson & Garn.-Jones	D
	Olearia hectorii Hook.f.	±
	Olearia laxiflora Kirk	D
	Olearia lineata (Kirk) Cockayne	±
	Olearia odorata Petrie	D
	Olearia polita H.D.Wilson & Garn.-Jones	D
	Olearia quinquevulnera Heenan	D
	Olearia solandri (Hook.f.) Hook.f.	±
	Olearia virgata (Hook.f.) Hook.f.	±
Elaeocarpaceae	*Aristotelia fruticosa* Hook.f.	D
	Elaeocarpus hookerianus Raoul	D, H
Fabaceae	*Sophora microphylla* Aiton	D, H
	Sophora prostrata Buchanan	D
Gesneriaceae	*Rhabdothamnus solandri* A.Cunn.	±
Lamiaceae	*Teucrium parvifolium* (Hook.f.) Kattari et Salmaki	±
Malvaceae	*Hoheria angustifolia* Raoul	D, H
	Hoheria sexstylosa Colenso	±, H
	Plagianthus divaricatus J.R.Forst. & G.Forst.	D
	Plagianthus regius (Poit.) Hochr. subsp. *regius*	D, H
Moraceae	*Streblus heterophyllus* (Blume) Corner	D, H
Myrtaceae	*Lophomyrtus obcordata* (Raoul) Burret	±
	Neomyrtus pedunculata (Hook.f.) Allan	±
Pennantiaceae	*Pennantia corymbosa* J.R.Forst. & G.Forst.	D, H
Pittosporaceae	*Pittosporum anomalum* Laing & Gourlay	D
	Pittosporum crassicaule Laing & Gourlay	D
	Pittosporum divaricatum Cockayne	D
	Pittosporum lineare Laing & Gourlay	D

	Pittosporum obcordatum Raoul	D
	Pittosporum rigidum Hook.f.	D
	Pittosporum turneri Petrie	D, H
Podocarpaceae	*Prumnopitys taxifolia* (Sol. ex D.Don) de Laub.	D, H
Polygonaceae	*Muehlenbeckia astonii* Petrie	D
	Muehlenbeckia axillaris (Hook.f.) Endl.	±
	Muehlenbeckia complexa (A.Cunn.) Meisn.	±
Primulaceae	*Myrsine divaricata* A.Cunn.	D
Rhamnaceae	*Discaria toumatou* Raoul	D
Rousseaceae	*Carpodetus serratus* J.R.Forst. & G.Forst.	D, H
Rubiaceae	*Coprosma acerosa* A.Cunn.	D
	Coprosma arborea Kirk	±, H
	Coprosma areolata Cheeseman	D
	Coprosma brunnea (Kirk) Cockayne ex Cheeseman	±
	Coprosma cheesemanii W.R.B.Oliv.	±
	Coprosma ciliata Hook.f.	D
	Coprosma crassifolia Colenso	D
	Coprosma cuneata Hook.f.	D
	Coprosma decurva Heads	D
	Coprosma depressa Colenso ex Hook.f.	D
	Coprosma distantia (de Lange & R.O.Gardner) de Lange	D
	Coprosma dumosa (Cheeseman) G.T.Jane	D
	Coprosma elatirioides de Lange & A.S.Markey	D
	Coprosma fowerakeri D.A.Norton & de Lange	±
	Coprosma intertexta G.Simpson	D
	Coprosma linariifolia Hook.f.	±
	Coprosma microcarpa Hook.f.	D
	Coprosma neglecta Cheeseman	±
	Coprosma obconica Kirk	D
	Coprosma parviflora Hook.f.	D
	Coprosma pedicellata Molloy, de Lange & B.D.Clarkson	D
	Coprosma polymorpha W.R.B.Oliv.	D
	Coprosma propinqua A.Cunn.	D
	Coprosma pseudociliata G.T.Jane	D
	Coprosma pseudocuneata W.R.B.Oliv. ex Garn.-Jones & Elder	±
	Coprosma rhamnoides A.Cunn.	D
	Coprosma rigida Cheeseman	D
	Coprosma rotundifolia A.Cunn.	D
	Coprosma rubra Petrie	D
	Coprosma rugosa Cheeseman	D
	Coprosma spathulata A.Cunn.	±
	Coprosma tenuicaulis Hook.f.	±
	Coprosma virescens Petrie	D
	Coprosma wallii Petrie in Cheeseman	D
Rutaceae	*Melicope simplex* A.Cunn.	D
Violaceae	*Melicytus alpinus* (Kirk) Garn.-Jones	D
	Melicytus crassifolius (Hook.f.) Garn.-Jones	D

	Melicytus drucei Molloy & B.D.Clarkson	D
	Melicytus flexuosus Molloy & A.P.Druce	D
	Melicytus micranthus (Hook.f.) Hook.f.	D
	Melicytus obovatus (Kirk) Garn.-Jones	±

Appendix 1.2. *List of 53 divaricate-like taxa outside New Zealand, compiled from published work and personal observations. This list is non-exhaustive and is proposed as an initial step towards more thorough local inventories. Names of families follow the nomenclature of the APG (Stevens, 2017a).*[1]

Family	Taxon	Native distribution	Source
Anacardiaceae	*Schinus fasciculatus* (Griseb.) I.M.Johnst.	Patagonia	McQueen (2000)
	Schinus johnstonii F.A.Barkley	Patagonia	McQueen (2000)
Asteraceae	*Amphipappus fremontii* Torr. & A. Gray	South-western USA	Tucker (1974)
	Tetradymia axillaris A. Nels.	South-western USA	Tucker (1974)
Bignoniaceae	*Rhigozum madagascariense* Drake	Madagascar/Africa	Bond & Silander (2007)
Burseraceae	*Commiphora brevicalyx* H. Perrier	Madagascar/Africa	Bond & Silander (2007)
Cannabaceae	*Celtis pallida* Torr.	Southern USA	Tucker (1974)
Combretaceae	*Terminalia seyrigii* (H. Perrier) Capuron	Madagascar	Bond & Silander (2007)
Ebenaceae	*Diospyros humbertiana* H. Perrier	Madagascar/Africa	Bond & Silander (2007)
Fabaceae	*Adesmia campestris* (Rendle) Rowlee	Patagonia	McQueen (2000)
	Adesmia echinus C.Presl	Chile	Pers. obs.
	Chadsia grevei Drake	Madagascar	Bond & Silander (2007)
	Pickeringia montana Nutt.	California	Tucker (1974)
	Psorothamnus emoryi (A.Gray) Rydb.	Southern USA/Northern Mexico	Tucker (1974)
	Psorothamnus polydenius (Torr.) Rydb.	South-western USA	Tucker (1974)
	Senna meridionalis (R. Vig.) Du Puy	Madagascar/Africa	Bond & Silander (2007)
Krameriaceae	*Krameria grayi* Rose & Painter	South-western USA	Tucker (1974)

[1] Input from one the examiners: *Schinus fasciculatus* is widespread in Argentina, in southern Brazil, and Paraguay (unless the divaricate forms are only found in Patagonia); *Adesmia campestris* is currently a synonym of *Adesmia volckmannii Phil.*; *Adesmia echinus* grows also in Argentina; *Bougainvillea spinosa* is widespread in Argentina; *Condalia microphylla* is widespread in Argentina; *Lycium chilense*, only the var. *comberi* appears to grow exclusively in Patagonia; *Lycium gilliesianum* is widespread in Argentina.

Nyctaginaceae	*Bougainvillea spinosa* (Cav.) Heimerl	Patagonia	McQueen (2000)
Olacaceae	*Ximenia perrieri* Cavaco & Keraudren	Madagascar/Africa	Bond & Silander (2007)
Oleaceae	*Menodora spinescens* A.Gray	South-western USA	Tucker (1974)
	Olea oleaster Hoffmanns. & Link	Europe	Pers. obs.
Picrodendraceae	*Tetracoccus hallii* Brandegee	South-western USA/Northern Mexico	Tucker (1974)
Pittosporaceae	*Pittosporum multiflorum* (A.Cunn. ex Loudon) L.Cayzer, Crisp &I.Telford	Australia	Relative to a pers. obs.
	Pittosporum spinescens (F.Muell.) L.Cayzer, Crisp & I.Telford	Australia	Pers. obs.
	Pittosporum viscidum L.Cayzer, Crisp & I.Telford	Australia	Relative to a pers. obs.
Rhamnaceae	*Adolphia californica* S. Watson	California/Northern Mexico	Tucker (1974)
	Ceanothus ferrisiae McMinn	California	Tucker (1974)
	Ceanothus jepsonii Greene	California	Tucker (1974)
	Condalia globosa I.M.Johnst.	South-western USA/Northern Mexico	Tucker (1974)
	Condalia microphylla Cav.	Patagonia	McQueen (2000)
Rosaceae	*Cercocarpus intricatus* S.Watson	South-western USA	Carlquist (1974)
	Coleogyne ramosissima Torr.	South-western USA	Tucker (1974)
	Cotoneaster atropurpureus Flinck & Hylmö	China	Relative to a pers. obs.
	Cotoneaster dammeri C.K.Schneid.	China	Relative to a pers. obs.
	Cotoneaster microphyllus Wall. ex Lindl.	Himalayas	Pers. obs.
	Cotoneaster perpusillus (C.K.Schneid.) Flinck & Hylmö	China	Pers. obs.
	Prunus fasciculata (Torr.) A.Gray	South-western USA	Tucker (1974)
	Prunus spinosa L.	Europe/Western Asia/North Africa	Pers. obs.
	Sarcopoterium spinosum (L.) Spach	Mediterranean Basin	Pers. obs.
Rubiaceae	*Coprosma nitida* Hook.f.	Australia/Tasmania	Thompson (2010)
	Coprosma quadrifida (Labill.) B.L.Rob.	Australia/Tasmania	Thompson (2010)
Salicaceae	*Azara microphylla* Hook.f.	Chile/Argentina	Pers. obs.
Solanaceae	*Lycium ameghinoi* Speg.	Patagonia	McQueen (2000)
	Lycium andersonii A. Gray	South-western USA/Northern Mexico	Tucker (1974)
	Lycium brevipes Benth.	California/Northern Mexico	Tucker (1974)

	Lycium californicum Nutt. ex Gray	California/Northern Mexico	Tucker (1974)
	Lycium chilense Miers ex Bertero	Patagonia	McQueen (2000)
	Lycium ferocissimum Miers	South Africa	Pers. obs.
	Lycium fremontii A.Gray	South-western USA/Northern Mexico	Tucker (1974)
	Lycium gilliesianum Miers	Patagonia	McQueen (2000)
	Lycium parishii A. Gray	South-western USA/Northern Mexico	Tucker (1974)
Violaceae	*Melicytus angustifolius* (DC.) Garn.-Jones subsp. *divaricatus*	Australia	Stajsic et al. (2015)
	Melicytus dentatus (DC.) Molloy & Mabb.	Australia	Stajsic et al. (2015)

Appendix 1.3. *Published divergence dates between New Zealand divaricate species and their closest sampled non-divaricate relatives. "+" = clade of species; "ca." = when no table with the date was available, it was estimated visually from the dated phylogeny; "or" = when different methods were used and gave different results.*

Divaricate species	**Sister non-divaricate species in the phylogeny**	**Estimated date of divergence (confidence interval if given)**	**Source**
Aristotelia fruticosa Hook.f.	*Aristotelia serrata* (J.R.Forst. & G.Forst.) Oliv.	3 Mya (standard deviation: 0 My)	Crayn et al. (2006)
Coprosma, 31 taxa	*Coprosma*, 73 taxa (incuding the 2 Australian divaricate-like species listed in Appendix 1.2)	Between about 11 Mya (95% HPD[2]: ca. 15-7 Mya) and 2.5 Mya (95% HPD: ca. 3-0.5 Mya)	Cantley et al. (2016)
Discaria toumatou Raoul	*Discaria chacaye* (G.Don) Tortosa	10.2 Mya (standard deviation: 3.7 My)	Wardle et al. (2001)
		3.94 Mya (95% HPD: 9.95-0.8 Mya)	Heenan & McGlone (2019)
Elaeocarpus hookerianus Raoul	*Elaeocarpus bancroftii* F.Muell. & F.M.Bailey + *Elaeocarpus arnhemicus* F.Muell.	4 Mya (standard deviation: 1 Mya)	Crayn et al. (2006)
Elaeocarpus hookerianus Raoul	*Elaeocarpus dentatus* (J.R.Forst. & G.Forst.) Vahl	13.13 Mya (95% HPD: 21.90-5.25 Mya)	Phoon (2015)
Lophomyrtus obcordata (Raoul) Burret + *Neomyrtus pedunculata* (Hook.f.) Allan	*Lophomyrtus bullata* Burret	ca. 4 Mya (95% HPD: ca. 9-1 Mya)	Thornhill et al. (2015)

[2] HPD = highest posterior density (confidence interval on the age).

Melicytus, 8 taxa	*Melicytus*, 15 taxa (including the 2 Australian divaricate-like species listed in Appendix 1.2)	From 6.41 Mya	Mitchell et al. (2009)
Muehlenbeckia (the 3 species listed in Appendix 1.1)	*Muehlenbeckia*, 16 taxa	From 20.5 Mya (95% HPD: 30.4-14.2 Mya), or from 22.3 Mya (95% HPD: 33.5-14.4 Mya)	Schuster et al. (2013)
Olearia solandri (Hook.f.) Hook.f.	*Olearia traversiorum* (F.Muell.) Hook.f.	ca. 1.8 Mya (95% HPD: ca. 3-1 Mya)	Wagstaff et al. (2011)
Pennantia corymbosa J.R.Forst. & G.Forst.	*Pennantia endlicheri* Reissek	0.9 Mya (95% HPD: 2.2-0.1 Mya)	Maurin (2020a)
Plagianthus divaricatus J.R.Forst. & G.Forst.	*Plagianthus regius* (Poit.) Hochr.	3.9 Mya (95% HPD: 8.2-1.9 Mya), or 5.4 Mya (standard deviation: 2.2 My)	Wagstaff & Tate (2011)
Prumnopitys taxifolia (Sol. ex D.Don) de Laub.	*Prumnopitys andina* (Poepp. ex Endl.) de Laub.	ca. 14 Mya (95% HPD: ca. 29-7 Mya)	Leslie et al. (2012)
		11.75 Mya (95% HPD: 27.2-4.73 Mya)	Heenan & McGlone (2019)
Raukaua anomalus (Hook.) A.D.Mitch., Frodin & Heads	*Raukaua simplex* (G.Forst.) A.D.Mitch., Frodin & Heads	0.88097 Mya	Nicolas & Plunkett (2014)
		0.2 Mya (95% HPD: 0.6-0 Mya)	Maurin (2020a)
Rhabdothamnus solandri A.Cunn.	*Coronanthera clarkeana* Schltr.	22.0 Mya (95% HPD: 29.5-18.0 Mya), or 17.9 Mya	Woo et al. (2011)
Rhabdothamnus solandri A.Cunn.	*Sinningia cooperi* (J. Paxton) Wiehler	26.44 Mya (95% HPD: 34.91-16.92 Mya)	Heenan & McGlone (2019)

1.2 Research questions and objectives

The core work of this research uses a molecular phylogenetic approach to date the age of the divaricate habit in the New Zealand flora, as suggested by Cooper et al. (1993). It uses 45 protein-coding DNA sequences drawn from complete and near-complete chloroplast genomes to build a phylogeny of 215 taxa (species and subspecies) calibrated with fossils and secondary calibrations. It is the first work to publish such an attempt. The sampling plan includes 73 of the 81 (90%) New Zealand divaricate taxa of the list in Appendix 1.1.

The goal of reconstructing this phylogeny is to identify the period when most divaricate species diverged from their closest non-divaricate relatives, in order to tease apart the different hypotheses about their evolutionary origins. Under the moa-browsing hypothesis, one may expect to find that many divergences occurred before the beginning of the Pliocene (ca. 5 Mya), since the ancestors of moa were large flightless birds at least as early as 16 Mya (Tennyson et al., 2010). Under the climate and the synthetic moa-climate hypotheses, one may expect to find divergences clustered within the last c. 5 My, because the climatic factors put forward to explain the evolution of the divaricates appeared only when the combination of global cooling (Hornibrook, 1992) and the rise of the Southern Alps in the Pliocene (Batt et al., 2000) created new droughty and frosty climates in New Zealand (particularly in the eastern South Island).

This work thus seeks to bring new and critical evidence to the intense debate that has been ongoing for more than 40 years over the selective pressures that promoted the evolution of the divaricate form. Resolving this controversy is vital for understanding the status of divaricate plants in contemporary New Zealand, for informing conservation management goals and strategies (Lee et al., 2010) and for predicting the likely impact of browsing mammals and climate change on their future abundance and distribution.

The genetic data that were generated for the purpose of this dating work are here also used to answer some questions surrounding the phylogeny of two New Zealand genera, *Corokia* A.Cunn. and *Pennantia* J.R.Forst. & G.Forst. These genera are among a few small angiosperm genera with New Zealand representatives that have received little attention regarding their species-level phylogeny. These studies were vital in the building of the core dating work of my PhD research: they allowed me to test phylogenetic

methods on a smaller scale in view of identifying the best-adapted method for a large phylogeny such as the one from the dating work.

Subsequent to the completion of this PhD, the results of the dating work will be used to identify patterns in the evolution of the traits associated with divarication, coupling the phylogeny with morphological data. It will explore questions such as the potential phylogenetic signal in divaricateness, or how the effect of climates and moa influenced the evolutionary rates of divaricate lineages. I will take part in the writing of this research article.

Finally, this research is part of a broader project which aims at testing a novel hypothesis about the evolution of anti-browsing defences. This hypothesis states that physical defences (such as divaricateness or spinescence) are of most value to young trees or shrubs where fertile soils coincide with climatic constraints preventing plants from quickly growing out of the reach of ground-dwelling herbivores (Lusk et al., 2016). This hypothesis could notably explain the abundance of divaricate plants on frosty and/or droughty, though fertile, lowland sites in New Zealand. The hypothesis is proposed as an alternative to the resource-availability hypothesis of plant defences (Coley et al., 1985) that fails to predict the geographic distribution of physical plant defences against browsing.

1.3 Thesis outline

This PhD thesis comprises five chapters:

- Chapter 1 provides the background of the thesis topic and introduces the research that has been carried out.
- Chapter 2 presents a dated phylogeny of the genus *Pennantia* J.R.Forst. & G.Forst. using whole chloroplast genome and investigating the 18S–26S nrDNA repeat region. For the latter, it notably introduces a technique to retrieve data that is rarely seen in the phylogenetic literature (only a few times, for viruses) despite the advantages it may offer.
- Chapter 3 presents a dated whole chloroplast phylogeny of the genus *Corokia* A.Cunn., and investigates the phylogeny of the ITS nuclear region to test its congruence with the chloroplast phylogeny.
- Chapter 4 is the core work of this PhD project. It presents the molecular dating of the emergence of the divaricate form in the New Zealand flora, using chloroplast genome.
- Chapter 5 summarises the thesis and provides it with a general conclusion and closing remarks.

Chapter 1 was published in *New Zealand Natural Sciences* in 2021. Chapter 2 and 3 were published, respectively, in *PhytoKeys* and *New Zealand Journal of Botany* in 2020. Chapter 4 is formatted with a view to facilitating fast submission to scientific journals after the submission of the thesis. The references cited in each chapter were not appended to each chapter individually; instead, a global list of references for the whole thesis is provided at the end, in the References section.

Two other publications resulted from this PhD but were not included in the thesis because they were tangential to the dating of the evolution of the New Zealand divaricates:

- The first is a short study investigating whether the New Zealand divaricates fit into a globally observed pattern in plant allometry: Corner's first rule (1949, p. 390, 4a). It was published in the journal *New Zealand Natural Sciences* in July 2020 under the following reference:

  Maurin, K. J. L., & Lusk, C. H. (2020). Do the New Zealand divaricates defy Corner's rules? *New Zealand Natural Sciences*, *45*. https://doi.org/10092/100730

- The second publication is a tutorial about how to use the software treePL (Smith & O'Meara, 2012) to build dated phylogenies. This software suffers from a lack of resources describing its practical use, so I set out to produce a tutorial based on my experience using it so that future users can benefit from some sort of help. I submitted it to *Briefings in Bioinformatics* in August 2020, but because the reviewers' commented that this work was more suited for a self-publication as a tutorial rather than as a journal article and given that the vast majority of tutorials in bioinformatics are self-published, I made it available as a preprint in August 2020 under the following reference:

  Maurin, K. J. L. (2020). An empirical guide for producing a dated phylogeny with treePL in a maximum likelihood framework. *ArXiv Preprint ArXiv:2008.07054*. http://arxiv.org/abs/2008.07054

Chapter 2 Phylogeny of *Pennantia* J.R.Forst. & G.Forst.

2.1 Preliminary note

This chapter was accepted for publication in *PhytoKeys* in July 2020 under the following reference:

Maurin, K. J. L. (2020). A dated phylogeny of the genus *Pennantia* (Pennantiaceae) based on whole chloroplast genome and nuclear ribosomal 18S–26S repeat region sequences. *PhytoKeys*, *155*, 15–32. https://doi.org/10.3897/phytokeys.155.53460

It was published under a CC-BY 4.0 International licence (https://creativecommons.org/licenses/by/4.0/). The content of the chapter is therefore an exact copy of the published article, except that (1) the heading and the lists of keywords and of references were omitted, (2) footnotes were added to add elements pointed out by the examiners without having to modify the published text, and (3) for consistency with the rest of the thesis, the citations were reformatted and the numbering of figures and tables adjusted.

2.2 Abstract

Pennantia, which comprises four species distributed in Australasia, was the subject of a monographic taxonomic treatment based on morphological characters in 2002. When this genus has been included in molecular phylogenies, it has usually been represented by a single species, *P. corymbosa* J.R.Forst. & G.Forst., or occasionally also by *P. cunninghamii* Miers. This study presents the first dated phylogenetic analysis encompassing all species of the genus *Pennantia* and using chloroplast DNA. The nuclear ribosomal 18S–26S repeat region is also investigated, using a chimeric reference sequence against which reads not mapping to the chloroplast genome were aligned. This mapping of off-target reads proved valuable in exploiting otherwise discarded data, but with rather variable success. The trees based on chloroplast DNA and the nuclear markers are congruent but the relationships among the members of the latter are less strongly supported overall, certainly due to the presence of ambiguous characters in the alignment

resulting from low coverage. The dated chloroplast DNA phylogeny suggests that *Pennantia* has diversified within the last 20 My, with the lineages represented by *P. baylisiana* (W.R.B.Oliv.) G.T.S.Baylis, *P. endlicheri* Reissek and *P. corymbosa* diversifying within the last 9 My. The analyses presented here also confirm previous molecular work based on the nuclear internal transcribed spacer region showing that *P. baylisiana* and *P. endlicheri*, which were sometimes considered synonyms, are not sister taxa and therefore support their recognition as distinct species.

2.3 Introduction

Pennantia J.R.Forst. & G.Forst. is the sole genus of the family Pennantiaceae J.Agardh, a member of Apiales that comprises four species in Australasia (Gardner & de Lange, 2002; Fig. 2.1). *Pennantia endlicheri* Reissek is a forest tree endemic to Norfolk Island, a small volcanic remnant located about 1400 km east of Australia's mainland. *Pennantia baylisiana* (W.R.B.Oliv.) G.T.S.Baylis (Three Kings Kaikomako/Kaikōmako Manawa Tāwhi) is a small tree originally known in the wild by only one plant, discovered in 1945 on Great Island/Manawa Tawhi (Three Kings Islands/Manawatāwhi, New Zealand, Baylis (1977)) and thought to be female. However, cuttings of the plant were induced to produce seeds in cultivation (Beever & Davidson, 1999; Gardner et al., 2004) and later the wild individual was observed seeding (A. Wright, 1989). It is nowadays planted throughout New Zealand in both residential and botanic gardens (Gardner & de Lange, 2002; pers. obs.) from cuttings of the original tree and from the seeds they produced (Peter J. de Lange, 2010). *Pennantia baylisiana* was regarded by Sleumer (1970) as synonymous with *P. endlicheri*, a view disputed by Baylis (1977, 1989); more recently, Gardner & de Lange (2002) maintained *P. baylisiana* on morphological grounds, while Mabberley (2017) still considered it a synonym of *P. endlicheri*. *Pennantia corymbosa* J.R.Forst. & G.Forst. is a tree endemic to the main islands of New Zealand (North Island, South Island and Stewart Island) and some outlying islands. It is a heteroblastic tree of coastal and lowland forests with a divaricating juvenile form (Dawson & Lucas, 2012). *Pennantia cunninghamii* Miers is an Australian endemic tree of subtropical to warm-temperate rainforest of the east coast. Miers (1852) initially placed this species in a monotypic section, *P.* sect. *Dermatocarpus* Miers, because of its fruits, which are different from those of *P. corymbosa* and *P. endlicheri*. In Miers' time, *P. baylisiana* had not yet been collected, and even though it has similar fruits to *P. cunninghamii*, Gardner

& de Lange (2002) maintained *P.* sect. *Dermatocarpus* on the basis of other morphological traits that distinguish *P. cunninghamii* from the other members of the genus, which they placed in *P.* sect. *Pennantia*.

The placement of Pennantiaceae within Apiales has been a matter of debate. Their morphology is consistent with Apiales in the inferior position of their ovary and their low number of carpels (Nicolas & Plunkett, 2014). On the molecular phylogenetics side, studies have mostly sampled *P. corymbosa* alone (Chandler & Plunkett, 2004; Qiu et al., 2010) or with *P. cunninghamii* (Byng et al., 2014; Kårehed, 2001, 2003; H.-T. Li et al., 2019; Magallón et al., 2015; Nicolas, 2009; Nicolas & Plunkett, 2009, 2014; Tank & Donoghue, 2010; Winkworth et al., 2008); Keeling et al. (2004), however, provided a phylogeny of the four species based on the nuclear ribosomal internal transcribed spacer (ITS) region. On one hand, analyses of nuclear markers proved rather ambiguous, sometimes showing that *Pennantia* falls among close sisters to Apiales, namely Dipsacales or Aquifoliales (Chandler & Plunkett, 2004; Nicolas, 2009), sometimes that it falls among Apiales (Keeling et al., 2004). On the other hand, sequence data from plastid (e.g. Kårehed, 2001; Li et al., 2019) and mitochondrial genes (albeit with poor support, Qiu et al. (2010)) placed them sister to the rest of the Apiales; this conclusion was strongly supported by studies that built a phylogeny combining both plastid genes and nuclear markers (e.g. Chandler & Plunkett, 2004; Magallón et al., 2015).

This study has three goals. (1) To propose the first molecular phylogeny that samples all four species of *Pennantia* for whole plastid DNA sequences, dated using two Apiales fossils and one secondary calibration. (2) To present and evaluate the relevance of a method I used to generate sequence data for nuclear markers at low marginal cost from the shotgun sequencing of genomic DNA: I mapped reads that were unmapped to the chloroplast DNA reference sequence ("off-target reads") against a chimeric 18S–26S nuclear ribosomal DNA repeat region reference sequence to build the sequences for a nuclear DNA phylogeny. (3) To use both the chloroplast DNA and nuclear DNA phylogenies to further examine proposals made by Gardner & de Lange (2002) regarding the relationships among the four *Pennantia* species based on morphological features alone, which have also been assessed by Keeling et al. (2004) using nuclear ribosomal sequences alone.

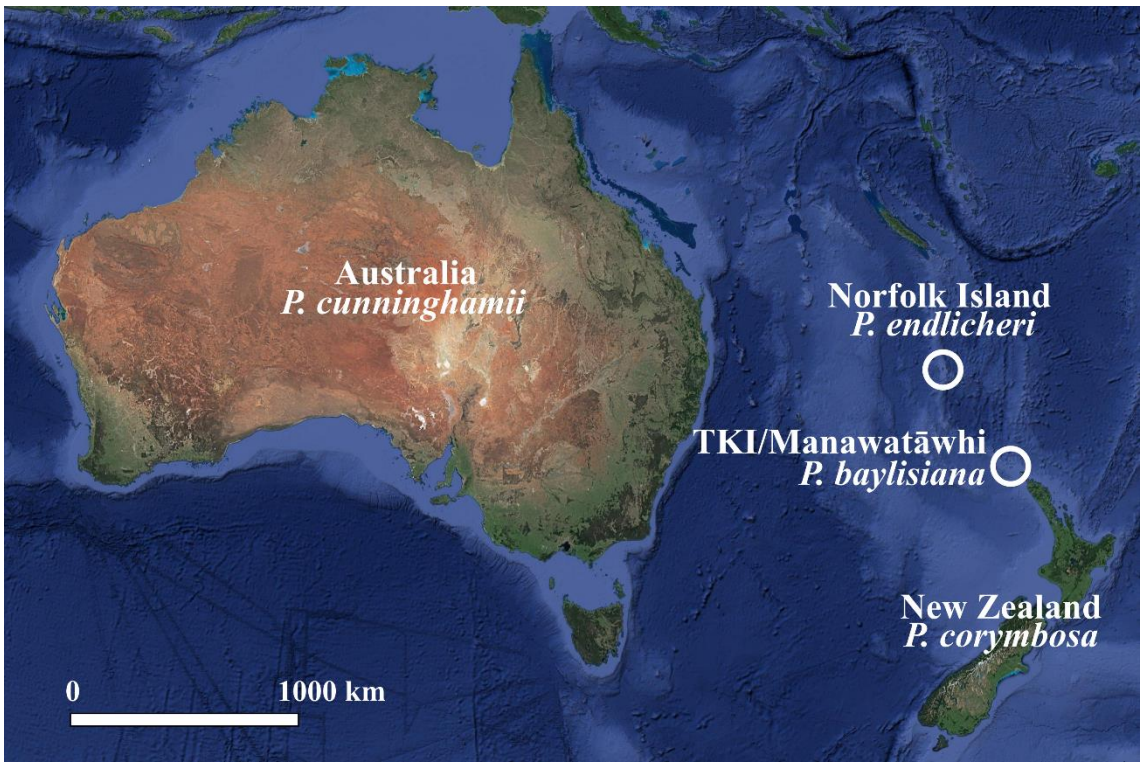

Figure 2.1. *General distribution of the four* Pennantia *species. TKI = Three Kings Islands. Generated in QGIS 3.0.1 from Google Satellite data obtained through the XYZ Tiles tool (https://mt1.google.com/vt/lyrs=s&x={x}&y={y}&z={z}).*

2.4 Methods

Sampling plan

Gardner & de Lange (2002) showed that all four *Pennantia* species are well defined morphologically, and that they have no morphologically divergent populations, a claim which still appears unchallenged today; therefore, it is reasonable in such a group to assume that morphological coherence is an accurate indication of monophyly within each species, and hence only one sample per species was considered. For the chloroplast DNA phylogeny, I also included representatives of five families of Apiales, and four closely related orders according to recent whole-plastid DNA phylogenies of land plants as an outgroup (e.g. Li et al., 2019; Magallón et al., 2015). I included newly generated sequences of the apialean families Araliaceae Juss. (6 species), Pittosporaceae R.Br. (1 species) and Torricelliaceae Hu (2 species), and of the order Asterales (1 species), along with previously published sequences downloaded from GenBank of two other families of Apiales, Apiaceae Lindl. and Torricelliaceae, and of three other orders, Aquifoliales, Dipsacales and Paracryphiales (1 species each); see Table 2.1 for details. I was not able

to generate nor could I find whole-plastid DNA sequences for the remaining two families of Apiales, Griseliniaceae Takht. and Myodocarpaceae Doweld. For the nuclear DNA phylogeny, newly generated sequences of the 18S–26S repeat region for *Pennantia* were obtained from the same samples used to generate the chloroplast DNA sequences. The sequences newly generated for this study were obtained either from field collections that were dried in silica gel and processed in the lab within three months of collection (Maurin collections in Table 2.1), or from herbarium specimens. I was not able to obtain sequences of the 18S–26S nuclear DNA repeat region from Torricelliaceae. The sampling plan for chloroplast DNA and the 18S–26S nuclear DNA repeat region is given in Table 2.1. At the time of submission of this paper, a whole chloroplast DNA sequence purported to be of *Torricellia angulata* Oliv. was available on GenBank (accession NC031509/KX648359); it was disregarded because it appears to derive from a member of Rosales. A second *Torricellia* chloroplast genome sequence (NC040944), from *T. tiliifolia* DC., was included.

DNA extraction

DNA from the samples of *Pennantia corymbosa*, *Raukaua anomalus* (Hook.) A.D.Mitch., Frodin & Heads and *Schefflera digitata* J.R.Forst. & G.Forst. was extracted using a CTAB-based protocol (J. J. Doyle & Dickson, 1987) modified as in (Smissen & Heenan (2007) to include a phenol:chloroform extraction and recovery using spin columns (Zymo IIC, Zymo Research, Orange County, California). The DNA of the other samples was extracted following the DNA tissue protocol of the Maxwell 16 instrument (Promega, Madison, Wisconsin) and further purified by phenol/chloroform extraction and recovery in spin columns. Detailed step-by-step protocols are available upon request. The DNA concentration of the extracts was measured using the Qubit (Thermo Fisher Scientific, Waltham, Massachusetts) dsDNA high-sensitivity assay protocol.

Table 2.1. *Sampling plan of this study, with voucher information and GenBank accession numbers. Samples sorted alphabetically by order name then species name. CANB = Australian National Herbarium (CANB), Canberra, Australia; CHR = Allan Herbarium (CHR), Lincoln, New Zealand; P = Herbarium of the Muséum National d'Histoire Naturelle, Paris, France. Newly generated sequences were formatted for submission to GenBank using the tool GB2Sequin (Lehwark & Greiner, 2019).*

Order	Family	Species	Distribution	Source of plant material or sequence	Herbarium accession #	Voucher or publication	Markers	GenBank accession #
Apiales	Araliaceae	*Cheirodendron bastardianum* (Decne.) Frodin	Marquesas Islands	P	P02800554	Perlman 19764	Chloroplast	MT385071
	Apiaceae	*Daucus carota* L.	Native to temperate Europe and south-west Asia	GenBank	-	Ruhlman et al. (2006)	Chloroplast	DQ898156
	Torricelliaceae	*Melanophylla alnifolia* Baker	Madagascar	P	P02529054	Ranirison 966	Chloroplast	MT385073
	Torricelliaceae	*Melanophylla modestei* G.E. Schatz, Lowry & A.-E. Wolf	Madagascar	P	P06233571	Bernard 1700	Chloroplast	MT385074
	Pennantiaceae	*Pennantia baylisiana* (W.R.B.Oliv.) G.T.S.Baylis	Three Kings Islands/Manawatāwhi (Great Island/Manawa Tawhi)	CHR	CHR 655088	Maurin 87	Chloroplast	MT385075
							Nuclear	MT434778
				GenBank	-	Rotherdam et al. (unpubl.)	Nuclear	EF660531
	Pennantiaceae	*Pennantia corymbosa* J.R.Forst. & G.Forst.	New Zealand's main islands and some neighbouring offshore islands	CHR	CHR 649661	Maurin 45	Chloroplast	MT385076
							Nuclear	MT434779
				GenBank	-	Rotherdam et al. (unpubl.)	Nuclear	EF635468
	Pennantiaceae	*Pennantia cunninghamii* Miers	East coast of Australia	CANB	CANB869762	Purdie 9229	Chloroplast	MT385077
							Nuclear	MT434780
				GenBank	-	Rotherdam et al. (unpubl.)	Nuclear	EF635470
	Pennantiaceae	*Pennantia endlicheri* Reissek	Norfolk Island	CANB	CBG8703383	Telford 10450	Chloroplast	MT385078
							Nuclear	MT434781
				GenBank	-	Rotherdam et al. (unpubl.)	Nuclear	EF635469
	Pittosporaceae	*Pittosporum eugenioides* A.Cunn.	North and South Islands of New Zealand	CHR	CHR 553618	Courtney, *s.n.*	Chloroplast	MT385079
	Araliaceae	*Raukaua anomalus* (Hook.) A.D.Mitch., Frodin & Heads	New Zealand's main islands	CHR	CHR 649673	Maurin 57	Chloroplast	MT385080
	Araliaceae	*Raukaua edgerleyi* (Hook.f.) Seem.	New Zealand's main islands	CHR	CHR 655508	Maurin 103	Chloroplast	MT385081
	Araliaceae	*Raukaua simplex* (G.Forst.) A.D.Mitch., Frodin & Heads	New Zealand's main islands, Auckland Islands	CHR	CHR 437312	Sykes 42/87	Chloroplast	MT385082
	Araliaceae	*Schefflera actinophylla* (Endl.) Harms	Northern and north-eastern coast of Australia	CANB	CANB874342	Lepschi 7083	Chloroplast	MT385083
	Araliaceae	*Schefflera digitata* J.R.Forst. & G.Forst.	New Zealand's main islands	CHR	CHR 649676	Maurin 60	Chloroplast	MT385084
	Torricelliaceae	*Torricellia tiliifolia* DC.	China, eastern Himalaya	GenBank	-	Yao et al. (2019)	Chloroplast	NC040944
Aquifoliales	Aquifoliaceae	*Ilex paraguariensis* A.St.-Hil.	South America	GenBank	-	Cascales et al. (2017)	Chloroplast	KP016928
Asterales	Argophyllaceae	*Corokia cotoneaster* Raoul	New Zealand's main islands	CHR	CHR 655097	Maurin 96	Chloroplast	MT385072
Dipsacales	Caprifoliaceae	*Dipsacus asper* Wallich ex Candolle	China, south-east Asia	GenBank	-	Park et al. (2018)	Chloroplast	MH074864
Paracryphiales	Paracryphiaceae	*Quintinia verdonii* F.Muell.	Eastern Australia	GenBank	-	Yao et al. (2019)	Chloroplast	MK397891

Library preparation and sequencing

Genomic DNA libraries of *Pennantia corymbosa*, *Raukaua anomalus* and *Schefflera digitata* were prepared using Illumina Nextera DNA Library Prep kits, following the manufacturer's instructions (Reference Guide, #15027987 v01, January 2016) except that I halved the quantities of reagents and the target amount of input DNA. Libraries of the other samples were prepared using Illumina TruSeq Nano DNA Library Prep kits, according to the manufacturer's instructions (Reference Guide, # 15041110 Rev. D, June 2015), again using halved reagent quantities and target input DNA; genomic DNA was fragmented using a Covaris ME220 Focused-ultrasonicator (settings: 75 s duration – 40 W peak power – 25% duty factor – 50 cycles per burst). The concentration and size range of libraries were measured with a LabChip GX Touch HT (Perkin Elmer). Libraries were enriched for chloroplast DNA using a custom MYBaits kit (Arbor Biosciences, Ann Arbor) modified from Stull et al. (2013) as detailed in Smissen et al. (unpubl.) using the manufacturer's instructions (version 3.02, July 2016 or version 4.01, April 2018). Illumina HiSeq shotgun sequencing was carried out by Otago Genomics using paired end 2 × 125 bp reads.

Chloroplast DNA assembly and annotation

Reads were first trimmed using Trimmomatic v. 0.38 (Bolger et al., 2014) with the following settings: ILLUMINACLIP:[path/to/NexteraPE-PE.fa for *Pennantia corymbosa*, *Raukaua anomalus* and *Schefflera digitata*, TruSeq3-PE-2.fa for the others]:1:30:10 SLIDINGWINDOW:10:20 MINLEN:40. The reads of the Pennantiaceae and Torricelliaceae samples were then mapped to *Torricellia tiliifolia* (NC040944), the closest sequence to *Pennantia* available in GenBank at the time the mappings were performed (July 2019) that was both verified and published. Mapping was performed with BWA, using the BWA-MEM algorithm (Li, 2013). The quality of the best resulting sequence, *P. cunninghamii*, was then improved (in terms of coverage, HQ% and number of ambiguous bases) by remapping its reads against a consensus sequence from the initial mapping against the *Torricellia* sequence. Finally, reads from all the other samples were mapped against the remapped *P. cunninghamii* sequence. The same process was followed for Araliaceae with the sequence of *Schefflera actinophylla* (Endl.) Harms, first mapped to the GenBank reference *Schefflera heptaphylla* (L.) Frodin (NC029764), *Pittosporum eugenioides* A.Cunn. first mapped to *Torricellia tiliifolia* (NC040944), and *Corokia cotoneaster* Raoul first mapped to *Llerasia caucana* (S.F.Blake) Cuatrec. (NC034821).

The resulting sequences, except *Melanophylla modestei* G.E. Schatz, Lowry & A.-E. Wolf, were of good overall quality (Suppl. material 1: Table S1): on average the HQ% was 98.4 (range: 93.7 – 99.9) and the percentage of ambiguous bases was 1.2% (range: 0.2% – 6.2%). Mean coverage ranged from 124 to 10,804. The *Melanophylla modestei* sequence was of lesser quality, with HQ% 63.6 and mean coverage of 16.5. However, its percentage of ambiguous bases was still low (4.8%), with the vast majority of them located outside the coding regions used in the phylogenetic analysis. The sequences were annotated by (1) aligning the improved references to the GenBank references used to map their reads against with the MAFFT algorithm v. 7.388 (Katoh et al., 2002; Katoh & Standley, 2013) plugin in Geneious Prime 2019.2.1, (2) transferring the annotations of the GenBank references to the improved references, and (3) aligning the other sequences to their corresponding improved references, again with MAFFT within Geneious Prime, and transferring the annotations across. Annotations were manually checked.

18S–26S nuclear ribosomal DNA repeat region assembly and annotation

In the absence of a complete 18S–26S nuclear ribosomal DNA repeat region for Apiales, I built a chimeric 18S–26S nuclear DNA repeat region from several GenBank sequences. I concatenated the 18S rRNA sequence of *Melanophylla alnifolia* Baker (AJ236002), the ITS1, 5.8 S RNA, and ITS2 sequences of *Pennantia cunninghamii* (EF635470), and the 26S rRNA sequence of *Pittosporum fairchildii* Cheeseman (AF479192), in that order. The structure of the resulting chimeric 18S–26S nuclear DNA repeat region is provided in Suppl. material 1: Fig. S1. I then mapped the off-target reads from the chloroplast DNA mappings of the shotgun sequencing data of my herbarium and fresh samples to this chimeric nuclear DNA reference.

The quality of the resulting assemblies was rather variable. There was no clear relationship between the number of reads available to map and the number of reads actually mapped to the chimeric reference (Suppl. material 1: Table S2). The mapping of the two *Melanophylla* species failed; the mapping of the four sequences of *Pennantia* was satisfactory for *P. baylisiana*, *P. cunninghamii* and *P. endlicheri* (HQ% > 86% and ambiguities < 7%), but less so for *P. corymbosa* (HQ% = 51.0%, and ambiguities = 29.1%). Because of the variable quality of my newly reconstructed 18S–26S nuclear DNA repeat region sequences, I aligned them together with the longest sequences of the 18S–26S nuclear DNA repeat region available on GenBank for the four *Pennantia* species, as a control of the identity of my newly generated sequences for the phylogenetic analyses.

Some statistics regarding these sequences discussed later in the paper were obtained with MEGA X (Kumar et al., 2018).

Data partitioning

Sixty protein-coding sequences (CDS, 46,051 sites) from the long and short single copy regions were used for the chloroplast DNA analyses (see list in Suppl. material 1: Table S3); coding rRNA, which was located in the inverted repeats, was not considered. CDS were partitioned into 1^{st} + 2^{nd} codon position on the one hand (30,701 sites), and 3^{rd} codon position on the other hand (15,350 sites). For the nuclear DNA analyses, the 18S–26S nuclear DNA repeat region alignment represented 810 sites, partitioned as ITS1 + ITS2 on the one hand (538 sites), a portion of 18S rRNA + whole 5.8S rRNA + a portion of 26S rRNA on the other hand (272 sites). The markers were aligned in Geneious Prime using the MAFFT plugin, and the alignments were manually checked.

Phylogenetic analyses and chloroplast DNA tree calibration

Phylogenetic analyses were conducted with the BEAST suite v. 2.5.2 (Bouckaert et al., 2019). Each of the four partitions was assigned its own evolutionary model using bModelTest (Bouckaert & Drummond, 2017) to average the best-fitted nucleotide models. A relaxed clock with rates drawn from an exponential distribution (Drummond et al., 2006) was associated to each partition. The MCMC chains were run for 250 million generations and sampled once every 25,000 generations for chloroplast DNA, and for 50 million generations sampled once every 5,000 generations for nuclear DNA. The influence of tree prior choice on the phylogeny and dating was assessed by repeating the analysis under both the Yule model (Yule, 1925) and the Birth-Death model (Gernhard, 2008). These analyses were run on the CIPRES platform (Miller et al., 2010). The proper convergence of the chains and the determination of the burnin that would maximise their effective sample size (ESS) was examined with Tracer v. 1.7.1 (Rambaut et al., 2018); the ESS of a parameter represents the number of effectively independent samples from the posterior distribution of the parameter, and therefore how strong its estimation is: values above 200 are considered satisfactory (BEAST Developers, 2017). Three independent runs per analysis (i.e. per combination of Birth-Death or Yule model with chloroplast DNA or nuclear DNA) were started from different seeds and combined with LogCombiner v. 2.5.2 (Bouckaert et al., 2019). The combined sampled trees from each analysis were then summarised in TreeAnnotator v. 2.5.2 (Bouckaert et al., 2019) with their selected burnin.

The chloroplast DNA phylogeny was calibrated using two fossils and one secondary calibration. Firstly, I assigned the age of the earliest confirmed fossils of *Torricellia*, which are ca. 48 My old (Manchester et al., 2017), to the minimum crown age of Torricelliaceae, using an offset exponential distribution (Mean = 20.0, Offset = 48.0), resulting in a wide prior with a 2.5% quantile of 48.5 My, a 97.5% quantile of 122 My, and a mean of 68 My. Secondly, I assigned the age of *Paleopanax oregonensis* Manchester fossils, which are considered from the Middle Eocene (Manchester, 1994), to the minimum crown age of Araliaceae, following Magallón et al. (2015) and Li et al. (2019); I used an offset exponential distribution (Mean = 20.0 and Offset = 37.8), resulting in a wide prior with a 2.5% quantile of 38.3 My, a 97.5% quantile of 112 My, and a mean of 57.8 My. Finally, the estimated age of Apiales in recent Angiosperm-wide phylogenies (Li et al., 2019; Magallón et al., 2015) is about 80–81 My old, with a maximum interval of about [70,95] My; I therefore assigned an offset lognormal distribution with M = 33.0, S = 0.2, and Offset = 48.0 to the crown age of the Apiales species, resulting in a prior with a 2.5% quantile of 69.9 My, a 97.5% quantile of 95.9 My, and a mean of 81.0 My.

The robustness of the Bayesian inference of tree topology for the phylogenies resulting from both the chloroplast DNA and the nuclear DNA sequence data was assessed with a maximum likelihood approach. RAxML v. 8.2.12 (Stamatakis, 2014) was run on CIPRES with the following settings for both phylogenies: GTRGAMMA model, rapid bootstrap analysis with search for best scoring tree (-f a -x) with 1,000 bootstrap replicates. The chloroplast DNA phylogeny was rooted by fixing the four non-Apiales sequences as outgroups, while no outgroup was set for the nuclear DNA phylogeny.

Finally, the six resulting trees (chloroplast DNA or nuclear DNA, with BEAST2/Birth-Death model, BEAST2/Yule model or RAxML) were first formatted in FigTree v. 1.4.4 (Rambaut, 2018) and then refined in Inkscape v. 0.92.3. Given the much larger number of sites in the chloroplast DNA dataset compared to the nuclear DNA dataset, a combined analysis was not conducted as its results would have been skewed towards what was observed with chloroplast DNA alone; moreover, the topologies of both phylogenies were congruent. The detailed settings and parameters used for the phylogenetic analyses are in the BEAST2 and RAxML files provided in Suppl. material 2.

2.5 Results

Dated chloroplast DNA phylogeny

The combination of the chains run under the Birth-Death model or the Yule model resulted in an Effective Sample Size (ESS) > 200 for all their parameters. The tree had the same topology and was very well supported within the ingroup Apiales under both models, all the node posterior probabilities (PP) being equal to 1. Moreover, the same topology was obtained for the chloroplast DNA tree built with RAxML, with 100% bootstrap support within Apiales. The tree resulting from the Birth-Death model is shown in Fig. 2.2, and the trees resulting from the Yule model and the RAxML analysis in Suppl. material 1: Fig. S2 and Fig. S3 respectively.

In the phylogeny presented in Fig. 2.2, the relationships between the families of Apiales that were included in the analysis conformed to contemporary ideas about the relationships among Apiales families (Stevens, 2017c). Here, the crown age of *Pennantia* was estimated at 9.5 My, with an HPD[3] of [2.6,19.5] My. Within *Pennantia*, the Australian species *P. cunninghamii* was sister to the rest of the genus. Then, *P. baylisiana*, from the Three Kings Islands/Manawatāwhi, was sister to a clade formed by the New Zealand species *P. corymbosa* and the Norfolk Island species *P. endlicheri*.

[3] HPD = highest posterior density (confidence interval on the age).

Node	Mean age	95% HPD
a	0.2	[0,0.6]
b	1.7	[0.3,4.0]
c	8.7	[2.2,19.0]
d	13.4	[3.5,30.3]
e	40.6	[37.8,46.1]
f	57.4	[47.2,68.6]
g	63.4	[52.4,75.5]
h	73.1	[61.1,85.5]
i	86.7	[73.3,100.9]
j	99.2	[77.8,121.0]
k	117.7	[76.8,178.9]
m	8.2	[1.3,20.9]
n	52.7	[48.0,61.2]
o	0.9	[0.1,2.2]
p	4.2	[1.1,8.8]
q	9.5	[2.6,19.5]
r	79.3	[44.8,111.8]
s	81.3	[48.0,114.0]

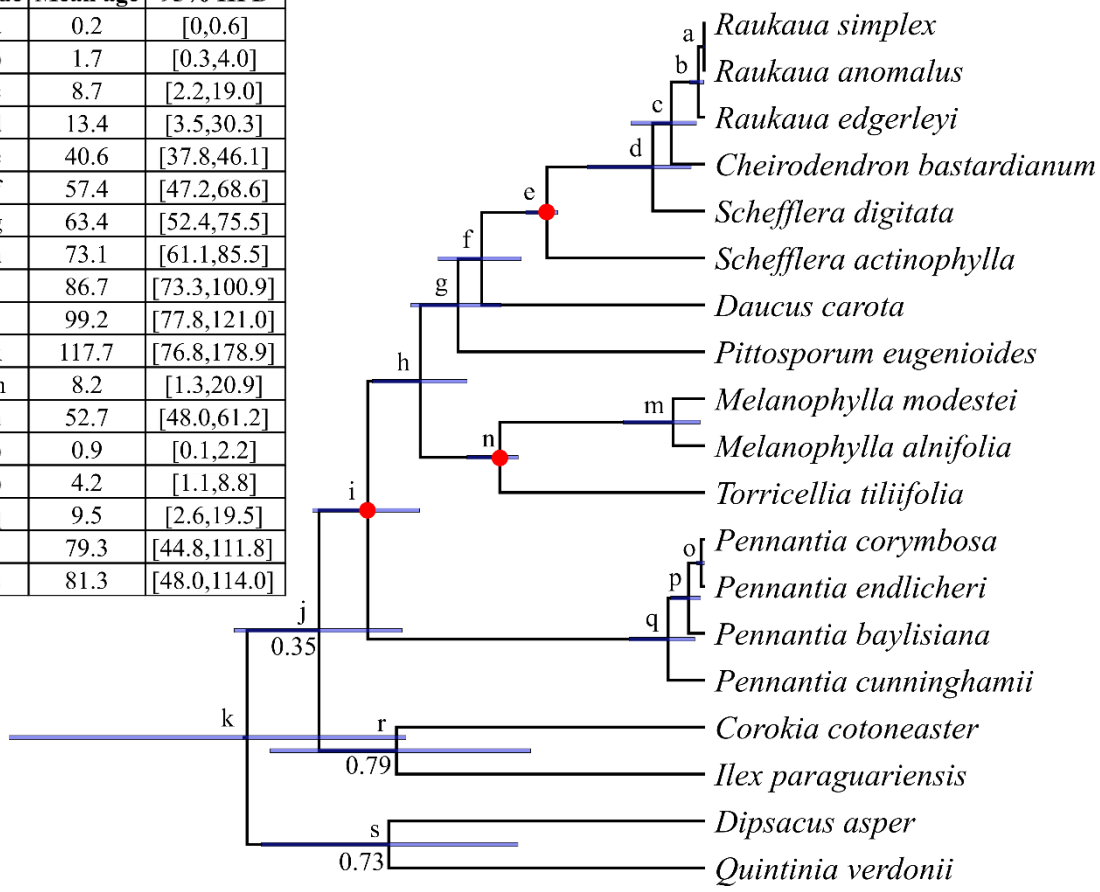

Figure 2.2. *Dated chloroplast DNA BEAST 2 phylogeny of* Pennantia, *under the Birth-Death model. Mean node age and 95% HPD (in My) is given in the table embedded in the figure under the corresponding letter code. 95% HPD is also represented by blue bars. All node posterior probabilities are equal to 1 except if indicated otherwise. The calibrated nodes (see text) are indicated by red dots.*

Undated 18S–26S nuclear DNA repeat region phylogeny

The chains yielded an ESS far greater than 200 even before they were combined under both the Birth-Death model and the Yule model. The resulting tree showed the same topology with comparable PP under both models, although the PP under the Yule model tended to be slightly lower than under the Birth-Death model. The topology of the tree produced from the RAxML analysis was congruent with the topology of the BEAST2 trees, with bootstrap values of 100% except for the node placing the two samples of *P. corymbosa* and *P. endlicheri* as sister to each other (bootstrap = 88%). For consistency with the chloroplast DNA phylogeny, I draw conclusions regarding the nuclear DNA phylogeny primarily by examining the Birth-Death model tree (Fig. 2.3), while providing the Yule model and RAxML trees in Suppl. material 1: Fig. S4 and Fig. S5 respectively. In the absence of suitable outgroup sequences, the RAxML nuclear DNA tree was rooted to make *P. cunninghamii* sister to the other species of Pennantia, in accordance with the

topology of the chloroplast DNA tree presented in this study and of the ITS tree of Keeling et al. (2004).

The percentage of identical sites between the two samples of each species was ≥ 98.7%. There were relatively few parsimony-informative sites in the nuclear DNA alignment: only 35 out of 538 (6.5%) sites in the ITS1/ITS2 partition and 0 out of 272 in the rRNA partition. The two samples of each species were recovered as sisters, usually with strong support: PP = 1 for *P. cunninghamii* and *P. baylisiana*, PP = 0.97 for *P. corymbosa*, but PP = 0.75 only for *P. endlicheri*. Moreover, the topology of this tree was congruent with that of the tree based on chloroplast DNA (Fig. 2.2), with strong support (PP = 0.99) for the clade *P. corymbosa* + *P. endlicheri* but weak support for the clade *P. corymbosa* + *P. endlicheri* + *P. baylisiana* (PP = 0.64), although the latter had 100% bootstrap support in the RAxML analysis. This phylogeny was also congruent with the one reported by Keeling et al. (2004), built with the maximum likelihood option of PAUP* 4.0b (Swofford, 2002), and showing comparable bootstrap values for equivalent nodes in the case of the present RAxML analysis.

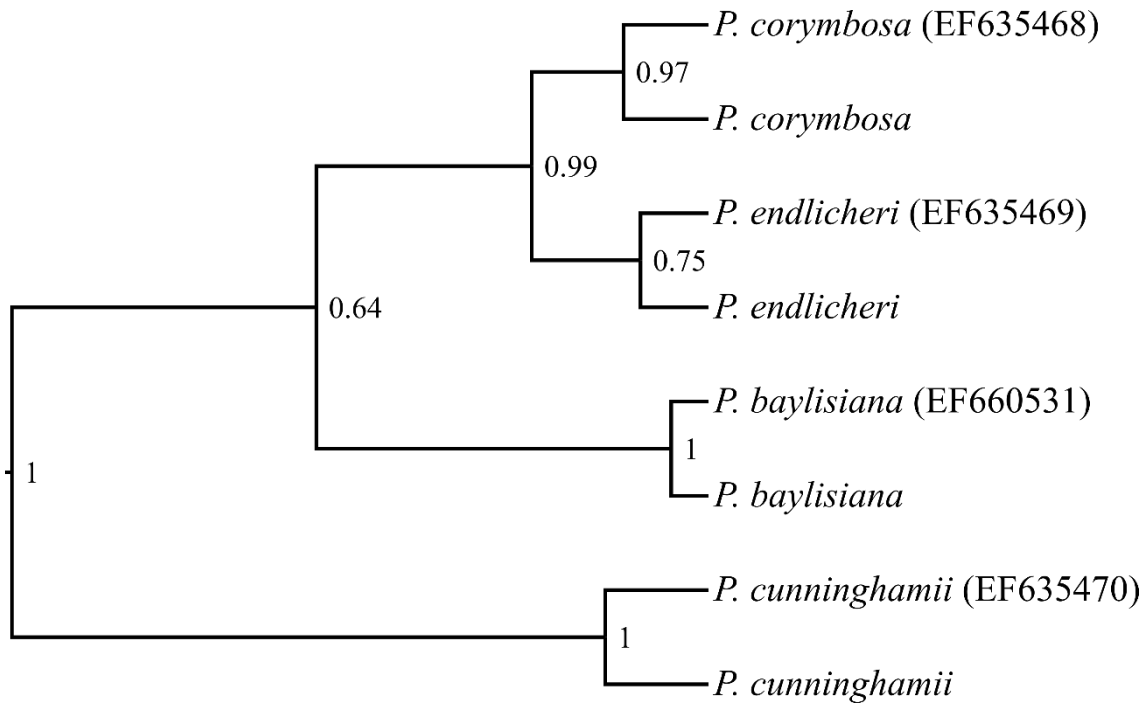

Figure 2.3. *Undated 18S–26S nuclear DNA repeat region BEAST 2 phylogeny of* Pennantia*, under the Birth-Death model. The tree was rooted to make* P. cunninghamii *sister to the other species of* Pennantia*, in accordance with the chloroplast DNA tree and the ITS tree of Keeling et al. (2004). Node posterior probability is shown next to the corresponding node. The sequences downloaded from GenBank have their accession number in round brackets; the others were generated from the samples used in this study.*

2.6 Discussion

Congruence between chloroplast and nuclear DNA phylogenies

Phylogenies based on chloroplast DNA markers and the 18S–26S nuclear DNA repeat region indicate the same relationships among the four species of *Pennantia*. They are also congruent with the ITS phylogeny of Keeling et al. (2004), confirming the relationships they inferred among the four species. The relatively low support values that were observed for some clades in the 18S–26S nuclear DNA repeat region could result from the limited amount of variation of this region, or more probably from the loss of sites during the phylogenetic analyses due to the presence of ambiguities: the sequences I generated from the samples of *P. endlicheri* and *P. corymbosa* have a percentage of ambiguities of 16.3% and 6.9% respectively (while all the other sequences have ≤ 0.5% of ambiguities), after trimming the sequences. Conflicting tree topologies did not seem to be in play in this case given the paucity of parsimony-informative sites in this 18S–26S nuclear DNA repeat region.

Crown age of Pennantiaceae and age of its most recent common ancestor (MRCA) with Torricelliaceae

The age of the MRCA of Pennantiaceae and Torricelliaceae (which is the crown age of Apiales) was estimated about 86.7 My, with an HPD of [73.3,100.9] My. This mean estimate is consistent with some of the previous dated phylogenies that include this MRCA: 73.6 My (Li et al., 2019), 80.8 My (Magallón et al., 2015) and 91.39 My (Tank et al., 2015); however, it is more recent than the 117.0 My indicated by Nicolas & Plunkett (2014), which might be explained by their use of an Araliaceae fossil about the same age as the one I used to date a node that is internal to Araliaceae.

The mean crown age of Pennantiaceae was estimated to be 9.5 My with an HPD of [2.6,19.5] My, which is slightly older than the previous estimate for *Pennantia* of 6.6 My with an HPD of ca. [1.6,15.8] My suggested by Nicolas & Plunkett (2014). The fact that I used more conservative priors than they did for the MRCAs of Araliaceae and Torricelliaceae may explain my older estimates. The difference in priors on the crown age of Araliaceae was mentioned above. Moreover, their priors were tightly constrained around old ages compared to mine, e.g. for the crown age of Torricelliaceae they used a prior with a 95% HPD of [55.8,58.7] My, while my prior had a 95% HPD of [48.5,122] My. I allowed the possibility for relatively older posterior dates than the estimated age of the fossils so as to account better for the fact that fossils can only represent the youngest possible age of the clade to which they are associated; older fossils might yet exist and be discovered. Nevertheless, the results of both sets of analyses suggest that *Pennantia* diversified within the last 20 My. The present analysis also shows that the diversification of the ancestors of the extant New Zealand, Three Kings Islands/Manawatāwhi and Norfolk Island species is much more recent, starting about 4.2 Mya with an HPD of [1.1,8.8] My.

Relationships within Pennantia

The phylogenies presented here significantly supported *Pennantia baylisiana* being a distinct species to *Pennantia endlicheri*, corroborating the conclusions Keeling et al. (2004) made from their ITS region phylogeny of the four species of *Pennantia*. Gardner & de Lange (2002) suggested that the closest relative of *P. baylisiana* may be *P. endlicheri* (p. 671) but maintained *P. baylisiana* distinct from *P. endlicheri* on morphological grounds: e.g. domatia developed and bearing trichomes in the former but hardly developed and glabrous in the latter. The chloroplast DNA phylogeny strongly supported the distinction between these species since they are not sister taxa, as it placed

P. baylisiana sister to the clade *P. endlicheri* + *P. corymbosa* with a PP of 1. In the nuclear DNA phylogeny, this node only had a PP of 0.64 but is strongly supported (bootstrap = 100%) in the phylogeny of Keeling et al. (2004). Characters shared between *P. endlicheri* and *P. corymbosa* that are not found in the two other species of the genus include the presence of uncinate trichomes (rather sparse and restricted to inflorescence axes in *P. endlicheri*) and a stigmatic ring being made of three distinct stigmas (Gardner & de Lange, 2002).

The present phylogenies also supported the placement by Miers (1852) of *Pennantia cunninghamii* in a monotypic section *Dermatocarpus*, which was maintained by Gardner & de Lange (2002) on morphological grounds. *P. cunninghamii* indeed has unique morphological features compared to the rest of the genus. For example, its domatia form pits while those of the other species are pockets (although shallow and sometimes absent in *P. endlicheri*), and its ovary is longitudinally ridged and thus appears to be formed by three carpels while the ovary of the other species is barrel-shaped and barely furrowed. Here, the results of the phylogeny based on chloroplast sequences were consistent with this infrageneric classification, placing *P. cunninghamii* sister to all the other *Pennantia* species with a posterior probability of 1. The nuclear DNA phylogeny presented here, in the absence of outgroups to *Pennantia*, does not explicitly support this idea, but it is consistent with it. The sister group relationship between *P. cunninghamii* and the rest of the genus was well supported by the ITS phylogeny of Keeling et al. (2004, bootstrap values ≥ 96%).

2.7 Conclusions

The analysis of chloroplast genome sequences supports previous phylogenetic results based on nuclear DNA in suggesting that *Pennantia cunninghamii* is sister to the rest of the genus. Moreover, it strongly supports previous nuclear DNA analyses in placing *P. baylisiana* as sister to the clade *P. endlicheri* + *P. corymbosa* rather than sister to *P. endlicheri* alone, with which it has sometimes been considered conspecific (e.g. Mabberley, 2017). This is consistent with previous studies based on morphology, which concluded that *P. baylisiana* should be recognised as a distinct species. The dated phylogeny presented here suggests that *Pennantia* diversified within the last 20 My, and possibly as recently as 2.6 My ago. It also suggests that divergences among the ancestors of the three species of section *Pennantia*, now distributed on Norfolk Island, Three Kings

Islands/Manawatāwhi and the main islands of New Zealand, happened over the last 9 My and as recently as 0.1 My ago. However, the island endemism of each *Pennantia* species and the lack of close outgroups and of information about ancestral distribution areas prevents the inference of confident biogeographical scenarios regarding the origin of the distribution of the extant species. Finally, this study has shown that the use of a chimeric reference sequence to utilise off-target reads from target enrichment libraries that are usually discarded can provide useful data for phylogenetic analysis. Although the quality of such mappings can be quite variable, as demonstrated here, the low marginal cost of this procedure makes it worth exploring in genome-based research using shotgun sequencing techniques.

2.8 Acknowledgments

I would like to thank Chris Lusk and Rob Smissen for commenting on the draft manuscripts; Peter de Lange and Porter Lowry II for commenting on the submitted manuscript; Otari Native Botanic Garden, the Pukemokemoke Bush Trust, the Department of Conservation, the Maungatautari Ecological Island Trust and the iwi Waikato Tainui, Ngāti Rangi and Mōkai Pātea for sampling permits and agreements in New Zealand; the herbaria CHR, CANB and P for leaf samples. This research is supported by the Royal Society of New Zealand – Te Apārangi through Marsden contract 16-UOW-029; and the Faculty of Science and Engineering of the University of Waikato through FSEN Student Trust Fund (# P102218 SoS/PG Support).

2.9 Supplementary material

Supplementary material 1: Figs S1–S5; Tables S1–S3

Author: Kévin J. L. Maurin

Data type: figures and tables

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Link: https://doi.org/10.3897/phytokeys.155.53460.suppl1

Supplementary material 2: BEAST2 and RAxML files

Author: Kévin J. L. Maurin

Data type: molecular data

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Link: https://doi.org/10.3897/phytokeys.155.53460.suppl2

Chapter 3 Phylogeny of *Corokia* A.Cunn.

3.1 Preliminary note

This chapter was accepted for publication in *New Zealand Journal of Botany* in March 2021 under the following reference:

Maurin, K. J., & Smissen, R. D. (2021). A dated phylogeny of Argophyllaceae (Asterales) is consistent with spread by long-distance dispersal. *New Zealand Journal of Botany*, 1–18. https://doi.org/10.1080/0028825X.2021.1905671

The publishing agreement signed with the publisher grants me "the right to include the article in a thesis or dissertation that is not to be published commercially, provided that acknowledegment to prior publication in the Journal is given". The second condition being fulfilled, the content of this chapter is therefore an exact copy of the published article, except that (1) the heading, the ORCID numbers of the authors, the lists of keywords and of references were omitted, (2) a Supplementary files section was added to provide the link to the supplementary material attached to the article, and that (3) for consistency with the rest of the thesis, the citations were reformatted and the numbering of figures and tables adjusted.

3.2 Abstract

Argophyllaceae is a small eudicot family of trees and shrubs of south-western Pacific distribution, comprising two genera: *Corokia* and *Argophyllum*. The phylogeny of *Corokia*, which contains six species, has attracted little attention so far, the genus being usually represented by a single species in studies looking at relationships at higher taxonomic levels. Here we bridge this knowledge gap with a complete phylogeny of the genus based on whole plastid DNA sequences. We also investigated nuclear ribosomal DNA markers, which yielded a poorly supported phylogeny. Comparing fossil-calibrated and biogeographic dating approaches, we conclude that extant Argophyllaceae species are probably not Gondwanan relicts, the timing of their divergences being better explained by long-distance dispersal after the break-up of Gondwana than by vicariance. The high level of endemicity of the species of *Corokia* prevents the reconstruction of a

precise biogeographic history of the genus, but our phylogenies suggest that the genus originated in Australia, then about 3.5 My ago started dispersing eastwards into the Pacific towards its present-day distribution.

3.3 Introduction

Argophyllaceae Takhtajan is a small family of Asterales confined to the south-west Pacific. Its two genera, *Corokia* A.Cunn and *Argophyllum* J.R.Forst & G.Forst., comprise six and 22 arborescent species, respectively (Bean & Forster, 2018; Eyde, 1966; Kårehed, 2007; Takhtajan, 2009). *Corokia buddleioides* A.Cunn. is found in the northern North Island of New Zealand. *Corokia carpodetoides* (F.Muell.) L.S.Sm. grows on Lord Howe Island, a small volcanic remnant located about 600 km east of Australia. *Corokia collenettei* L.Riley is found on Rapa Iti, an island of French Polynesia. *Corokia cotoneaster* Raoul is a divaricate (small-leaved with tangled branches) shrub that is widely distributed throughout the New Zealand archipelago. *Corokia macrocarpa* Kirk occurs on the Chatham Islands, a small group of islands located c. 850 km east of the mainland of New Zealand (P. B. Heenan et al., 2010). Finally, *C. whiteana* L.S.Sm. is found in north-eastern New South Wales, Australia. A natural hybrid is known between *C. buddleioides* and *C. cotoneaster*: *C.* ×*virgata* Turrill, whose diversity of leaf and fruit colour makes it a popular ornamental (sometimes referred to by its synonym, *C.* ×*cheesemanii* Carse). *Argophyllum* is represented by 11 species in mainland Australia and 11 species in New Caledonia, all endemic to their respective landmasses (Bean & Forster, 2018).

As far as we are aware, no molecular phylogenetic study has included all species of either *Corokia* and *Argophyllum* (Bean & Forster, 2018), let alone of both genera. *Corokia cotoneaster* alone has often been used to represent *Corokia* in studies focusing on higher taxonomic levels (Albach et al., 2001; Gustafsson et al., 1996; Kårehed et al., 1999; Li et al., 2019; Lundberg, 2001; Magallón et al., 2015; Qiu et al., 2010; Xiang et al., 1993). Heenan et al. (2010) produced sequences of the internal transcribed spacer (ITS) region for at least one sample of each of the six species of *Corokia*, and built a phylogenetic network. Although they did not present a rooted phylogeny, they interpreted their analysis as suggesting that *C. whiteana* is sister to the rest of the genus. *Argophyllum* is less frequently represented than *Corokia* in phylogenies of higher taxonomic levels,

and only one to three sequences have been used used, often from samples that were not identified to the species level (e.g. Bremer et al., 2002; Gustafsson et al., 1996; Magallón et al., 2015).

Vicariance and long-distance dispersal are two processes that have been put forward to explain the current distribution of extant organisms across the landmasses formerly forming Gondwana (e.g. Heads, 2005, 2013, 2016; Pole, 1994; Sanmartín & Ronquist, 2004; Waters & Craw, 2006; Winkworth et al., 2002). The former proposes that most lineages have been inhabiting the landmasses they currently occupy since the break-up of Gondwana: in contrast, the latter proposes that most lineages reached their present distributions via long-distance dispersal after the break-up of Gondwana. In many cases, studies suggested that a combination of both processes probably took place to produce the current distribution of species on these landmasses, particularly in plants (e.g. Korall & Pryer, 2014; Noben et al., 2017). The current distribution of Argophyllaceae includes three landmasses of Gondwanan origin (Australia, New Zealand and New Caledonia), which makes it suitable to estimate whether it is better explained by vicariance or long-distance dispersal.

In this study, we present the first molecular phylogeny of Argophyllaceae that includes all six species of *Corokia* – we however could include only a few species of *Argophyllum*. We use whole-plastid DNA alone for Argophyllaceae and its outgroups, and address the congruence between plastid DNA and nuclear DNA markers within Argophyllaceae. We evaluate the historical biogeographic hypothesis accounting for the extant distribution of *Argophyllum* and *Corokia* species. We compared results from dated phylogenies using biogeographic calibrations (Ho et al., 2015) to results from dated phylogenies using fossil calibrations to ask whether the timing of the distribution of Argophyllaceae is better explained by vicariance from the break-up of Gondwana or by long-distance dispersal after the break-up.

3.4 Material and methods

Sampling plan for plastid DNA

All six species of *Corokia* are well-circumscribed, even though two species have infra-specific taxa. A morphological variant of *C. buddleioides* has been recognised as a variety: *C. buddleioides* var. *linearis* Cheeseman. It has narrower leaves (< 1 cm wide; Allan, 1961) than the type (1-3 cm; Allan, 1961). Several local forms of *C. cotoneaster* have been suggested as departing morphologically from the type enough to merit taxonomic recognition (Eagle, 2006). One of these, a population growing on the central west coast of the North Island of New Zealand, known as *C.* aff. *cotoneaster* "Paritutu" (CHR 497632: de Lange et al. (2018)), has larger leaves (> 3 cm; Eagle, 2006) than the type (< 1 cm, Allan, 1961) while retaining divaricating branching; this variant is currently categorised as a nationally endangered unnamed species (de Lange et al., 2018) under the New Zealand Threat Classification System (Townsend et al., 2008). We therefore included one sample of the type of each species, and one sample of *C. buddleioides* var. *linearis* and *C.* aff. *cotoneaster* "Paritutu".

We were unable to include representatives of all species of *Argophyllum*. We chose two species from Australia (*A. lejourdanii* F.Muell. and *A. nullumense* R.T.Baker) and two from New Caledonia (*A. latifolium* Vieill. ex Zemann and *A. montanum* Schltr.) to represent the distribution of the genus. We also added a species of the New Caledonian endemic genus *Phelline* Labill. (*P. comosa* Labill.), sole genus of Phellinaceae Takhtajan, the sister family to Argophyllaceae (Li et al., 2019). Finally, we added representatives of as many of the other families of Asterales as we could find a sequence of on GenBank (10 species) or generate from our samples (five species), as well as two species of Aquifoliales from GenBank as outgroups. The total number of plastid DNA sequences included in our analyses was therefore 30 (Table 3.1). Sequences that were not downloaded from GenBank were obtained from herbarium specimens or field collections that were dried in silica gel and processed in the lab within ten months of collection.

Sampling plan for nuclear DNA markers

The sequences of the 18S–26S nrDNA repeat region that we generated from the Argophyllaceae samples listed in Table 3.1 were not of sufficient quality for use in a phylogenetic analysis. We therefore used all 12 sequences of Argophyllaceae available on GenBank in October 2019 (Supplementary Table 3.1). They include at least one sample per recognised species of *Corokia*, and two species of *Argophyllum*. The ITS and

rRNA portions of the 18S–26S nrDNA repeat region were not annotated on these sequences. We therefore aligned them to the annotated GenBank sequence *Llerasia caucana* (S.F.Blake) Cuatrec. (Asteraceae, accession # KX064001) using MUSCLE v. 3.8.425 (Edgar, 2004) in-built in Geneious Prime 2019.2.1 (https://www.geneious.com), then transferred its annotations across to our sequences of interest.

DNA extraction

DNA extractions of the newly sequenced specimens were performed following the DNA tissue protocol of the robot Maxwell 16 (Promega), followed by a phenol:chloroform extraction and recovery using spin columns (Smissen & Heenan, 2007). The DNA concentration of the extracts was measured using a Qubit (Thermo Fischer Scientific) dsDNA high-sensitivity assay.

Plastid DNA library preparation and sequencing

Indexed DNA libraries of all the samples were produced using Illumina TruSeq Nano DNA Library Prep kits following the manufacturer's protocol (# 15041110 Rev. D, June 2015), except that reagent quantities were halved. The DNA fragmentation step was performed on a Covaris ME220 Focused-ultrasonicator, with the settings 75 s duration – 40 W peak power – 25% duty factor – 50 cycles per burst. Library concentration was estimated with a LabChip GX Touch HT (Perkin Elmer), and up to eight libraries pooled into "pre-pools" prior to enrichment, aiming for equimolarity. Libraries were enriched for plastid DNA following the protocol of the Arbor Biosciences myBaits Hybridization Capture for Targeted NGS Manual (v. 4.01, April 2018). The RNA baits were designed by RDS in conjunction with Arbor Biosciences (Ann Arbor, Michigan), based on Stull et al. (2013) but using an adjusted set of reference sequences. After a final library quantification of the enriched pre-pools, they were pooled into a final library then sequenced on an Illumina HiSeq 2500 by Otago Genomics Facility using 2 x 125 bp paired-end chemistry.

Table 3.1. *Sampling plan of this study for plastid DNA.*

Order	Family	Species	Native distribution	GenBank accession numbers
Aquifoliales	Aquifoliaceae	*Ilex wilsonii* Loes.	East Asia	KX426471 (Yao et al., 2016)
Aquifoliales	Cardiopteridaceae	*Gonocaryum lobbianum* (Miers) Kurz	East and South-East Asia	NC041492 (Jo et al., 2019)
Asterales	Argophyllaceae	*Argophyllum latifolium* Vieill. ex Zemann	New Caledonia (Grande Terre)	MW255586 (this study)
Asterales	Argophyllaceae	*Argophyllum lejourdanii* F.Muell.	Australia (northern Queensland)	MW255587 (this study)
Asterales	Argophyllaceae	*Argophyllum montanum* Schltr.	New Caledonia (Grande Terre)	MW255588 (this study)
Asterales	Argophyllaceae	*Argophyllum nullumense* R.T.Baker	Australia (eastern)	MW255589 (this study)
Asterales	Argophyllaceae	*Corokia buddleioides* A.Cunn.	New Zealand (northern North Island)	MW194049 (Maurin et al. unpublished)[4]
Asterales	Argophyllaceae	*Corokia buddleioides* var. *linearis* Cheeseman	New Zealand (northern North Island)	MW255590 (this study)
Asterales	Argophyllaceae	*Corokia carpodetoides* (F.Muell.) L.S.Sm.	Lord Howe Island	MW194050 (Maurin et al. unpublished)
Asterales	Argophyllaceae	*Corokia collenettei* L.Riley	French Polynesia (Rapa Iti)	MW194051 (Chapter 4)
Asterales	Argophyllaceae	*Corokia cotoneaster* Raoul	New Zealand (North Island and South Island)	MT385072 (Maurin, 2020a)
Asterales	Argophyllaceae	*Corokia* aff. *cotoneaster* "Paritutu"	New Zealand (central west coast of North Island)	MW255591 (this study)
Asterales	Argophyllaceae	*Corokia macrocarpa* Kirk	Chatham Islands (Chatham Island)	MW194052 (Maurin et al. unpublished)
Asterales	Argophyllaceae	*Corokia whiteana* L.S.Sm.	Australia (north-eastern New South Wales)	MW194053 (Maurin et al. unpublished)
Asterales	Asteraceae	*Archidasyphyllum excelsum* (D. Don) P.L.Ferreira, Saavedra & Groppo	Chile, Argentina	MH899017 (Gruenstaeudl & Jenke, 2020)
Asterales	Asteraceae	*Cichorium intybus* L.	Europe and northern Africa	NC043842 (Yang et al., 2019)
Asterales	Asteraceae	*Olearia pachyphylla* Cheeseman	New Zealand (northern North Island)	MW229258 (Maurin et al. unpublished)
Asterales	Asteraceae	*Saussurea przewalskii* Maxim.	China, Bhutan	NC044732 (Zhang et al., 2019)
Asterales	Campanulaceae	*Adenophora divaricata* Franch. & Sav.	East Asia	NC036221 (Cheon et al., 2017)
Asterales	Campanulaceae	*Campanula takesimana* Nakai	Korea	NC026203 (Cheon et al., 2016)
Asterales	Campanulaceae	*Codonopsis lanceolata* (Siebold & Zucc.) Benth. & Hook.f. ex Trautv.	East Asia	MH018574 (Lee et al., 2018)
Asterales	Campanulaceae	*Platycodon grandiflorus* (Jacq.) A.DC.	East Asia	NC035624 (Hong et al., 2017)
Asterales	Goodeniaceae	*Scaevola taccada* (Gaertn.) Roxb.	Indo-Pacific	NC040933 (Yao et al., 2019)
Asterales	Menyanthaceae	*Menyanthes trifoliata* L.	Asia, Europe, North America	NC041436 (Njuguna et al., 2019)
Asterales	Menyanthaceae	*Nymphoides hydrophylla* (Lour.) Kuntze	Asia	NC041482 (Njuguna et al., 2019)
Asterales	Phellinaceae	*Phelline comosa* Labill.	New Caledonia	MW255592 (this study)
Asterales	Rousseaceae	*Abrophyllum ornans* (F.Muell.) Hook.f.	Australia (eastern)	MW246782 (Maurin et al. unpublished)
Asterales	Rousseaceae	*Carpodetus arboreus* (Lauterb. & K.Schum.) Schltr.	Papua New Guinea	MW246783 (Maurin et al. unpublished)
Asterales	Rousseaceae	*Carpodetus serratus* J.R.Forst. & G.Forst.	New Zealand (main islands)	MW246786 (Maurin et al. unpublished)
Asterales	Rousseaceae	*Cuttsia viburnea* F.Muell.	Australia (eastern)	MW246787 (Maurin et al. unpublished)

Note: GenBank accession numbers are provided, with the reference of the original publication of the sequence. Newly generated sequences were formatted for submission to GenBank using the tool GB2Sequin (Lehwark & Greiner, 2019).

[4] Corresponds to Chapter 4 of the present thesis.

Plastid DNA assembly and annotation

Reads were first trimmed using Trimmomatic (v. 0.38, Bolger et al. (2014)) with the following settings: ILLUMINACLIP:path/to/TruSeq3-PE-2.fa:1:30:10 SLIDINGWINDOW:10:20 MINLEN:40. We built the sequences of the newly generated Phellinaceae and Argophyllaceae species by mapping their reads to a sequence of *Corokia cotoneaster* from GenBank (accession # MT385072). Mappings were performed with BWA, using the BWA-MEM algorithm (Li, 2013). These sequences were annotated by aligning them to the C. cotoneaster sequence from GenBank using the MAFFT algorithm (v. 7.388, Katoh et al. (2002), Katoh & Standley (2013)) plugin in Geneious Prime 2019.2.1 and transferring the annotation of this sequence to the newly generated sequences. Annotations were manually checked.

Data partitioning and alignment building

Sequences of 52 protein coding regions (44,446 sites; see list in Supplementary Table 2) of the long and short single copies were extracted from the plastid genome alignment and concatenated for phylogenetic analyses. They were partitioned into 1st + 2nd codon position on the one hand (29,631 sites), 3rd codon position on the other hand (14,815 sites). Nuclear ribosomal sequences were partitioned as ITS1 + a portion of ITS2 on the one hand (376 sites), 5.8S rRNA + a portion of 18S rRNA on the other hand (185 sites). Alignments were built using MAFFT and partitions generated in Geneious Prime 2019.2.1.

Reconstruction of the phylogeny of *Corokia*

Phylogenetic analyses were performed with the BEAST suite v. 2.5.2 (Bouckaert et al., 2019). We used bModelTest (Bouckaert & Drummond, 2017) to average the best-fitting site models for each of the partitions. We used a relaxed clock with rates drawn from a lognormal distribution (Drummond et al., 2006). The MCMC chain was run for 50 million generations sampled one every 5,000 for nuclear markers, and 250 million generations sampled one every 25,000 for plastid markers. In order to test the robustness of our phylogeny to our choice of tree prior model, we ran the same analysis with one of the two most appropriate priors given our dataset: Yule model (Yule, 1925) and Birth-Death model (Gernhard, 2008).

Tracer v. 1.7.1 (Rambaut et al., 2018) was used to check the proper convergence of the chains and determine the burnin that would maximise their effective sample size (ESS). We combined the results of two to five chains starting from different seeds for

each analysis in order to reach satisfying ESS (≥ 200), using LogCombiner v. 2.5.2 (Bouckaert et al., 2019). The combined sampled trees were then summarised into a consensus tree for each analysis in TreeAnnotator v. 2.5.2 (Bouckaert et al., 2019). The resulting consensus trees were first formatted in FigTree v. 1.4.4 (Rambaut, 2018), then refined in Inkscape v. 0.92.3.

Fossil calibration of the plastid DNA phylogeny

We assigned a lognormal distribution with a mean of 72.1 My (M = 8.0, S = 0.75, Offset = 64.1, mean in real space) to the age of the crown of Asteraceae, following Li et al. (2019) in their use of the *Tubulifloridites lilliei* (Couper) Farabee & Canright fossil (Barreda et al 2015; Fig. 3.1). Moreover, we assigned a lognormal distribution with a mean of 90 My (M = 20.0, S = 0.5, Offset = 70.0, mean in real space) to the crown age of Asterales, as the latest Angiosperm-wide whole-plastid DNA phylogenies suggest (Li et al., 2019; Magallón et al., 2015; Fig. 3.1). We then assigned a uniform prior on [0,90] My to the crown ages of *Corokia* and *Argophyllum*.

Biogeographic calibration of the plastid DNA phylogeny

Zealandia became totally separated from Australia about 55 Mya (P. B. Heenan & McGlone, 2019); in order to test whether Argophyllaceae is a Gondwanan relict or if its distribution is better explained by more recent long-distance dispersal, we assigned this age to the crown age of *Argophyllum* in a first approach. We chose a gamma distribution with a vertical hardbound around 50 My and a long tail towards older ages (Alpha = 1.0, Beta = 50.0, Offset = 48.0; Fig. 3.2), changed the priors on the crown age of Asteraceae and Asterales for a uniform distribution on [0,10000] My, and kept the uniform prior on [0,90] My for the crown age of *Corokia*. In a second approach, we used the same priors and parameters except that we assigned the prior on the crown age of *Argophyllum* to *Corokia* as well. This age of 55 My is conservative: because Gondwana and Zealandia started to split about 82 Mya (Wallis & Trewick, 2009), these landmasses may have been biogeographically isolated earlier than 55 Mya.

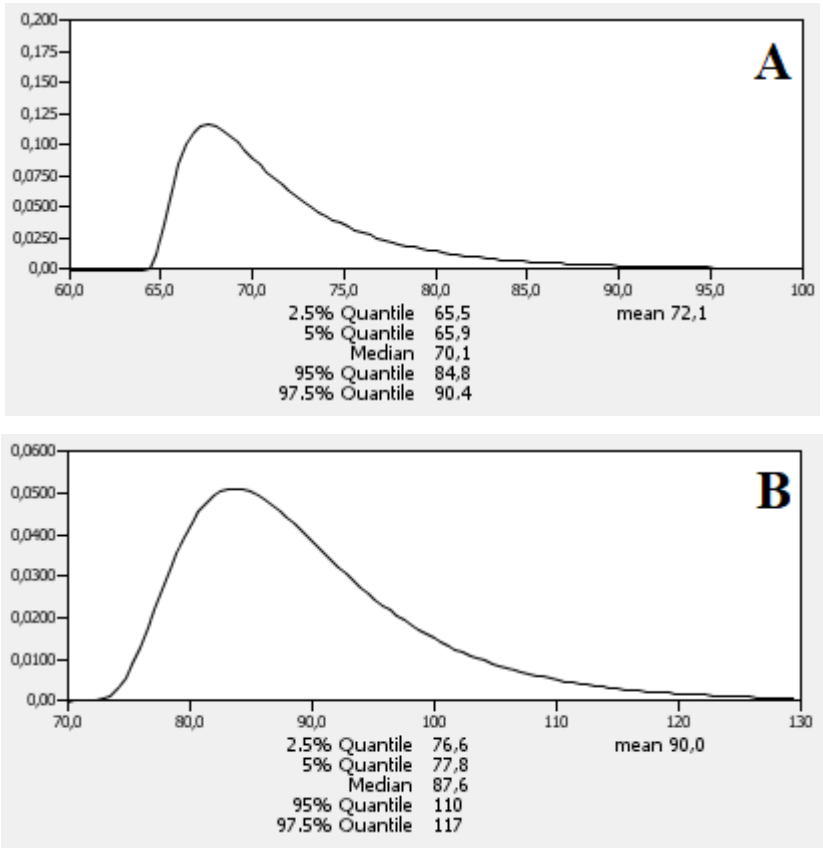


Figure 3.1. *Priors on the crown ages of Asteraceae (A) and Asterales (B) for the fossil-calibrated dating of the plastid DNA phylogeny.*

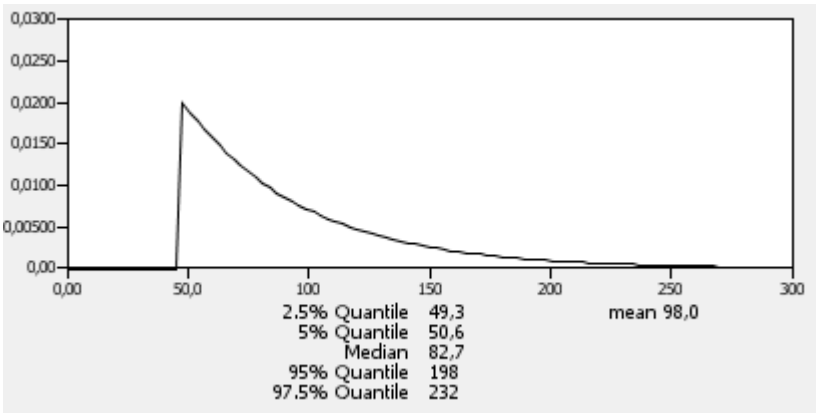


Figure 3.2. *Prior on the crown age of* Argophyllum *for the biogeographic dating of the plastid DNA phylogeny under the first approach. This prior was also assigned to the crown age of* Argophyllum *and to the crown age of* Corokia *under the second approach of the biogeographic dating of the plastid DNA phylogeny.*

This process produced eight trees: six based on plastid DNA with a unique combination of fossil-calibrated dating or biogeographic dating first approach or biogeographic dating second approach, and Birth-Death or Yule model; two based on nuclear markers, one built with the Birth-Death model, the other with the Yule model. Given the differences in sampling plan between plastid DNA and nuclear DNA markers,

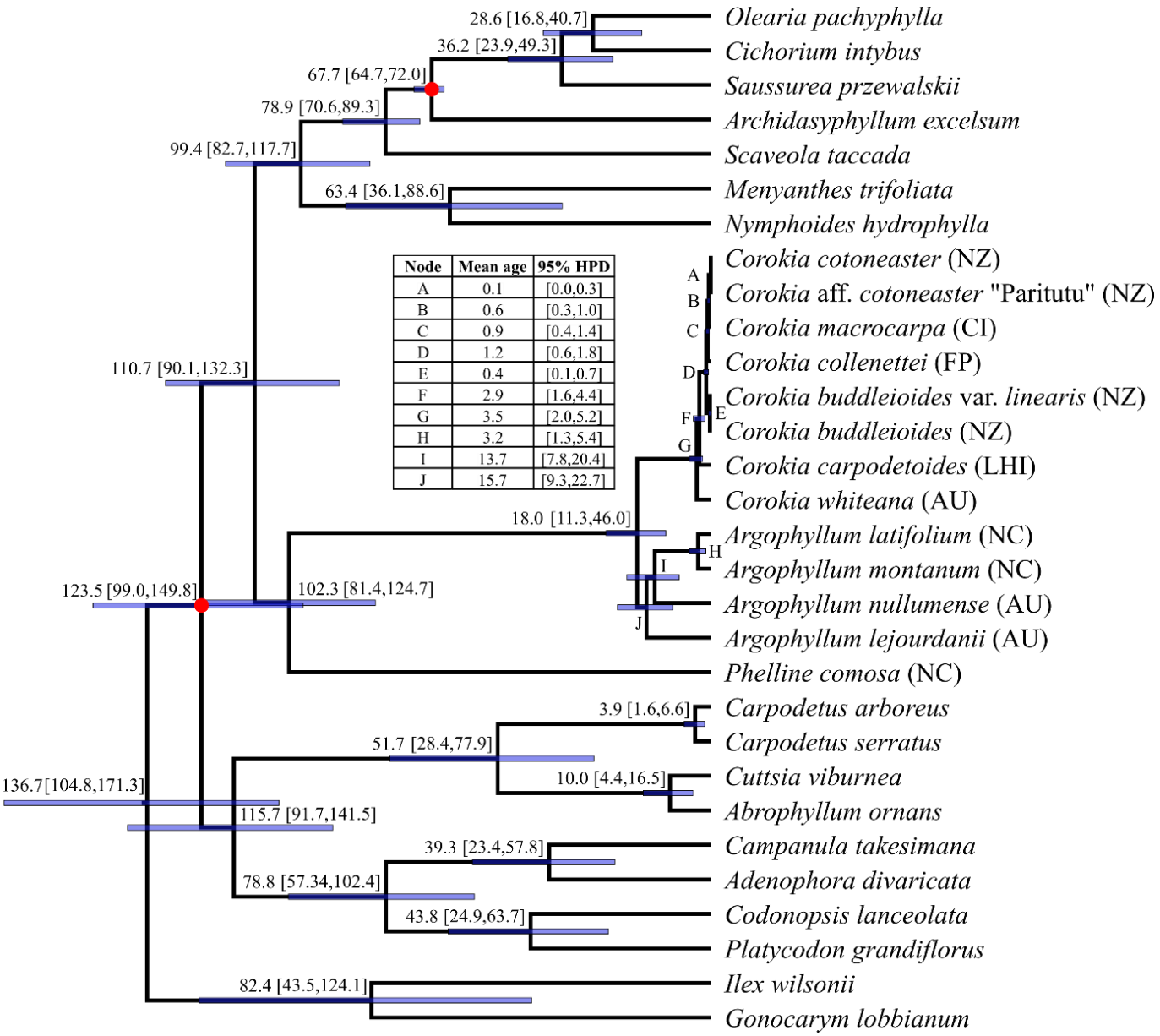
we did not perform an analysis combining the two genomes. Moreover, the amount of plastid DNA sites compared to nuclear DNA sites would have biased the results of a combined analysis towards the phylogeny we observed with the plastid DNA alone, masking potential discrepancies between the evolution of these two different genomes. The detailed settings and parameters used for the phylogenetic analyses are in the XML files provided in Supplementary Material – Supplementary Files 1–8.

3.5 Results

Plastid DNA phylogeny – fossil-calibrated dating

The chains run under the Birth-Death model converged rapidly, and their combination yielded ESS > 200 except for a parameter of limited interest in our study: the mean of the clock rate for the 1st + 2nd codons partition. The chains run under the Yule model converged overall slowly, nonetheless yielding ESS > 200 for all parameters once combined. The phylogenies resulting from both models showed the same topology with similar dates and are strongly supported, all the nodes having a posterior probability (PP) = 1. The 95% high posterior density (HPD) of the ages were relatively wide, especially near the root of the phylogeny. Figure 3.3 shows the tree built under the Birth-Death model, while the tree built under the Yule model is provided in Supplementary Material – Supplementary Figure 1.

Within *Corokia*, the phylogeny places the Australian species *C. whiteana* as sister to the rest of the genus. Within the rest of the genus, the Lorde Howe Island species *C. carpodetoides* is placed as sister to the other species. The taxa *C. cotoneaster* and *C.* aff. *cotoneaster* "Paritutu" are resolved as sister taxa, and so are *C. buddleioides* and *C. buddleioides* var. *linearis*, but these four taxa do not unite to form an endemic New Zealand clade. Instead, *C. cotoneaster* is sister to the Chatham Islands species *C. macrocarpa*. This clade is sister to the French Polynesian species *C. collenettei*, and finally *C. buddleioides* is sister to this latter clade.



Node	Mean age	95% HPD
A	0.1	[0.0,0.3]
B	0.6	[0.3,1.0]
C	0.9	[0.4,1.4]
D	1.2	[0.6,1.8]
E	0.4	[0.1,0.7]
F	2.9	[1.6,4.4]
G	3.5	[2.0,5.2]
H	3.2	[1.3,5.4]
I	13.7	[7.8,20.4]
J	15.7	[9.3,22.7]

Figure 3.3. *Fossil-calibrated dated phylogeny of Argophyllaceae built under the Birth-Death model on plastid DNA markers. Numbers near the nodes are their mean age with highest posterior density in square brackets. The red dots indicate the calibrated nodes. The posterior probabilities of all the nodes are equal to 1. Letters in round brackets indicate areas of native distribution for Phellinaceae and Argophyllaceae: AU = mainland Australia, CI = Chatham Islands, FP = French Polynesia, LHI = Lord Howe Island, NC = New Caledonia, NZ = New Zealand archipelago.*

Plastid DNA phylogeny – biogeographic dating

For both of our biogeographic dating approaches, the chains run under the Birth-Death model and the Yule model converged and their combination yielded ESS > 200 for all parameters. The phylogenies resulting from both models showed the same topology, with wide HPD especially near the root, and all their nodes had a PP = 1. The topology of these trees was identical to the topology of the trees from the fossil-calibrated dating analysis. Figure 3.4 and Figure 3.5 show the trees of the first and second approach respectively, built under the Birth-Death model, while the corresponding trees built under the Yule model are provided in Supplementary Material – Supplementary Figure 2 and 3, respectively.

In the first biogeographic dating approach (Figure 3.4), the estimated ages of deeper nodes are all considerably older than their counterparts in the fossil-calibrated tree, still with wide HPD. For example, the root of the tree (divergence of Aquifoliales and Asterales) has a mean age of 311.4 Myo (HPD: [177.9,470.8] My), while it is ca 136.7 Myo (HPD: [104.8,171.3] My) in the fossil-calibrated tree (Figure 3.3). Inevitably, these ages are even older in our second biogeographic dating approach (Figure 3.5), in which we set the prior on the age of the most recent common ancestor of *Corokia* before the isolation of Zealandia and Australia c. 55 Mya: for example, the age of the root is 966.4 Myo (HPD: [522.2,1473.9] My).

Node	Mean age	95% HPD
A	0.3	[0.0,0.7]
B	1.4	[0.5,2.5]
C	2.0	[0.8,3.4]
D	2.8	[1.3,4.7]
E	0.8	[0.1,1.8]
F	6.8	[3.1,11.2]
G	8.3	[3.8,13.3]
H	8.2	[2.8,14.8]
I	46.5	[35.8,58.3]
J	52.3	[48.0,61.4]

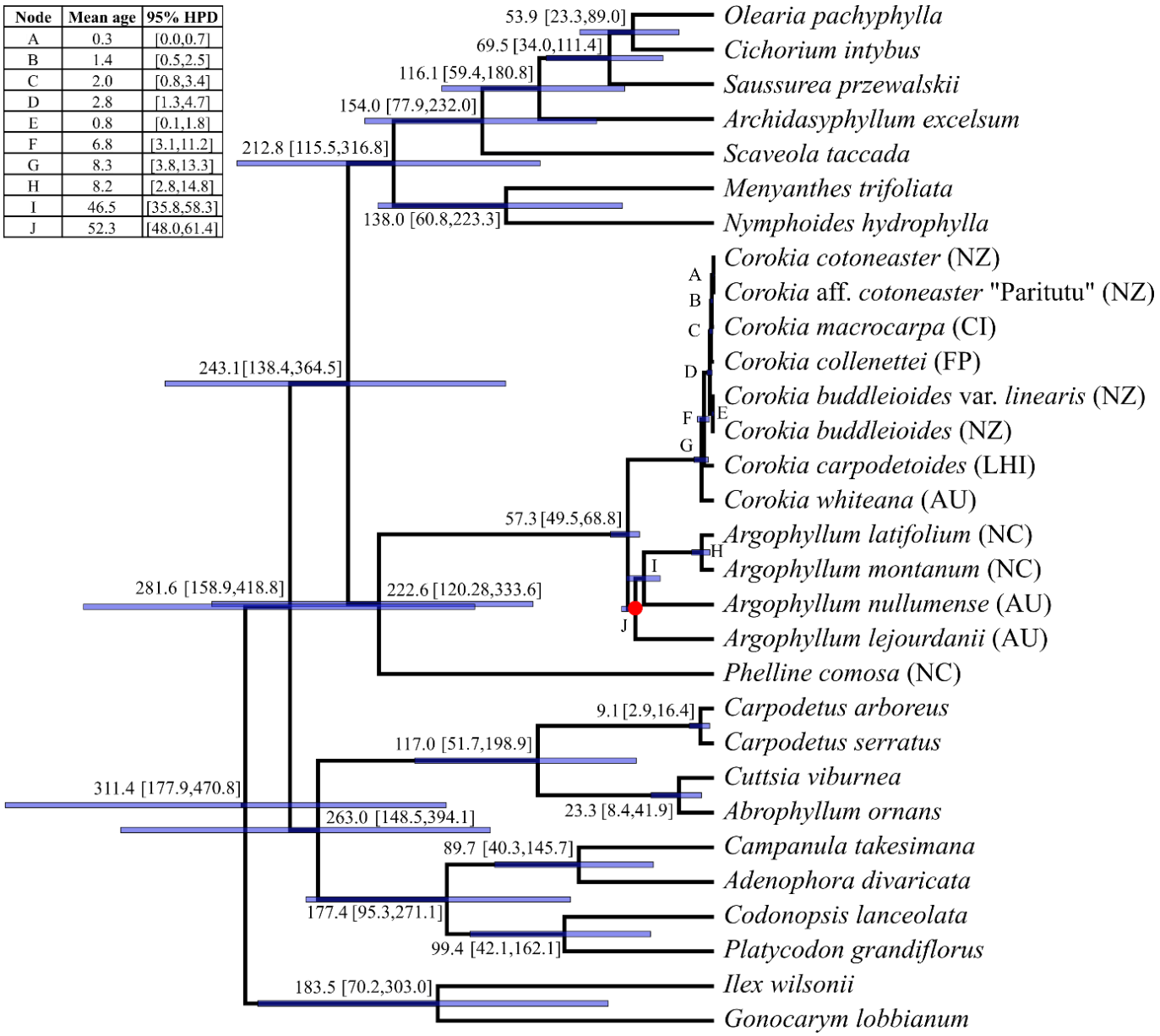


Figure 3.4. *Biogeographically dated phylogeny of Argophyllaceae; first approach, built under the Birth-Death model on plastid DNA markers. Numbers near the nodes are their mean age with highest posterior density in square brackets. The red dot indicates the calibrated node. The posterior probabilities of all the nodes are equal to 1. Letters in round brackets indicate areas of native distribution for Phellinaceae and Argophyllaceae: AU = mainland Australia, CI = Chatham Islands, FP = French Polynesia, LHI = Lord Howe Island, NC = New Caledonia, NZ = New Zealand archipelago.*

Node	Mean age	95% HPD
A	1.1	[0.1,2.5]
B	4.8	[1.5,8.4]
C	6.9	[2.5,11.7]
D	10.0	[4.0,16.9]
E	2.7	[0.4,6.0]
F	39.6	[20.0,53.0]
G	51.2	[48.0,57.8]
H	23.8	[8.3,43.6]
I	97.8	[48.6,151.2]
J	113.1	[62.1,171.6]

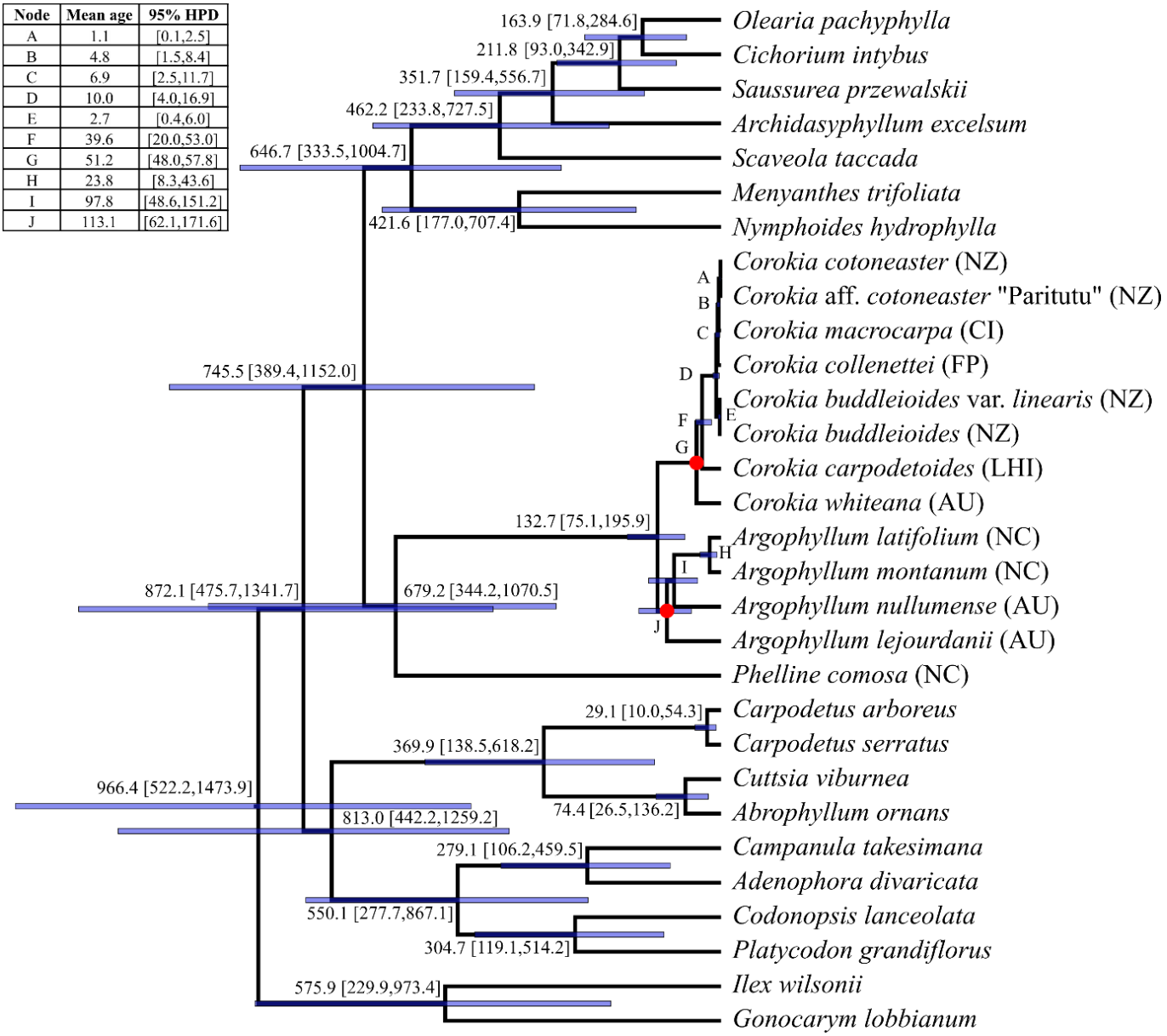


Figure 3.5. *Biogeographically dated phylogeny of Argophyllaceae; second approach, built under the Birth-Death model on plastid DNA markers. Numbers near the nodes are their mean age with highest posterior density in square brackets. The red dots indicate the calibrated nodes. The posterior probabilities of all the nodes are equal to 1. Letters in round brackets indicate areas of native distribution for Phellinaceae and Argophyllaceae: AU = mainland Australia, CI = Chatham Islands, FP = French Polynesia, LHI = Lord Howe Island, NC = New Caledonia, NZ = New Zealand archipelago.*

Nuclear ribosomal DNA phylogeny

Both the chains run with the Birth-Death model or the Yule model converged in spite of short local bumps, and the combined chains of each model gave ESS > 200 for all the parameters. Irrespective of whether the data was run with a Birth-Death model or a Yule model, the resulting trees had the same topology with similar dates, even though the phylogeny resulting from the Yule model is slightly less well supported. Figure 3.6

therefore shows the phylogeny resulting from the Birth-Death model, while the Yule model phylogeny is provided in Supplementary Material – Supplementary Figure 4.

As one might expect from highly conserved sequences, the rRNA alignment showed only two parsimony-informative sites (1.1%) and the ITS1 + ITS2 alignment showed 39 parsimony-informative sites (10.4%; statistics obtained with MEGA X; Kumar et al 2018). None of these sites brought phylogenetic information about the *Corokia* clade sister to *C. whiteana*. *Corokia* is nonetheless shown as a monophyletic group with strong support (posterior probability (PP) = 1 under the Birth-Death model, 0.97 under the Yule model); *C. whiteana* is placed as a sister to the rest of *Corokia*, again with strong support (PP = 0.99 under the Birth-Death model, 0.95 under the Yule model); the two sequences of *C. buddleioides* form a strongly supported clade (PP = 1 under both models); the resolution within the rest of the genus is however unsupported (PP < 0.5 under both models).

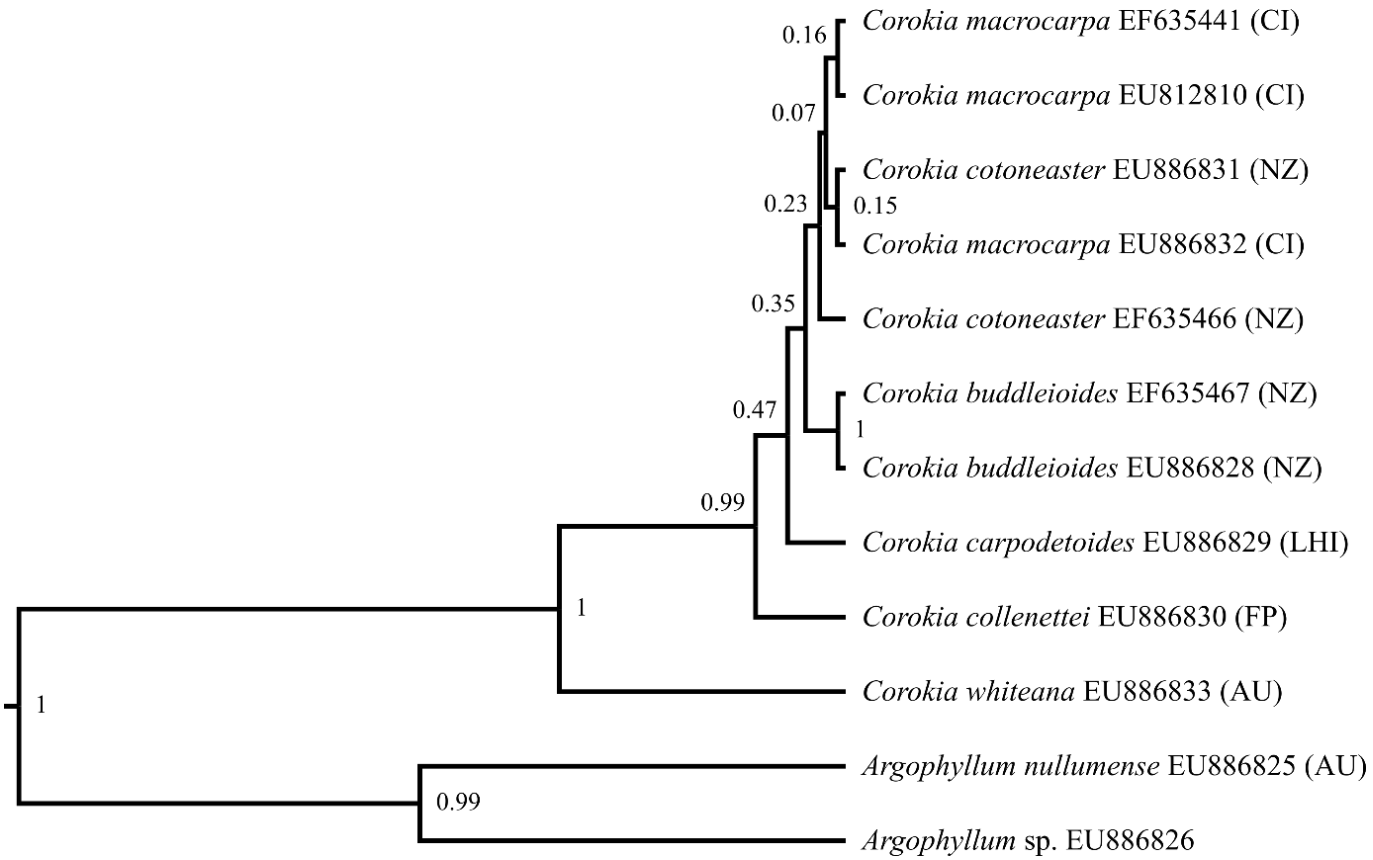


Figure 3.6. *Phylogeny of Argophyllaceae built under the Birth-Death model from nuclear DNA markers. Numbers at the nodes are their posterior probabilities. EF/EU numbers are the GenBank accession numbers corresponding to the sequences. Letters in round brackets indicate areas of native distribution (when known): AU = mainland Australia, CI = Chatham Islands, FP = French Polynesia, LHI = Lord Howe Island, NC = New Caledonia, NZ = New Zealand archipelago.*

3.6 Discussion

The phylogeny of *Corokia* is resolved at the species level in plastid DNA analyses

Our fossil-calibrated phylogeny of Argophyllaceae based on plastid DNA is fully resolved and strongly supported (all PP = 1, Fig. 3.3). As a result, the phylogeny of *Corokia* is resolved at the specific and infra-specific level, placing the Australian species *C. whiteana* as sister to the rest of the genus. At the family level, this phylogeny is congruent with previous work on Asterales, despite the absence of families we could not obtain a sequence for (e.g. Li et al., 2019; Lundberg & Bremer, 2003; Magallón et al., 2015). The clade [Menyanthaceae + [Asteraceae + Goodeniaceae]] is resolved as sister to the clade [Phellinaceae + Argophyllaceae], and the clade [Rousseaceae + Campanulaceae] is sister to the rest of the Asterales. The ages of our nodes however tend to be older than most estimates (e.g. Li et al., 2019; Magallón et al., 2015; Stevens, 2017b), the more so as older nodes are considered: e.g. the crown age of the clade [Rousseaceae + Campanulaceae] was estimated between c. 81 My and c. 76 My, while in our tree it is estimated at 115.7 My (HPD: c. [91.7,141.5] My). This is probably because we used conservative priors, with old mean ages and probability distributions extending well back in time (Figures 3.1–3.2).

The paucity of parsimony-informative sites among the different nrDNA sequences is a sufficient explanation of the poor overall support for relationships within *Corokia* (Figure 3.6). This outcome is not surprising given the results obtained by Hennan et al. (2010), whose ITS phylogenetic network did not resolve relationships among *C. buddleioides*, *C. cotoneaster*, *C. macrocarpa* and *C. collenettei*. Nonetheless, our phylogenies based on plastid DNA markers and nuclear markers are congruent in placing the Australian species *C. whiteana* as sister to the rest of the genus (Figures 3.3–3.6), even though the other relationships within *Corokia* are not supported in the nrDNA phylogeny.

Did the genera of Argophyllaceae diversify prior to the breakup of Gondwana?

The results of our analyses using biogeographic calibrations are inconsistent with a vicariance scenario as an explanation of the distribution of extant Argophyllaceae. Our first biogeographic dating approach placed a minimum age of 55 Mya, the approximate time when Zealandia and Australia became biogeographically isolated, for the divergence between Australian and New Caledonian *Argophyllum* species. Under this scenario, the mean estimate for the crown age of Asterales is 281.6 My, with an HPD of

[158.9,418.8] My (Figure 3.4), corresponding to the interval between the Oxfordian (in the Upper Jurassic) and the Lochkovian (in the Lower Devonian). These ages might be considered reasonable if angiosperm clades are accepted to generally be older than their first appearance in the fossil record, although these estimates far exceed most estimates of the age of the Angiosperms (e.g. Li et al., 2019; Magallón et al., 2015) and any fossils widely accepted to represent Angiosperms. The oldest fossils widely accepted to be of angiospermous plants are no older than c. 140 Myo (Magallón et al. (2015) and references therein), and molecular clock estimations of the age of the most recent common ancestor of extant Angiosperms resulted in dates mostly up to the Permian (reviewed in Magallón et al. (2015)). Under this first scenario, the most recent common ancestor age estimates for extant species of *Corokia* are still considerably younger (HPD: [3.8,13.3] My, Figure 3.4) than the period when Zealandia and Australia became biogeographically isolated, and are therefore inconsistent with vicariance explanations of the distribution of these species. Our second biogeographic dating approach constrained both the most recent common ancestors of extant *Argophyllum* species and extant *Corokia* species to predate the biogeographical isolation of Zealandia from Australia (55 Mya). Under this scenario the crown age of Asterales is estimated to have a mean of 872.1 My, with an HPD of [475.7,1341.7] My (Figure 3.5), corresponding to the period Lower Ordovician–Precambrian. Since this age predates the evolution not just of Angiosperms, but of vascular plants in general, it is inconsistent with vicariance as an explanation of the distribution of extant species of *Corokia* and *Argophyllum*.

Biogeographic history of Argophyllaceae based on plastid DNA

Given the long period of time since the divergence of Argophyllaceae and the New Caledonian family Phellinaceae, it is difficult to draw inferences about the distribution of their most recent common ancestor. Both Phellinaceae and *Argophyllum* are known from the Miocene fossil record of New Zealand (Bean & Forster, 2018; Pole, 2010), but have become extinct in New Zealand since then. The small number of species in *Corokia* and their high level of endemicity, as well as the small number of *Argophyllum* species we included in our analyses, do not allow the identification of precise biogeographic scenarios within Argophyllaceae. However, looking at our plastid DNA phylogeny we can suggest several dispersal scenarios that try to minimise the number of dispersal events:

(1) Ancestral area of the family in New Caledonia. Dispersal of Argophyllum and independently of Corokia to Australia. Then, extinction of Corokia in New

Caledonia, dispersal of Corokia to Lord Howe Island, New Zealand and subsequently to French Polynesia and the Chatham Islands. This scenario implies six dispersal and one extinction events.

(2) Ancestral area of the family in Australia. Dispersal of Argophyllum to New Caledonia, dispersal of Corokia to Lord Howe Island, New Zealand and subsequently to French Polynesia and the Chatham Islands. This scenario implies five dispersal events.

(3) Ancestral area of the family in Australia. Dispersal of Argophyllum to New Caledonia, dispersal of Corokia to Lord Howe Island and separately to New Zealand, French Polynesia and the Chatham Islands. This scenario implies five dispersal events.

(4) Ancestral area of the family in New Zealand. Dispersal of Argophyllum to New Caledonia and Australia, and extinction of Argophyllum from New Zealand. Dispersal of Corokia to Lord Howe Island, Australia, the Chatham Islands and French Polynesia. This scenario implies six dispersal and one extinction events, but the extinction of *Argophyllum* from New Zealand is indicated by the presence of a Miocene fossil so does not require an ad hoc hypothesis.

An Australian origin of *Corokia* and *Argophyllum* is biogeographically parsimonious, as reflected by the sister relationship of Australian species to the others in both genera (*C. whiteana* for *Corokia*, *A. lejourdanii* and *A. nullumense* for *Argophyllum*). New Zealand experienced a gradual submergence beginning in the Upper Cretaceous and peaking c. 25-23 Mya (Cooper, 1989; Landis et al., 2008), at which point the land mass is estimated to have been reduced to about 18% of its present-day land area (Cooper & Cooper, 1995); in parallel, New Caledonia experienced periods of complete submergence during the Paleocene and Eocene, only to resurface c. 37 Mya (Grandcolas et al., 2008). These events make New Zealand or New Caledonian origins of both genera less likely than an Australian origin, if our relatively recent fossil-calibrated date estimates are accepted. Besides being more parsimonious than scenarios 1 and 4, scenarios 2 and 3 are therefore the most likely of the scenarios we propose. Between these two scenarios, 2 could be even more likely than 3 if we consider shorter-distance dispersal more likely than dispersal between more distant landmasses. Finally, the fossil-calibrated phylogeny suggests that the diversification of *Corokia* began no earlier than Pliocene, since the divergences between the species are concentrated within the last 5.2 My (the lower bound of the HPD of the most recent common ancestor of extant *Corokia* species).

3.7 Conclusions

Our plastid DNA phylogeny was strongly supported and fully resolved, offering the first dated phylogeny of all six species of *Corokia* resolved at the specific level. Although the reconstruction of precise biogeographic scenarios within Argophyllaceae is hampered by the small number of species in *Corokia* and their high level of endemicity, as well as the small number of *Argophyllum* species that we considered, our results suggest an Australian origin of both genera. Moreover, our results from fossil-calibrated dating analyses suggest that the extant lineages of *Corokia* diversified no earlier than the early Pliocene, and the extant species of *Argophyllum* diversified no earlier than the lower Miocene – although in the latter case more species would need to be included in a future dated phylogeny to make a stronger inference. These periods of radiation are inconsistent with either groups having diversified prior to the break-up of Gondwana. This conclusion is supported by our results from biogeographically calibrated dating analyses on the plastid DNA phylogeny, as they show that if a vicariant scenario from a Gondwanan origin might be plausible for *Argophyllum* if angiosperm clades are much older than their first appearance in the fossil record (with Asterales indicated to have a most recent common ancestor in the late Carboniferous), it is implausible for *Corokia* (with Asterales indicated to have a most recent common ancestor in the Precambrian/early Ordovician, prior to any fossil evidence of land plants). Finally, our phylogeny of all *Corokia* species based on the nuclear 18S–26S nrDNA repeat region is congruent with the plastid DNA phylogeny in placing the Australian species *C. whiteana* as sister to the rest of the genus, but it is otherwise poorly resolved.

3.8 Acknowledgements

The authors would like to thank Chris Lusk and two anonymous reviewers for commenting on the draft manuscript; the Direction de l'Environnement of the Province Sud of New Caledonia for local sampling permits and David Bruy, Vanessa Hequet, Sandrine Isnard and Hervé Vandrot for their help in preparing and conducting the sampling there; the Department of Conservation, Otari Native Garden, Ngāti Rangi and Mōkai Pātea for the sampling permits and agreements in New Zealand; Patricio Saldivia Perez for DNA samples; CHR, NOU, CANB, MEL, HO and P for leaf samples; Mary Korver (CHR) for assistance with herbarium loans.

3.9 Disclosure statement

No potential conflict of interest was reported by the author(s).

3.10 Funding

This research is supported by the Royal Society of New Zealand (Te Apārangi) [Marsden contract 16-UOW-029]; and the Faculty of Science and Engineering of the University of Waikato through FSEN Student Trust Fund [grant number # P102218 SoS/PG Support].

3.11 Supplementary material

All supplementary files are available from the online version of the article: https://www.tandfonline.com/doi/suppl/10.1080/0028825X.2021.1905671

Chapter 4 Dating the emergence of the divaricate habit in New Zealand

4.1 Preliminary note

This chapter, co-authored with Chris Lusk and Rob Smissen, is being prepared for submission to a high-impact international journal. Its topic therefore had to be broadened towards cage architectures in general—the divaricate habit being the New Zealand example of this phenomenon—to better appeal to a wider readership. The general style of writing followed the guidelines and customs for publication in such journals. The additional information that was necessary to make this chapter better stand on its own was extracted from the supplementary files: Supplementary File 1 (sampling plan) became Appendix 4.1, Supplementary File 2 (calibration strategy) became Appendix 4.2, and Supplementary Files 3 to 5 (resulting phylogenies) are substituted for a summarised and more reader-friendly phylogeny in the chapter. The fully detailed trees of Supplementary Files 3 to 5, as well as Supplementary Files 6 to 9 (files for the phylogenetic analyses and the Rubiaceae sequences) will be made available with the published article only.

4.2 Abstract

Plants have evolved various physical defenses against herbivores, including densely-branched cage architectures with small widely-separated leaves. The convergent evolution of "divaricate" cage architectures in many New Zealand plant lineages was initially interpreted as a response to cold, dry Pliocene-Pleistocene climates, consistent with their current abundance on frosty and droughty sites. Recent experiments have shown the divaricate form deters extant browsers, supporting an alternative interpretation as a response to now-extinct avian browsers whose ancestors arrived during the Paleogene. We present a dated phylogeny based on 45 chloroplast genes from 215 eudicots, showing that the great majority of extant divaricate plants diverged from non-divaricate sisters within the last 5 My, implicating Pliocene-Pleistocene climates in the proliferation of cage architectures in New Zealand. This may indicate that cage architectures were favored

as an anti-browsing defense only when Pliocene-Pleistocene climatic constraints prevented young trees and shrubs from quickly growing out of reach of ground-dwelling herbivores. This conclusion is consistent with the abundance of cage architectures in other parts of the world where plant growth is restricted by climatic constraints like aridity or short frost-free periods.

4.3 Introduction

Herbivory is a major driver of plant diversification (Ehrlich & Raven, 1964; Maron et al., 2019), selecting for a wide array of chemical and physical defences (Hanley et al., 2007; Koricheva et al., 2004). Although spinescence is the best known type of structural defence against vertebrate browsers (e.g. Charles-Dominique et al., 2016), densely branched ”cage” architectures with small widely separated leaves have also been shown to deter browsing (Charles-Dominique et al., 2017). Cage architectures are unusually common in New Zealand, occurring there in as many as 80 eudicot and one gymnosperm species from 20 families, representing about 13% of the indigenous woody flora (Maurin & Lusk, 2021). Cage architectures in other regions are often accompanied by spinescence (e.g. Cavagnaro & Golluscio, 2017; Charles-Dominique et al., 2017), but most New Zealand cage-like plants are remarkably non-spinescent, and have historically been termed “divaricate” or “divaricating” plants (Greenwood & Atkinson, 1977).

The putative selective forces driving this celebrated case of convergent evolution in the New Zealand flora have been the subject of intense debate. Early botanists, noting its prominence in the rain shadow of New Zealand’s Southern Alps, interpreted the divaricate form as an adaptation to dry, cold climates that arose during the Pliocene-Pleistocene (Cockayne, 1912; Diels, 1896), a hypothesis developed further by McGlone & Webb (1981). Greenwood & Atkinson (1977) proposed an alternative hypothesis, that the divaricate form arose as a defence against now-extinct avian browsers, the moa. Although experiments have since confirmed that the divaricate form can deter browsing by both avian and ungulate herbivores (Bond et al., 2004; Pollock et al., 2007), there is also experimental evidence that the small leaves of divaricate plants help them cope with frost (Lusk et al., 2018). The climatic and moa-browsing hypotheses are not mutually exclusive (A. Cooper et al., 1993), and a recent synthetic hypothesis proposes that the divaricate habit did not become advantageous as an anti-browsing defence until climatic

adversity restricted growth rates of young trees and shrubs, preventing them from growing quickly out of reach of ground-dwelling herbivores (Lusk et al., 2016).

Cooper et al. (1993) suggested phylogenetic dating of divergences between New Zealand divaricates and their non-divaricate relatives to help test hypotheses. Fossils date moa presence in New Zealand to at least 16-19 My ago (Tennyson et al., 2010), and genetic evidence suggests their volant ancestors arrived c. 60 My (Phillips et al., 2010); thus, under the moa-browsing hypothesis, one might expect the divaricate form to date at least as far back as the early Miocene. The cold, dry climates purported to have favoured selection for the divaricate form under the climate hypothesis arose from the Pliocene-Pleistocene combination of global cooling (Hornibrook, 1992) and uplift of the Southern Alps (Batt et al., 2000); thus, under both the climate hypothesis and the synthetic browsing-climate hypothesis, one might expect the divaricate form to be at most c. 5 My old. Dated phylogenies including divaricate species indicate ages from < 1 Ma to > 20 Ma for such divergences (reviewed in Appendix 1.3), but these studies did not necessarily sample the most closely related pairs of extant non-divaricate and divaricate species, since it was not their objective; the resulting phylogenies may therefore overestimate some divergence dates.

Here, we present the first study with the objective of dating the origin of the divaricate habit in extant lineages of the New Zealand flora. We propose a dated phylogeny of 215 species, with an extensive sampling of New Zealand divaricates (91%). It is based on DNA sequences of protein-coding chloroplast genes extracted from complete or near-complete plastid genomes, built under a maximum likelihood framework and calibrated using ten fossils and one secondary calibration.

4.4 Methods

Sampling plan

We sampled 215 eudicot species. This included 73 divaricate species, representing 91% of the list of eudicot divaricate species of New Zealand compiled in Maurin & Lusk (2021). We managed to include 100% of the divaricate species from 21 out of the 23 eudicot genera with divaricate representatives listed in Maurin & Lusk, (2021); 82% of divaricate *Coprosma* species were included (Table 4.1). We aimed at including the most

closely related non-divaricate species as possible to each divaricate species we considered based on morphological characters and previous phylogenetic studies. 190 of the sequences we used were newly generated for this study from herbarium specimens, while the others were sourced from GenBank. Appendix 4.1 presents the sampling plan with the relevant information regarding the specimens.

DNA extraction

The DNA of the samples was extracted following one of two methods:

(1) using a CTAB-based protocol (Doyle & Dickson, 1987) modified as in Smissen & Heenan (2007) to include a phenol:chloroform extraction and recovery using spin columns (Zymo IIC, Zymo Research, Orange County, California).

(2) following the DNA tissue protocol of the Maxwell 16 instrument (Promega, Madison, Wisconsin) and further purified by phenol/chloroform extraction and recovery in spin columns.

Detailed step-by-step protocols are available upon request. The DNA concentration of the extracts was measured using the Qubit (Thermo Fisher Scientific, Waltham, Massachusetts) dsDNA high-sensitivity assay protocol.

Library preparation and sequencing

Genomic DNA libraries were prepared following one of two methods:

(1) using Illumina Nextera DNA Library Prep kits, following the manufacturer's instructions (Reference Guide, #15027987 v01, January 2016) except that we halved the quantities of reagents and the target amount of input DNA.

(2) using Illumina TruSeq Nano DNA Library Prep kits, according to the manufacturer's instructions (Reference Guide, # 15041110 Rev. D, June 2015) again using halved reagent quantities and target input DNA; genomic DNA was fragmented using a Covaris ME220 Focused-ultrasonicator (settings: 75 s duration - 40 W peak power - 25% duty factor - 50 cycles per burst).

The concentration and size range of libraries was measured with a LabChip GX Touch HT (Perkin Elmer). Libraries were enriched for chloroplast DNA using a custom MYBaits kit (Arbor Biosciences, Ann Arbor) modified from Stull et al. (2013) as detailed in Smissen et al. (unpubl.) using the manufacturer's instructions (version 3.02, July 2016 or version 4.01, April 2018). Illumina HiSeq sequencing was carried out by Otago Genomics Facility (Dunedin, New Zealand) using paired end 2 × 125 bp reads.

Chloroplast DNA assembly and annotation

Reads were first trimmed using Trimmomatic v. 0.38 (Bolger et al., 2014) with the following settings: ILLUMINACLIP:[path/to/NexteraPE-PE.fa or TruSeq3-PE-2.fa according to the library preparation method]:1:30:10 SLIDINGWINDOW:10:20 MINLEN:40. For each family we included, a complete chloroplast genome as closely related as possible to our samples was selected from GenBank as a reference against which to map the reads of samples from that family. Then, in each family, the best-quality (i.e. best compromise between highest HQ%, lowest percentage of ambiguous bases and highest coverage) resulting consensus sequence was selected and its reads mapped against itself to create an assembly of improved quality. Finally, the reads of the other members of the family were mapped against this new improved consensus. Mappings were performed with BWA, using the BWA-MEM algorithm (Li, 2013).

Sequence annotation was carried out as follows: (1) the improved reference of each family was aligned to the corresponding GenBank reference used to map its reads against with the MAFFT algorithm v. 7.388 (Katoh et al., 2002; Katoh & Standley 2013) plugin in Geneious Prime 2019.2.1, (2) the annotations of the GenBank references were transferred to the improved references and manually checked, and (3) the other sequences of each family were aligned to their corresponding improved references, again with MAFFT within Geneious Prime, and annotations transferred across and manually checked.

After making test phylogenies of the resulting sequences, unexpected relationships within the genus *Coprosma* led us to investigate these sequences more deeply. It appeared that a non-negligible proportion of the ambiguous bases found in their protein-coding sequences was caused by pseudogene sequences, in that they contained frame-shift mutations and substitutions leading to inferred stop codons. Non-functional nuclear or mitochondrial genes resulting from gene transfer from the plastid genome are both well documented (e.g. Cummings et al., 2003; Goremykin et al., 2009; G.-J. Zhang et al., 2020), although we are not aware of other reports of them impacting assemblies of massively parallel sequence data. Because mitochondrial genomes occur in plant cells at much higher copy number than nuclear genomes, they are likely to be the source of the pseudogenes in our *Coprosma* data, but proliferation of a plastid derived sequence or sequences within the nuclear genome cannot be excluded. To minimise this issue, we mapped these reads against the GenBank sequence of *Anthospermum spathulatum* (accession #KY378687), a chloroplast sequence missing one copy of the inverted repeat

region, with custom mapping parameters in Geneious Prime (available upon request to the authors).

Data partitioning

We used 45 protein-coding sequences from the long and short single copy regions. They were aligned in Geneious Prime using the MAFFT plugin, and the alignment manually checked. We partitioned this alignment into 1^{st} + 2^{nd} codon position on the one hand and 3^{rd} codon position on the other hand. Sites containing at least one unresolved base were removed prior to conducting the phylogenetic analyses described below, resulting in a final alignment of 31,248 sites.

Reconstruction and dating of the phylogeny

We reconstructed the phylogeny of our samples with RAxML v. 8.2.12 (Stamatakis, 2014), run on CIPRES (Miller et al., 2010), following the guide by Maurin (2020b). The search for the best ML tree was conducted with the following settings: 10 random alternative starting trees, 1,000 bootstrap (BS) replicates, GTRGAMMA model, and fixing the sequence of *Ranunculus sceleratus* (Ranunculales) as an outgroup to the rest of the Eudicots. From this best ML tree, we generated 1,000 BS replicates to produce a dated phylogeny with 95% CI (confidence interval on the age) at the nodes using treePL (Smith & O'Meara, 2012), again following the guide by Maurin (2020b). To calibrate the tree, we used ten fossils for internal nodes and one secondary calibration for the root of the tree. These calibrations are explained in Appendix 4.2.

This treePL phylogeny was compared to a phylogeny built under Bayesian inference. We used BEAST 2.6.2 (Bouckaert et al., 2019) with the following settings: Birth-Death model (Gernhard, 2008), bModelTest (Bouckaert & Drummond, 2017) and relaxed clock with rates drawn from a lognormal distribution (Drummond et al., 2006) for each partition. We combined the results of three chains started from different seeds, which we sampled once every 50k generations and ran until their combination resulted in effective sampling size > 200 for all parameters. We calibrated the same nodes as for the treePL-built phylogeny, and provide details about the calibration strategy in Supplementary File 2.

Finally, the resulting trees were first formatted in FigTree v. 1.4.4 (Rambaut, 2018) then refined in Inkscape v. 0.92.3. The treePL and BEAST2 files used for the phylogenetic analyses are provided as Supplementary Files 6 and 7 respectively.

4.5 Results

Congruence between the treePL and the BEAST2 phylogenies

The Bayesian inference and maximum likelihood approaches produced virtually the same result. The treePL and BEAST2 phylogenies (Supplementary Files 3 and 4 respectively) were almost identical in topology, the differences being within genera with poorly supported relationships among species (e.g. *Pittosporum*, *Olearia*) and that the BEAST2 phylogeny places Gunnerales as sister to Ranunculales instead of sister to the Pentapetalae (albeit with poor support: posterior probability = 0.82). These phylogenies were also very similar in dates, the differences in age estimates are of little significance. In particular, both these phylogenies lead to the same conclusions regarding our research question. Because the three BEAST2 chains had not produced satisfactory effective sampling sizes (> 200) for all their parameters individually even though they had converged towards the same solution, we hereafter discuss our results and draw our conclusions by examining the treePL phylogeny.

Congruence between our treePL phylogeny and prior knowledge

The dated phylogeny we built with treePL is robust to the choice of parameters. Even though this phylogeny was built using the best optimisation and smoothing parameters suggested by treePL, we tested other sensible values: they resulted in trees that had the same dates as the tree built from the best parameters. The differences in values (10^{-1}-10^{0} order of magnitude) are non-significant four our purpose, and can at least partly be attributed to the small degree of stochasticity of the treePL dating process (Maurin, 2020b).

The topology of our treePL phylogeny from the root to the crown of the families is consistent with modern ideas of the relationships about the corresponding clades (e.g. Stevens, 2017a; The Angiosperm Phylogeny Group, 2016; Fig. 4.1). This backbone has a BS support ≥ 94%, with all nodes but two having BS support of 100%. Furthermore, the great majority of genera are monophyletic with BS support of 100%, the exceptions being a few genera of doubtful or contentious monophyly, as suggested by previous studies of said groups (e.g. *Coprosma* (Cantley et al., 2016) and *Teucrium* (Salmaki et al., 2016)).

The ages of the orders and higher taxonomic ranks are largely consistent with previous knowledge (e.g. Stevens, 2017a; Fig. 4.1). Similarly, our age estimates of family and genus crowns are consistent with previous studies with comparable taxon sampling,

although there is one notable difference: the split between *Coprosma moorei* and the clade composed of *Nertera* and the rest of *Coprosma*. Our date is significantly younger than Cantley et al. (2016), most likely because their calibration was conservatively old but also perhaps because of our much increased sampling of nucleotides.

Figure 4.1 (on the next page). *Dated phylogeny of our 215 species, built with treePL from RAxML-generated trees. Names of families, orders and selected higher taxonomic ranks are indicated at the crown node of said ranks. Blue names indicate divaricate species. Bootstrap support values < 95 are indicated. Some genera are collapsed at their crown node because of overall poorly supported relationships within: the number of non-divaricate (ND) and divaricate (D) species they contain are indicated. Purple horizontal bars represent the 95% confidence interval on node ages. Green dots indicate calibrations, the letter referring to the corresponding letter in Appendix 4.2. Blue dots indicate nodes that we conservatively chose as the earliest divergence between divaricate and non-divaricate species or clades for each genus with divaricate representatives (cf. Table 1); a red vertical bar is added to indicate 5 My.*

Divergence time between divaricate species and their closest non-divaricate relatives

The older CI bound of the age of the > 90% BS-supported MRCA of a divaricate species (or clade) and its closest non-divaricate sister is ≤ 5 My in all but three cases (Fig. 4.1, summarised in Table 4.1). Because our discussion about the evolution of divaricate habit in New Zealand is centred around whether or not the divaricate habit proliferated within the last c. 5 Mya, we considered the older CI bounds of these ages instead of the more natural mean ages, to ensure a conservative treatment of the question. The only three cases for which older dates are suggested are *Teucrium*, *Neomyrtus* and *Rhabdothamnus*.

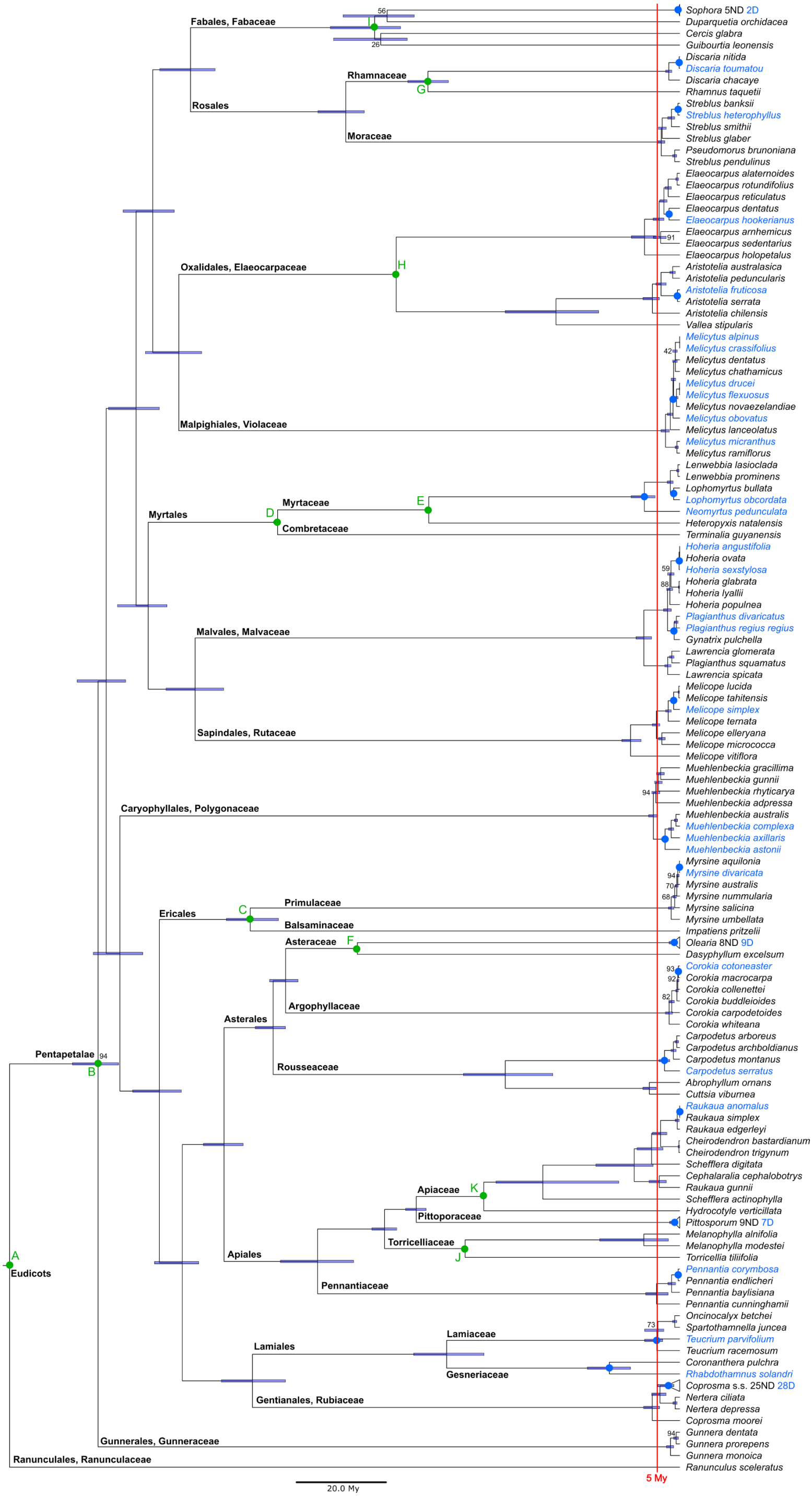

Sophora 5ND 2D
Duparquetia orchidacea
Cercis glabra
Guibourtia leonensis
Discaria nitida
Discaria toumatou
Discaria chacaye
Rhamnus taquetii
Streblus banksii
Streblus heterophyllus
Streblus smithii
Streblus glaber
Pseudomorus brunoniana
Streblus pendulinus
Elaeocarpus alaternoides
Elaeocarpus rotundifolius
Elaeocarpus reticulatus
Elaeocarpus dentatus
Elaeocarpus hookerianus
Elaeocarpus arnhemicus
Elaeocarpus sedentarius
Elaeocarpus holopetalus
Aristotelia australasica
Aristotelia peduncularis
Aristotelia fruticosa
Aristotelia serrata
Aristotelia chilensis
Vallea stipularis
Melicytus alpinus
Melicytus crassifolius
Melicytus dentatus
Melicytus chathamicus
Melicytus drucei
Melicytus flexuosus
Melicytus novaezelandiae
Melicytus obovatus
Melicytus lanceolatus
Melicytus micranthus
Melicytus ramiflorus
Lenwebbia lasioclada
Lenwebbia prominens
Lophomyrtus bullata
Lophomyrtus obcordata
Neomyrtus pedunculata
Heteropyxis natalensis
Terminalia guyanensis
Hoheria angustifolia
Hoheria ovata
Hoheria sexstylosa
Hoheria glabrata
Hoheria lyallii
Hoheria populnea
Plagianthus divaricatus
Plagianthus regius regius
Gynatrix pulchella
Lawrencia glomerata
Plagianthus squamatus
Lawrencia spicata
Melicope lucida
Melicope tahitensis
Melicope simplex
Melicope ternata
Melicope elleryana
Melicope micrococca
Melicope vitiflora
Muehlenbeckia gracillima
Muehlenbeckia gunnii
Muehlenbeckia rhyticarya
Muehlenbeckia adpressa
Muehlenbeckia australis
Muehlenbeckia complexa
Muehlenbeckia axillaris
Muehlenbeckia astonii
Myrsine aquilonia
Myrsine divaricata
Myrsine australis
Myrsine nummularia
Myrsine salicina
Myrsine umbellata
Impatiens pritzelii
Olearia 8ND 9D
Dasyphyllum excelsum
Corokia cotoneaster
Corokia macrocarpa
Corokia collenettei
Corokia buddleioides
Corokia carpodetoides
Corokia whiteana
Carpodetus arboreus
Carpodetus archboldianus
Carpodetus montanus
Carpodetus serratus
Abrophyllum ornans
Cuttsia viburnea
Raukaua anomalus
Raukaua simplex
Raukaua edgerleyi
Cheirodendron bastardianum
Cheirodendron trigynum
Schefflera digitata
Cephalaralia cephalobotrys
Raukaua gunnii
Schefflera actinophylla
Hydrocotyle verticillata
Pittosporum 9ND 7D
Melanophylla alnifolia
Melanophylla modestei
Torricellia tiliifolia
Pennantia corymbosa
Pennantia endlicheri
Pennantia baylisiana
Pennantia cunninghamii
Oncinocalyx betchei
Spartothamnella juncea
Teucrium parvifolium
Teucrium racemosum
Coronanthera pulchra
Rhabdothamnus solandri
Coprosma s.s. 25ND 28D
Nertera ciliata
Nertera depressa
Coprosma moorei
Gunnera dentata
Gunnera prorepens
Gunnera monoica
Ranunculus sceleratus
Fabales, Fabaceae
Rosales
Rhamnaceae
Moraceae
Oxalidales, Elaeocarpaceae
Malpighiales, Violaceae
Myrtales
Myrtaceae
Combretaceae
Malvales, Malvaceae
Sapindales, Rutaceae
Caryophyllales, Polygonaceae
Ericales
Primulaceae
Balsaminaceae
Asteraceae
Argophyllaceae
Asterales
Rousseaceae
Pentapetalae
Apiaceae
Pittoporaceae
Torricelliaceae
Apiales
Pennantiaceae
Lamiaceae
Lamiales
Gesneriaceae
Gentianales, Rubiaceae
Gunnerales, Gunneraceae
Ranunculales, Ranunculaceae
Eudicots
A
B
C
D
E
F
G
H
J
K
56
26
91
42
59
88
94
70
68
93
92
82
94
73
94
5 My
20.0 My

Table 4.1. *Older confidence interval (CI) bound of the age of the most recent common ancestor (MRCA) of divaricate species or clade and their non-divaricate sister for each genus represented in our phylogeny, ordered by increasing age. Also provided is the proportion of divaricate species of each genus (according to the list in Appendix 1.1) that we sampled.*

Genus	**Proportion of divaricate species sampled**	**Older CI bound of divaricate/ non-divaricate MRCA (My)**
Raukaua	1/1 (100%)	0.0
Myrsine	1/1 (100%)	0.1
Discaria	1/1 (100%)	0.2
Hoheria	2/2 (100%)	0.3
Corokia	1/1 (100%)	0.5
Pennantia	1/1 (100%)	0.6
Sophora	2/2 (100%)	0.6
Streblus	1/1 (100%)	0.7
Aristotelia	1/1 (100%)	0.9
Lophomyrtus	1/1 (100%)	1.8
Melicope	1/1 (100%)	1.8
Plagianthus	2/2 (100%)	1.8
Melicytus	6/6 (100%)	2.0
Pittosporum	7/7 (100%)	2.2
Olearia	9/9 (100%)	2.4
Elaeocarpus	1/1 (100%)	3.1
Muehlenbeckia	3/3 (100%)	4.1
Carpodetus	1/1 (100%)	4.6
Coprosma	28/34 (82%)	4.9
Teucrium	1/1 (100%)	7.8
Neomyrtus	1/1 (100%)	10.9
Rhabdothamnus	1/1 (100%)	20.6

4.6 Discussion

The maximum divergence dates we obtained indicate a proliferation of the divaricate habit in New Zealand within the last 5 My (Table 4.1; Figs. 4.1 and 4.2). The only three species associated with divergences older than 5 Mya (*Teucrium parvifolium*, *Neomyrtus pedunculata*, *Rhabdothamnus solandri*) have only weakly developed cage architectures, and were described by Greenwood & Atkinson (1977) as "semi-divaricate". In contrast, divergences that gave rise to highly developed cage architectures occurred exclusively after this date. *Neomyrtus* and *Rhabdothamnus* are both monospecific; even if we sampled their closest extant non-divaricate relatives, it is likely that extinctions of closer non-divaricate relatives have occurred. In the case of *Neomyrtus*, phylogenies of nuclear internal transcribed spacer sequences suggest that the monospecific and New-Caledonian endemic genus *Myrtastrum*, for which limited chloroplast DNA sequence is available, is a closer relative than any taxa we sampled (Smissen, unpublished: see GenBank HQ225439 and KM064787). The difference in the case of *Teucrium* may not be significant: the placement of *T. parvifolium* is only supported by a BS value of 73 (Fig. 4.1); a better resolved and more thoroughly sampled phylogeny based on similar genetic markers and dating approach to ours may find a younger MRCA of *T. parvifolium* and a non-divaricate relative.

This Pliocene-Pleistocene concentration of divergences implicates climatic adversity as at least partly responsible for the evolution of the divaricate form in many New Zealand lineages. The combined effects of the rise of the Southern Alps (Batt et al., 2000) and global cooling (Hornibrook, 1992) created new frosty and droughty environments in New Zealand during the last 5 My, especially in the eastern South Island (Fig. 4.2).

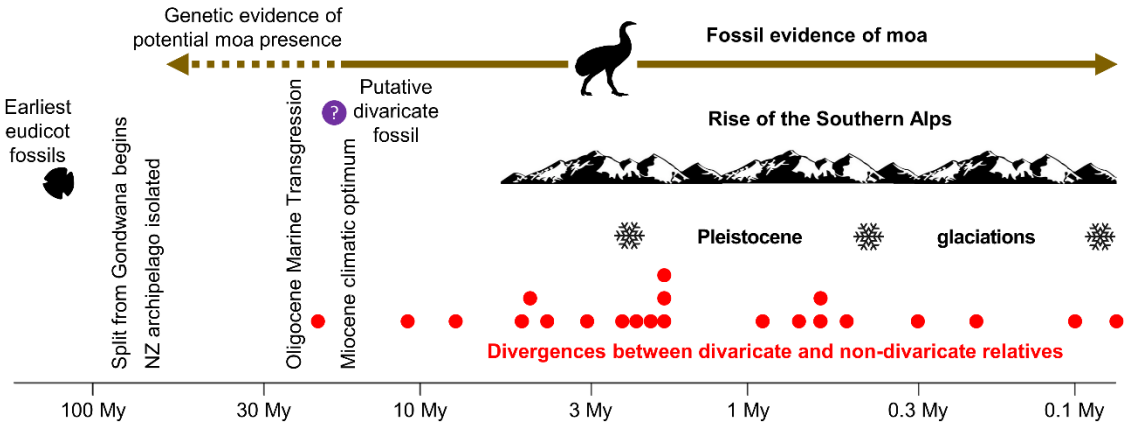


Figure 4.2. *Log time-scale plot of the maximum estimated ages of the divergences between divaricate and non-divaricate species (cf. Table 4.1), alongside the selective pressures purported to have favoured the evolution of the divaricate habit in New Zealand. Key elements of the geological history of New Zealand (NZ) are also shown.*

Our results are difficult to reconcile with an explanation of divaricate evolution based solely on avian browsing (Greenwood & Atkinson, 1977). Fossil evidence indicates that moa were present in New Zealand at least 16 My ago (Tennyson et al., 2010), and the date of divergence of moa from their closest extant relatives (South American tinamous) has been estimated at c. 60 Mya (Phillips et al., 2010; see Fig. 4.2). If moa browsing alone had driven the development of the divaricate habit, we might therefore expect to find highly developed cage architectures associated with many divergences before 5 Mya and possibly whole clades of species showing cage architectures, as seen in the Miocene proliferation of spinescence in Africa, coincident with the rise of bovids (Charles-Dominique et al., 2016).

Although it is possible that Neogene plant extinctions have obscured evidence of earlier proliferation of cage architectures in New Zealand, overall the plant fossil record is not consistent with this scenario. The New Zealand flora in general has undergone massive turnover since the mid-Miocene, with an estimated 89 % of the extant vascular flora species of New Zealand originating within the last 15 My (Heenan & McGlone, 2019). Large moa were present at least as early as 16 My ago (Tennyson et al., 2010; see Fig. 4.2), and probably much longer. Earlier divaricate lineages might conceivably have been present during the Miocene, only to have died out and been replaced by new lineages that independently evolved the divaricate habit anew in response to moa browsing. According to this scenario, the cold and dry climates arising since 5 Mya would not have been critical for the local evolution of cage architectures. A macrofossil of a divaricate-like plant with nanophyll leaves has been reported from the early Miocene of New

Zealand (Campbell et al., 2000), though the equivalence of this plant with contemporary divaricates is by no means certain. More conclusively, the typical leaf class sizes of extent divaricate species (nanophylls and leptophylls, sensu Wolfe (1993)) make up only 1.5 % of New Zealand macrofossil assemblages from the warmer climates of the early to mid-Miocene (Reichgelt et al., 2017). This palaeobotanical evidence seems telling, as small leaves are normally an integral part of contemporary cage architectures in other regions (Bond & Silander, 2007; Charles-Dominique et al., 2017; McQueen, 2000). Cage architectures are therefore unlikely to have been widespread in early to mid-Miocene New Zealand, although Pole & Moore (2011) reported fossil leaves very similar to those of the extant New Zealand divaricate *Myrsine divaricata* from near the end of the Miocene (6.5 - 6.0 My), by which time global temperatures had cooled considerably.

Although our chronology of divergences (Table 4.1; Fig. 4.2) is compatible with an explanation based solely on climate (Diels, 1896; McGlone & Webb, 1981), other studies implicate browsing in the evolution of cage architectures. Experiments have shown New Zealand divaricate plants to be less attractive to both avian and ungulate browsers than larger-leaved, more sparsely-branched relatives (Pollock et al., 2007), as also seen with cage architectures in southern Africa (Charles-Dominique et al., 2017). These studies indicate a selective advantage of the divaricate habit, and of cage architecture in general, in deterring browsers.

The balance of evidence from this and previous research is therefore best explained by the synthetic hypothesis that cage architectures were not strongly selected in New Zealand until cold, dry Pliocene-Pleistocene climates prevented juvenile trees from growing quickly out of the browse zone (Lusk et al., 2016). This hypothesis is consistent with the abundance of cage architectures in other regions where plant growth is restricted by aridity or short frost-free periods, such as Patagonian steppe (McQueen, 2000), African savannas (Charles-Dominique et al., 2017), and Malagasy thickets (Bond & Silander, 2007). It is also consistent with the low incidence of spinescence (a more well-known structural defence) in moist tropical forests (Charles-Dominique et al., 2016; da Silva-Luz et al., 2019), where juvenile pioneer trees can rapidly escape from ground-based browsers by growing several metres in height per year beneath treefall gaps (Brokaw, 1987). As well as reconciling the two competing explanations of the divaricate form in New Zealand, this study thus adds evidence that climate modulates the adaptive value of structural defences against browsing, worldwide. The especially high incidence of spinescence in fertile savannas (Scholes, 1990), and of cage architectures on alluvial soils in New Zealand (Lusk et al., 2020), suggest selection for structural defences is

strongest where high nutrient availability coincides with strong climatic constraints on plant growth rates (Lusk et al., 2016).

4.7 Acknowledgments

This research is supported by the Royal Society of New Zealand through Marsden contract 16-UOW-029 and the Faculty of Science and Engineering of the University of Waikato through FSEN Student Trust Fund (# P102218 SoS/PG Support). We thank Ana, Carina, Caroline and Kat for technical lab support; Ines Schönberger and Mary Korver for herbarium support at CHR; the herbaria BRI, CANB, CHR, HO, MEL, NOU, OTA, P, and Jason Cantley (San Francisco State University) for samples; the Direction de l'Environnement of Province Sud of New Caledonia for local sampling permits; the Department of Conservation of New Zealand for local sampling permits, and the Sanctuary Mountain Maungatautari, the Waipa District Council, the Pukemokemoke Bush Trust, Otari Native Botanic Garden and the iwi Mōkai Pātea, Ngāti Apa, Ngāti Hauiti, Ngāti Rangi, Pirirakau Hapū, Taumutu, Waikato-Tainui for local sampling agreements.

4.8 Appendix

Appendix 4.1. *Sampling plan for this study. GenBank numbers are provided when available, with the reference to the study where they were first published. If no GenBank number is available, the herbarium accession number is provided instead—some samples are still awaiting accession. Species highlighted in blue are divaricates. Rubiaceae sequences are provided in a Geneious file (Supplementary File 8) because they could not be deposited in GenBank.*

Order	Family	Taxon	Source
Apiales	Araliaceae	*Cephalaralia cephalobotrys* (F.Muell.) Harms	MW183403 (this study)
Apiales	Araliaceae	*Cheirodendron bastardianum* (Decne.) Frodin	MT385071 (Maurin, 2020a)
Apiales	Araliaceae	*Cheirodendron trigynum* (Gaudich.) A.Heller	MW183404 (this study)
Apiales	Araliaceae	*Hydrocotyle verticillata* Thunb.	HM596070 (Downie & Jansen, 2015)
Apiales	Araliaceae	*Raukaua anomalus* (Hook.) A.D.Mitch., Frodin & Heads	MT385080 (Maurin, 2020a)
Apiales	Araliaceae	*Raukaua edgerleyi* (Hook.f.) Seem.	MT385081 (Maurin, 2020a)
Apiales	Araliaceae	*Raukaua gunnii* (Hook.f.) Frodin	MW183405 (this study)
Apiales	Araliaceae	*Raukaua simplex* (G.Forst.) A.D.Mitch., Frodin & Heads	MT385082 (Maurin, 2020a)
Apiales	Araliaceae	*Schefflera actinophylla* (Endl.) Harms	MT385083 (Maurin, 2020a)

Apiales	Araliaceae	*Schefflera digitata* J.R.Forst. & G.Forst.	MT385084 (Maurin, 2020a)
Apiales	Pennantiaceae	*Pennantia baylisiana* (Oliv.) G.T.S.Baylis	MT385075 (Maurin, 2020a)
Apiales	Pennantiaceae	*Pennantia corymbosa* J.R.Forst. & G.Forst.	MT385076 (Maurin, 2020a)
Apiales	Pennantiaceae	*Pennantia cunninghamii* Miers	MT385077 (Maurin, 2020a)
Apiales	Pennantiaceae	*Pennantia endlicheri* Reissek	MT385078 (Maurin, 2020a)
Apiales	Pittosporaceae	*Pittosporum anomalum* Laing & Gourlay	MW191866 (this study)
Apiales	Pittosporaceae	*Pittosporum bracteolatum* Endl.	MW191867 (this study)
Apiales	Pittosporaceae	*Pittosporum colensoi* Hook. f.	MW191868 (this study)
Apiales	Pittosporaceae	*Pittosporum crassicaule* Cockayne ex Laing & Gourlay	MW191869 (this study)
Apiales	Pittosporaceae	*Pittosporum crassifolium* Banks & Sol. ex A.Cunn.	MW191870 (this study)
Apiales	Pittosporaceae	*Pittosporum divaricatum* Cockayne	MW191871 (this study)
Apiales	Pittosporaceae	*Pittosporum eugenioides* A. Cunn.	MT385079 (Maurin, 2020a)
Apiales	Pittosporaceae	*Pittosporum lineare* Laing & Gourlay	MW191872 (this study)
Apiales	Pittosporaceae	*Pittosporum obcordatum* Raoul	MW191873 (this study)
Apiales	Pittosporaceae	*Pittosporum patulum* Hook. f.	MW191874 (this study)
Apiales	Pittosporaceae	*Pittosporum ralphii* Kirk	MW191875 (this study)
Apiales	Pittosporaceae	*Pittosporum rigidum* Hook. f.	MW191876 (this study)
Apiales	Pittosporaceae	*Pittosporum tenuifolium* Banks & Sol. ex Gaertn.	MW191877 (this study)
Apiales	Pittosporaceae	*Pittosporum turneri* Petrie	MW191878 (this study)
Apiales	Pittosporaceae	*Pittosporum umbellatum* Banks & Sol. ex Gaertn.	MW191879 (this study)
Apiales	Pittosporaceae	*Pittosporum virgatum* Kirk	MW191880 (this study)
Apiales	Torricelliaceae	*Melanophylla alnifolia* Baker	MT385073 (Maurin, 2020a)
Apiales	Torricelliaceae	*Melanophylla modestei* G.E.Schatz & al.	MT385074 (Maurin, 2020a)
Apiales	Torricelliaceae	*Torricellia tiliifolia* DC.	NC040944 (Yao et al., 2019)
Asterales	Argophyllaceae	*Corokia buddleioides* A.Cunn.	MW194049 (this study)
Asterales	Argophyllaceae	*Corokia carpodetoides* (F.Muell.) L.S.Sm.	MW194050 (this study)
Asterales	Argophyllaceae	*Corokia collenettei* L.Riley	MW194051 (this study)
Asterales	Argophyllaceae	*Corokia cotoneaster* Raoul	MT385072 (Maurin, 2020a)
Asterales	Argophyllaceae	*Corokia macrocarpa* Kirk	MW194052 (this study)
Asterales	Argophyllaceae	*Corokia whiteana* L.S.Sm.	MW194053 (this study)
Asterales	Asteraceae	*Dasyphyllum excelsum* (D.Don) Cabrera	MH899017 (Gruenstaeudl & Jenke, 2020)
Asterales	Asteraceae	*Olearia alpicola* (F.Muell.) F.Muell. ex Benth.	MW229247 (this study)
Asterales	Asteraceae	*Olearia arborescens* (G.Forst.) Cockayne & Laing	MW229248 (this study)
Asterales	Asteraceae	*Olearia bullata* H.D.Wilson & Garn.-Jones	MW229249 (this study)
Asterales	Asteraceae	*Olearia cheesemanii* Cockayne & Allan	MW229250 (this study)
Asterales	Asteraceae	*Olearia fragrantissima* Petrie	MW229251 (this study)
Asterales	Asteraceae	*Olearia hectorii* Hook.f.	MW229252 (this study)
Asterales	Asteraceae	*Olearia ilicifolia* Hook.f.	MW229253 (this study)
Asterales	Asteraceae	*Olearia laxiflora* Kirk	MW229254 (this study)
Asterales	Asteraceae	*Olearia lineata* (Kirk) Cockayne	MW229255 (this study)
Asterales	Asteraceae	*Olearia obcordata* (Hook.f.) Benth.	MW229256 (this study)
Asterales	Asteraceae	*Olearia odorata* Petrie	MW229257 (this study)
Asterales	Asteraceae	*Olearia pachyphylla* Cheeseman	MW229258 (this study)

Asterales	Asteraceae	*Olearia polita* H.D.Wilson & Garn.-Jones	MW229259 (this study)
Asterales	Asteraceae	*Olearia quinquevulnera* Heenan	MW229260 (this study)
Asterales	Asteraceae	*Olearia solandri* (Hook.f.) Hook.f.	MW229261 (this study)
Asterales	Asteraceae	*Olearia traversiorum* (F.Muell.) Hook.f.	MW229262 (this study)
Asterales	Asteraceae	*Olearia virgata* (Hook.f.) Hook.f.	MW229263 (this study)
Asterales	Rousseaceae	*Abrophyllum ornans* (F.Muell.) Hook.f.	MW246782 (this study)
Asterales	Rousseaceae	*Carpodetus arboreus* (Lauterb. & K.Schum.) Schltr.	MW246783 (this study)
Asterales	Rousseaceae	*Carpodetus archboldianus* Reeder	MW246784 (this study)
Asterales	Rousseaceae	*Carpodetus montanus* (Ridl.) Reeder	MW246785 (this study)
Asterales	Rousseaceae	*Carpodetus serratus* J.R.Forst. & G.Forst.	MW246786 (this study)
Asterales	Rousseaceae	*Cuttsia viburnea* F.Muell.	MW246787 (this study)
Caryophyllales	Polygonaceae	*Muehlenbeckia adpressa* Meisn.	MW148933 (this study)
Caryophyllales	Polygonaceae	*Muehlenbeckia astonii* Petrie	MW148934 (this study)
Caryophyllales	Polygonaceae	*Muehlenbeckia australis* (G. Forst.) Meisn.	MW148935 (this study)
Caryophyllales	Polygonaceae	*Muehlenbeckia axillaris* (Hook.f.) Endl.	MW148936 (this study)
Caryophyllales	Polygonaceae	*Muehlenbeckia complexa* (A.Cunn.) Meisn.	MW148937 (this study)
Caryophyllales	Polygonaceae	*Muehlenbeckia gracillima* Meisn.	MW148938 (this study)
Caryophyllales	Polygonaceae	*Muehlenbeckia gunnii* Walp.	MW148939 (this study)
Caryophyllales	Polygonaceae	*Muehlenbeckia rhyticarya* F.Muell. ex Benth.	MW148940 (this study)
Ericales	Balsaminaceae	*Impatiens pritzelii* Hook. f.	MN418389 (Q. Wang et al., 2019)
Ericales	Primulaceae	*Myrsine aquilonia* de Lange & Heenan	MW246776 (this study)
Ericales	Primulaceae	*Myrsine australis* (A.Rich.) Allan	MW246777 (this study)
Ericales	Primulaceae	*Myrsine divaricata* A. Cunn.	MW246778 (this study)
Ericales	Primulaceae	*Myrsine nummularia* (Hook. f.) Hook. f.	MW246779 (this study)
Ericales	Primulaceae	*Myrsine salicina* Heward ex Hook.f.	MW246780 (this study)
Ericales	Primulaceae	*Myrsine umbellata* Mart.	MW246781 (this study)
Fabales	Fabaceae	*Cercis glabra* Pamp.	KY806281 (Wang et al., 2017)
Fabales	Fabaceae	*Duparquetia orchidacea* Baill.	MN709829 (R. Zhang et al., 2020)
Fabales	Fabaceae	*Guibourtia leonensis* J.Leonard	MG564755 (Tosso et al., 2018)
Fabales	Fabaceae	*Sophora chathamica* Cockayne	MW191851 (this study)
Fabales	Fabaceae	*Sophora fulvida* (Allan) Heenan & de Lange	MW191852 (this study)
Fabales	Fabaceae	*Sophora godleyi* Heenan & de Lange	MW191853 (this study)
Fabales	Fabaceae	*Sophora microphylla* Aiton	MW191854 (this study)
Fabales	Fabaceae	*Sophora molloyi* Heenan & de Lange	MW191855 (this study)
Fabales	Fabaceae	*Sophora prostrata* Buchanan	MW191856 (this study)
Fabales	Fabaceae	*Sophora tetraptera* J.F.Mill.	MW191857 (this study)
Gentianales	Rubiaceae	*Coprosma* "decipiens"	AK 236876
Gentianales	Rubiaceae	*Coprosma acerosa* A.Cunn.	PTBG
Gentianales	Rubiaceae	*Coprosma acutifolia* Hook. f.	AK 305635
Gentianales	Rubiaceae	*Coprosma arborea* Kirk	AK 304876
Gentianales	Rubiaceae	*Coprosma areolata* Cheeseman	PTBG
Gentianales	Rubiaceae	*Coprosma autumnalis* Colenso	PTBG
Gentianales	Rubiaceae	*Coprosma brunnea* (Kirk) Cockayne ex Cheeseman	PTBG

Gentianales	Rubiaceae	*Coprosma cheesemanii* W.R.B.Oliv.	PTBG
Gentianales	Rubiaceae	*Coprosma ciliata* Hook.f.	PTBG
Gentianales	Rubiaceae	*Coprosma cordicarpa* J. Cantley, Sporck-Koehler & Chau	PTBG
Gentianales	Rubiaceae	*Coprosma crassifolia* Colenso	PTBG
Gentianales	Rubiaceae	*Coprosma crenulata* W.R.B.Oliv.	PTBG
Gentianales	Rubiaceae	*Coprosma cuneata* Hook.f.	PERTH
Gentianales	Rubiaceae	*Coprosma distantia* (de Lange & R.O.Gardner) de Lange	AK 322617
Gentianales	Rubiaceae	*Coprosma dodonaeifolia* W.R.B.Oliv.	CHR 606211
Gentianales	Rubiaceae	*Coprosma dumosa* (Cheeseman) G.T.Jane	PTBG
Gentianales	Rubiaceae	*Coprosma elatirioides* de Lange & A.S.Markey	PTBG
Gentianales	Rubiaceae	*Coprosma ernodeoides* A.Gray	SFSU
Gentianales	Rubiaceae	*Coprosma foetidissima* J.R.Forst. & G.Forst.	PTBG
Gentianales	Rubiaceae	*Coprosma foliosa* A.Gray	PTBG
Gentianales	Rubiaceae	*Coprosma fowerakeri* D.A.Norton & de Lange	PTBG
Gentianales	Rubiaceae	*Coprosma intertexta* G.Simpson	PTBG
Gentianales	Rubiaceae	*Coprosma linariifolia* Hook.f.	CHR 639402
Gentianales	Rubiaceae	*Coprosma lucida* J.R.Forst. & G.Forst.	CHR 639372
Gentianales	Rubiaceae	*Coprosma meyeri* W.L.Wagner & Lorence	PTBG
Gentianales	Rubiaceae	*Coprosma moorei* F.Muell. ex Rodway	PERTH
Gentianales	Rubiaceae	*Coprosma obconica* Kirk	PTBG
Gentianales	Rubiaceae	*Coprosma ochracea* W.R.B.Oliv.	PTBG
Gentianales	Rubiaceae	*Coprosma oliveri* Fosberg	WU
Gentianales	Rubiaceae	*Coprosma parviflora* Hook.f.	PTBG
Gentianales	Rubiaceae	*Coprosma pedicellata* Molloy, de Lange & B.D.Clarkson	AK 316376
Gentianales	Rubiaceae	*Coprosma perpusilla* Colenso	PTBG
Gentianales	Rubiaceae	*Coprosma petriei* Cheeseman	PTBG
Gentianales	Rubiaceae	*Coprosma pilosa* Endl.	AK 297246
Gentianales	Rubiaceae	*Coprosma propinqua* A.Cunn.	CHR 639412
Gentianales	Rubiaceae	*Coprosma pseudociliata* G.T.Jane	AK 229039
Gentianales	Rubiaceae	*Coprosma pseudocuneata* W.R.B.Oliv. ex Garn.-Jones & Elder	PTBG
Gentianales	Rubiaceae	*Coprosma repens* "Poor Knights form"	PTBG
Gentianales	Rubiaceae	*Coprosma repens* A.Rich.	CHR 595644
Gentianales	Rubiaceae	*Coprosma rhamnoides* A.Cunn.	PTBG
Gentianales	Rubiaceae	*Coprosma rigida* Cheeseman	CHR 639416
Gentianales	Rubiaceae	*Coprosma robusta* Raoul	PTBG
Gentianales	Rubiaceae	*Coprosma rotundifolia* A.Cunn.	CHR 639409
Gentianales	Rubiaceae	*Coprosma rubra* Petrie	PTBG
Gentianales	Rubiaceae	*Coprosma rugosa* Cheeseman	PTBG
Gentianales	Rubiaceae	*Coprosma serrulata* Hook.f. ex Buchanan	PTBG
Gentianales	Rubiaceae	*Coprosma spathulata* A.Cunn.	CHR 649665
Gentianales	Rubiaceae	*Coprosma tahitensis* A.Gray	PAP
Gentianales	Rubiaceae	*Coprosma talbrockiei* L.B.Moore & R.Mason	CHR 476107
Gentianales	Rubiaceae	*Coprosma tenuifolia* Cheeseman	PTBG

Gentianales	Rubiaceae	*Coprosma ternata* W.R.B.Oliv.	SFSU
Gentianales	Rubiaceae	*Coprosma virescens* Petrie	PTBG
Gentianales	Rubiaceae	*Coprosma waima* A.P.Druce	PTBG
Gentianales	Rubiaceae	*Coprosma wallii* Petrie	PTBG
Gentianales	Rubiaceae	*Nertera ciliata* Kirk	PERTH
Gentianales	Rubiaceae	*Nertera depressa* Banks & Sol. ex Gaertn.	PTBG
Gunnerales	Gunneraceae	*Gunnera dentata* Kirk	MW218452 (this study)
Gunnerales	Gunneraceae	*Gunnera monoica* Raoul	MW218453 (this study)
Gunnerales	Gunneraceae	*Gunnera prorepens* Hook.f.	MW218454 (this study)
Lamiales	Gesneriaceae	*Coronanthera pulchra* C.B.Clarke	MW242810 (this study)
Lamiales	Gesneriaceae	*Rhabdothamnus solandri* A.Cunn.	MW242811 (this study)
Lamiales	Lamiaceae	*Oncinocalyx betchei* F.Muell.	MW238399 (this study)
Lamiales	Lamiaceae	*Spartothamnella juncea* (A.Cunn. ex Walp.) Briq.	MW238400 (this study)
Lamiales	Lamiaceae	*Teucrium parvifolium* (Hook.f.) Kattari & Salmaki	MW238401 (this study)
Lamiales	Lamiaceae	*Teucrium racemosum* R.Br.	MW238402 (this study)
Malpighiales	Violaceae	*Melicytus alpinus* (Kirk) Garn.-Jones	MW238803 (this study)
Malpighiales	Violaceae	*Melicytus chathamicus* (F. Muell.) Garn.-Jones	MW238804 (this study)
Malpighiales	Violaceae	*Melicytus crassifolius* (Hook.f.) Garn.-Jones	MW238805 (this study)
Malpighiales	Violaceae	*Melicytus dentatus* (R.Br. ex DC.) Molloy & Mabb.	MW238806 (this study)
Malpighiales	Violaceae	*Melicytus drucei* Molloy & B.D.Clarkson	MW238807 (this study)
Malpighiales	Violaceae	*Melicytus flexuosus* Molloy & A.P.Druce	MW238808 (this study)
Malpighiales	Violaceae	*Melicytus lanceolatus* Hook.f.	MW238809 (this study)
Malpighiales	Violaceae	*Melicytus micranthus* Hook.f.	MW238810 (this study)
Malpighiales	Violaceae	*Melicytus novae-zelandiae* (A.Cunn.) P.S.Green	MW238811 (this study)
Malpighiales	Violaceae	*Melicytus obovatus* (Kirk) Garn.-Jones	MW238812 (this study)
Malpighiales	Violaceae	*Melicytus ramiflorus* J.R.Forst. & G.Forst.	MW238813 (this study)
Malvales	Malvaceae	*Gynatrix pulchella* (Willd.) Alef.	MW194054 (this study)
Malvales	Malvaceae	*Hoheria angustifolia* Raoul	MW194055 (this study)
Malvales	Malvaceae	*Hoheria glabrata* Sprague & Summerh.	MW194056 (this study)
Malvales	Malvaceae	*Hoheria lyallii* Hook. f.	MW194057 (this study)
Malvales	Malvaceae	*Hoheria ovata* Simpson & J.S.Thomson	MW194058 (this study)
Malvales	Malvaceae	*Hoheria populnea* A.Cunn.	MW194059 (this study)
Malvales	Malvaceae	*Hoheria sexstylosa* Colenso	MW194060 (this study)
Malvales	Malvaceae	*Lawrencia glomerata* Hook.	MW194061 (this study)
Malvales	Malvaceae	*Lawrencia spicata* Hook.	MW194062 (this study)
Malvales	Malvaceae	*Plagianthus divaricatus* J.R.Forst. & G.Forst.	MW194063 (this study)
Malvales	Malvaceae	*Plagianthus regius* (Poit.) Hochr. subsp. *regius*	MW194064 (this study)
Malvales	Malvaceae	*Plagianthus squamatus* (Nees) Benth.	MW194065 (this study)
Myrtales	Combretaceae	*Terminalia guyanensis* Eichler	MK726027 (Gonçalves et al., 2019)
Myrtales	Myrtaceae	*Heteropyxis natalensis* Harv.	MK726014 (Gonçalves et al., 2019)
Myrtales	Myrtaceae	*Lenwebbia lasioclada* (F.Muell.) N.Snow & Guymer	MW214667 (this study)
Myrtales	Myrtaceae	*Lenwebbia prominens* N.Snow & Guymer	MW214668 (this study)
Myrtales	Myrtaceae	*Lophomyrtus bullata* Burret	MW214669 (this study)

Myrtales	Myrtaceae	*Lophomyrtus obcordata* (Raoul) Burret	MW214670 (this study)
Myrtales	Myrtaceae	*Neomyrtus pedunculata* (Hook.f.) Allan	MW214671 (this study)
Oxalidales	Elaeocarpaceae	*Aristotelia australasica* F.Muell.	MW218455 (this study)
Oxalidales	Elaeocarpaceae	*Aristotelia chilensis* (Molina) Stuntz	MW218456 (this study)
Oxalidales	Elaeocarpaceae	*Aristotelia fruticosa* Hook.f.	MW218457 (this study)
Oxalidales	Elaeocarpaceae	*Aristotelia peduncularis* (Labill.) Hook.f.	MW218458 (this study)
Oxalidales	Elaeocarpaceae	*Aristotelia serrata* (J.R.Forst. & G.Forst.) Oliv.	MW218459 (this study)
Oxalidales	Elaeocarpaceae	*Elaeocarpus alaternoides* Brongn. & Gris	MW218460 (this study)
Oxalidales	Elaeocarpaceae	*Elaeocarpus arnhemicus* F.Muell.	MW218461 (this study)
Oxalidales	Elaeocarpaceae	*Elaeocarpus dentatus* (J.R.Forst. & G.Forst.) Vahl	MW218462 (this study)
Oxalidales	Elaeocarpaceae	*Elaeocarpus holopetalus* F.Muell.	MW218463 (this study)
Oxalidales	Elaeocarpaceae	*Elaeocarpus hookerianus* Raoul	MW218464 (this study)
Oxalidales	Elaeocarpaceae	*Elaeocarpus reticulatus* Sm.	MW218465 (this study)
Oxalidales	Elaeocarpaceae	*Elaeocarpus rotundifolius* Brongn. & Gris	MW218466 (this study)
Oxalidales	Elaeocarpaceae	*Elaeocarpus sedentarius* Maynard & Crayn	MW218467 (this study)
Oxalidales	Elaeocarpaceae	*Vallea stipularis* L.f.	MW218468 (this study)
Ranunculales	Ranunculaceae	*Ranunculus sceleratus* L.	MK253452 (He et al., 2019)
Rosales	Moraceae	*Pseudomorus brunoniana* (Endl.) Bureau	MW238797 (this study)
Rosales	Moraceae	*Streblus banksii* (Cheeseman) C.J.Webb	MW238798 (this study)
Rosales	Moraceae	*Streblus glaber* (Merr.) Corner	MW238799 (this study)
Rosales	Moraceae	*Streblus heterophyllus* (Blume) Corner	MW238800 (this study)
Rosales	Moraceae	*Streblus pendulinus* (Endl.) F.Muell.	MW238801 (this study)
Rosales	Moraceae	*Streblus smithii* (Cheeseman) Corner	MW238802 (this study)
Rosales	Rhamnaceae	*Discaria chacaye* (G.Don) Tortosa	MW148941 (this study)
Rosales	Rhamnaceae	*Discaria nitida* Tortosa	MW148942 (this study)
Rosales	Rhamnaceae	*Discaria toumatou* Raoul	MW148943 (this study)
Rosales	Rhamnaceae	*Rhamnus taquetii* (H.L,v.) H.L,v.	MN901522 (Jin et al., 2020)
Sapindales	Rutaceae	*Melicope elleryana* (F. Muell.) T.G. Hartley	MW221968 (this study)
Sapindales	Rutaceae	*Melicope lucida* (A. Gray) A.C. Sm.	MW221969 (this study)
Sapindales	Rutaceae	*Melicope micrococca* (F. Muell.) T.G. Hartley	MW221970 (this study)
Sapindales	Rutaceae	*Melicope simplex* A.Cunn.	MW221971 (this study)
Sapindales	Rutaceae	*Melicope tahitensis* Nadeaud	MW221972 (this study)
Sapindales	Rutaceae	*Melicope ternata* J.R.Forst. & G.Forst.	MW221973 (this study)
Sapindales	Rutaceae	*Melicope vitiflora* (F.Muell.) T.G.Hartley	MW221974 (this study)

Appendix 4.2. Calibration strategy we designed for our phylogeny.

When chosen calibration ages correspond to boundaries of geological stages, the values were taken from Cohen et al. (2013, v. 2020/01). Our choice of fossils and their placement is inspired by Beaulieu et al. (2015), Janssens et al. (2020), Li et al. (2019), Magallón et al. (2015), Ramírez-Barahona et al. (2020), with adjustments made to better suit our dataset. CG = crown group.

In treePL, all node ages chosen as minimum ages (i.e. all nodes but A) were implemented without defining maximum node ages. The age of the root of the tree (node A) was implemented as a maximum age without a defining a minimum node age.

In BEAST2, all node ages priors were implemented as exponential distributions. Node ages chosen as minimum ages were set as the Offset values of the distributions, and the Mean values were chosen so that the 97.5% quantile of the distributions were about 20% of the minimum age. The age of the root (node A) was implemented with an Offset value of 125 My (corresponding to the boundary between the Barremian and the Aptian, see below) and a Mean value chosen so that the 97.5% quantile of the distribution was 150 My (see below).

A. Node: root of the tree (CG Eudicotyledons).

Secondary calibration.

Reference: Stevens (2017)

Calibration: Even though the earliest fossils attributed to the Eudicotyledons date from the Barremian–Aptian (see Magallón et al. (2015) and references therein), we decided to use a secondary calibration for this node. We chose this approach because our tests of treePL suggested that the root of the tree should be calibrated with its oldest sensible age: 150 My is the common oldest boundary of age estimates of the crown of Eudicots (Stevens, 2017b), so we chose this date as the maximum age for this node.

B. Node: CG Pentapetalae.

Fossil: Unnamed pentamerous flower

Stratigraphy and locality: Rose Creek locality of the Dakota Formation, Nebraska, USA.

Reference: Basinger & Dilcher (1984)

Calibration: The age of the Rose Creek locality is estimated to be Late Albian–Early Cenomanian (J. A. Doyle & Endress, 2010; Friis et al., 2011). We therefore chose the upper boundary of the Albian, 100.5 My, as a minimum age for this node.

C. Node: CG Ericales.

Fossil: (1) *Paleoenkianthus sayrevillensis* Nixon & Crepet, (2) *Pentapetalum trifasciculandricus* Martínez-Millán, Crepet & Nixon

Stratigraphy and locality: Old Crossman Clay Pit locality of the Raritan Formation, New Jersey, USA.

Reference: (1) Nixon & Crepet (1993), (2) Martínez-Millán et al. (2009)

Calibration: The Raritan formation is estimated to be of Turonian age (Martínez-Millán et al., 2009; Nixon & Crepet, 1993). We therefore chose the upper boundary of the Turonian, 89.8 My, as a minimum age for this node.

D. Node: CG Myrtales.

Fossil: *Esgueiria futabensis* Takahashi, Crane & Ando

Stratigraphy and locality: Kamikitaba assemblage, Asamigawa Member of the Ashizawa Formation, Fukushima Prefecture, Japan.

Reference: Takahashi et al. (1999)

Calibration: The Asamigawa Member is estimated to be from the Lower Coniacian (Takahashi et al., 1999). We therefore chose the lower boundary of the Coniacian, 89.8 My, as a minimum age for this node.

E. Node: CG Myrtaceae.

Fossil: *Paleomyrtinaea* Pigg, Stockey & Maxwell

Stratigraphy and locality: Princeton Chert, British Columbia, Canada and Sentinel Butte Formation of the Fort Union Group, Almont, North Dakota, USA.

Reference: Pigg et al. (1993), Crane et al. (1990)

Calibration: The Princeton Chert and the Sentinel Butte Formation are estimated to be of Middle Eocene and Mid/Upper Paleocene age (Crane et al., 1990; Pigg et al., 1993), respectively. We therefore chose the upper boundary of the Paleocene, 56 My, as a minimum age for this node.

F. Node: CG Asteraceae.

Fossil: *Tubulifloridites lilliei* (Couper) Farabee & Canright

Stratigraphy and locality: Snow Hill Island Formation and López de Bertodano Formation, James Ross Island and Vega, Antarctica.

Reference: Barreda et al. (2015)

Calibration: The age of the López de Bertodano Formation and the Snow Hill Island Formation cover, respectively, the Maastrichtian and the Late Campanian (Olivero, 2012). We therefore chose the upper boundary of the Campanian, 72.1 My, as a minimum age for this node.

G. Node: CG Rhamnaceae.

Fossil: *Paliurus* sp.

Stratigraphy and locality: Wind River Formation, Wyoming, USA.

Reference: Manchester (1999)

Calibration: This fossil was dated at the Early Eocene within the Wind River Formation (Manchester, 1999). We therefore chose the upper boundary of the earliest stage of the Eocene (the Ypresian), 47.8 My, as a minimum age for this node.

H. Node: CG Elaeocarpaceae.

Fossil: *Sloanea ungeri* (Heer) Manchester & Kvaček

Stratigraphy and locality: Great Plains regions, North America

Reference: Manchester & Kvaček (2009)

Calibration: The fossils of *Sloanea ungeri* range from Puercan to Early Eocene (Manchester & Kvaček, 2009), so we chose the upper boundary of the Puercan, 63.3 My (Fossilworks, n.d.), as a minimum age for this node.

I. Node: CG Fabaceae.

Fossil: diverse Fabaceae

Stratigraphy and locality: various localities

Reference: Herendeen et al. (1992)

Calibration: Herendeen et al. (1992) report that the earliest occurrences of reliable fossils of different Fabaceae subfamilies were found in the Upper Paleocene. We therefore chose the upper boundary of the Paleocene, 56 My, as a minimum age for this node.

J. Node: CG Torricelliaceae.

Fossil: *Torricellia bonesii* (Manch.) Manch.

Stratigraphy and locality: Messel maar lake, Darmstadt, Germany.

Reference: Collinson (1988), Collinson et al. (2012) (cited in Manchester et al. (2017))

Calibration: Upon re-examining fossils of *Torricellia*—the only fossil *Torricelliaceae* genus until the fossil published by Manchester et al. (2020) (Plunkett et al., 2018)—Manchester et al. (2017) concluded that the oldest confirmed fossil of Torricelliaceae is from the Messel maar lake; we therefore assigned this fossil to the crown node of the family. The age of the Messel maar lake is considered to be ca. 48 Ma years old (Lenz et al., 2015), so we chose this value as a minimum age for this node. We disregarded a recently published Torricelliaceae fossil species from the Maastrichtian because it was tentatively considered a "potential member" of the family whose occurrence "would be" the earliest known fossil of the family (Manchester et al., 2020).

K. Node: CG Araliaceae.

Fossil: *Paleopanax oregonensis* Manchester

Stratigraphy and locality: Nut Beds locality of the Clarno Formation, Oregon, USA.

Reference: Manchester (1994)

Calibration: The Nut Beds is estimated to be no younger than 43.8 My old (Dillhoff et al. (2009) and references therein); we therefore chose this value as a minimum age for this node.

Chapter 5 General conclusions

With the phylogenies of *Pennantia* J.R.Forst. & G.Forst. and *Corokia* A.Cunn. presented in this thesis (Chapter 2 and Chapter 3, respectively), both genera now have a dated phylogeny of all their respective species based on whole plastid genome sequences. The phylogenies of each genus presented in Chapters 2 and 3 were congruent with the broader analysis presented in Chapter 4, both in terms of topology and date estimates. Most importantly, these analyses all suggested that, in each genus, the divaricate species diverged from their non-divaricate sisters in the Pleistocene. Each genus has few species (< 10), and they are endemic to the landmasses where they are currently found, with no more than two species of each genus per landmass; it is therefore difficult to propose more than a tentative attempt at reconstructing biogeographic scenarios for either genus, even more so for *Pennantia* than for *Corokia*. Nonetheless, it seems possible to infer a pattern where, in both genera, the most recent common ancestor of the extant species probably lived in Australia, and from there some of its descendants dispersed eastwards—a common pattern in the flora of the south and south-west Pacific (Sanmartín et al., 2007).

The core work of this research (Chapter 4) brings new and crucial evidence to the debate over the evolution of the divaricate habit in New Zealand. This work has shown that at least the great majority of species exhibiting the divaricate habit diverged from non-divaricate relatives within the last 5 My. It is therefore now very difficult to argue that avian browsing alone was responsible for its over-representation in the New Zealand flora. Instead, this age is consistent with a strong influence of Plio-Pleistocene climates, although it does not exclude a role of avian browsing. The evidence from this work, in conjunction with previous research demonstrating the anti-browsing properties of the divaricate habit, is best explained by the synthetic hypothesis that cage architectures were not strongly selected in New Zealand until cold and dry Plio-Pleistocene climates prevented juvenile trees and shrubs from growing quickly out of the reach of ground-dwelling avian browsers (Lusk et al., 2016).

The core work of this PhD research thus adds to the evidence that climate modulates the adaptive value of plant structural defences against browsing, worldwide. The especially high incidence of spinescence in fertile savannas (Charles-Dominique et al., 2016; Scholes, 1990), and of the divaricate habit—a type of cage architecture—on

alluvial soils in New Zealand (Lusk et al., 2020), suggest selection for structural defences is strongest where high nutrient availability coincides with strong climatic constraints on plant growth rates (Lusk et al., 2016).

In the future, New Zealand divaricates are likely to survive browsing pressure but might be challenged by climate change. The divaricate habit seems effective in deterring browsing by introduced mammals, as suggested by the experimental results of Pollock et al. (2007) and by a study of the regeneration of divaricate and non-divaricate species in a forest remnant that had been subject to ungulate browsing for a century (Lusk, 2014). Ungulates tend to avoid some (though not all) divaricate species, at least until depletion of more attractive plants (Forsyth et al., 2002; Lusk, 2014). All climate change scenarios proposed by NIWA (2016) based on the IPCC's 5th Assessment predict, by the end of the 21st century, (1) an increase in mean, maximum and minimum temperature, especially at high elevations and/or during warm seasons, and a decrease in the number of days of frost; (2) an increase in severity and frequency of droughts in areas that are already dry (e.g. eastern South Island), more dry days (throughout North Island and in inland South Island), and lower relative humidity (especially in the South Island in spring and summer). If the divaricates may have evolved to adapt to drier climates, it is not sure how far their tolerance could stretch when facing even drier conditions; however, since they seem to have evolved to adapt to colder, frostier climates, an increase in temperatures may threaten their survival unless their architecture becomes and exaptation (Gould & Vrba, 1982) to hotter climates.

Observational studies have suggested that divaricates tolerate frosty and droughty environments (Lusk et al., 2016, 2020), which is also consistent with the results of this thesis showing proliferation of the divaricate habit in response to frosty and droughty Plio-Pleistocene climates. However, as far as I am aware, there are no published physiological studies of how the peculiar architecture of the divaricate habit actually influences the response of the plants to environmental stresses—the only studies about this were made on detached leaves or short leafy twigs (e.g. Bannister et al., 1995; Darrow et al., 2001, 2002), a method which tell us little about the response of the whole plant. The response of divaricate plants to the predicted effects of climate change in New Zealand is therefore difficult to anticipate: they might be able to tolerate the drier climates—although it is hard to estimate how far their tolerance could stretch—but it is

impossible to speculate whether or not their adaptation to frosty climates could become a selective disadvantage in the face of a general increase in temperatures.

Future research could usefully take one of two directions:

- To help better evaluate the survival of New Zealand divaricates as climate change unfolds, it would be useful to evaluate their maximum resistance to high temperatures and droughts. Physiological studies of how the architecture of the divaricate habit influences the response of the plants (e.g. death, tolerance) to such environmental stresses may not have been conducted yet because they require examining whole plants under controlled conditions—and not just detached branches or leaves as past physiological studies have done—and would therefore be difficult experiments to carry out.
- Conducting a similar study in other regions of the world where divaricate-like species are found could be interesting in:
    - Understanding the origins of cage architectures globally. By evaluating the relative roles of climatic and browsing selective pressures in their evolution, such research may explain, for example, how cage architectures could be favoured over spines as an anti-browsing defence.
    - Consequently, further examining the new theory about the distribution and abundance of plant structural defences against herbivory proposed by Lusk et al. (2016). If the evolution of cage architectures and other divaricate-like structures outside New Zealand (e.g. wire plants in Madagascar, Bond & Silander (2007)) presents characteristics of a combined effect of past climatic and browsing selective pressures, this new theory would gain support. Besides cage architectures, future work could weigh the relative roles of past climatic and past browsing pressures in favouring the evolution of other plant structural defences, such as spines (e.g. in the vein of the study of South African spiny plants by Charles-Dominique et al. (2016)), to evaluate how widespread on the globe, across ecosystems or across the land plant phylogeny this new theory applies.

Such studies would first need thorough local inventories of plants displaying cage architectures, to identify the actual extent of the phenomenon.

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Thesis appendix: Co-authorship forms

These are the co-authorship forms for Chapters 1, 3 and 4.

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Co-Authorship Form

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work**. Completed forms should be included in your appendices for all the copies of your thesis submitted for examination and library deposit (including digital deposit).

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work. Chapter 1, section 1.1 – Literature review.

Nature of contribution by PhD candidate	Researched and read all the papers; wrote the review; made the tables and figures.
Extent of contribution by PhD candidate (%)	90

CO-AUTHORS

Name	Nature of Contribution
Chris Lusk	Helped sharpening the writing style and the ideas.

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
Chris Lusk		17/05/21

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Chapter 3 - Phylogeny of *Corokia* A.Cunn.

Nature of contribution by PhD candidate	Fieldwork (prep + conducting); paperwork for ordering herbarium samples; the great majority of lab work and data processing; phylogenetic analyses; writing + figures.
Extent of contribution by PhD candidate (%)	90

CO-AUTHORS

Name	Nature of Contribution
Rob Smissen	Some lab work; writing.

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
ROB SMISSEN		16/11/20

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Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 4 - Dating the emergence of the divaricate habit in New Zealand

Nature of contribution by PhD candidate	Fieldwork (prep + conducting); paperwork for ordering herbarium samples; the majority of lab work and data processing; phylogenetic analyses; writing + figures.
Extent of contribution by PhD candidate (%)	85

CO-AUTHORS

Name	Nature of Contribution
Rob Smissen	Some lab work and data processing; writing.
Chris Lusk	Writing

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
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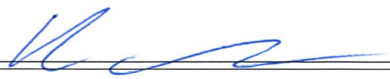
ROB SMISSEN		16/11/20