1. Introduction

1.1 TAXONOMIC BACKGROUND

As flowers in the family Calceolariaceae are easily spotted from a distance, and the overall shape and color of the corolla attracts both humans and pollinators alike, they have received much attention throughout the centuries. First decribed by Linneaus (1770) *Calceolaria* L. (Fig. 1) became subject to noticable additions and subdivisions by botanist like Ruíz and Pavón (1798), Bentham (1846), Kränzlin (1907), and Pennell (1945, 1951a, 1951b). Modern revisions of the genus Calceolaria was made by Molau (1988) for neotropic species, and Ehrhart (2000, 2005) for Chilean species. A modern revision is still lacking for Argentinean species, and the total number of species (approximately 300) could potentially be more than currently recognised.
Jovellana, the much smaller sister genus of *Calceolaria*, has not received as much historical attention as *Calceolaria*. It was first described by Ruíz and Pavón (1798) during their long field trip to South America, and was subject to attention from botanists like G. Don (1838), Colenso (Colenso, 1894; Hooker, 1843), and Kränzlin (1907). A modern revision has been lacking for *Jovellana* Ruiz & Pav., but is provided and declares a phylogenetically supported monophyletic sister group relationship to *Calceolaria* (Paper II), while recognising four species, disjunctly distributed across the Pacific Ocean. Apart from a few widespread annual weeds from *Calceolaria*, the two species of *Jovellana* present in New Zealand are the only species located outside the immediate Andean mountain range in South America.

A third genus in the family, the monotypic *Porodittia* (Cav.) G. Don, has been hypothesised to be related to ancestors of the tribe Calceolarieae (Molau, 1988). The flower differ from those of *Calceolaria* and *Jovellana* in having an embryology resulting in a different flower shape (Mayr and Weber, 2006), and is a narrow endemic in central Peru, only found between departments of Ancash and Ayacucho. The species was originaly described as a member of *Calceolaria* (*C. triandra* Cav.) but was later speculated to be a member of *Jovellana* (Don, 1838). In the same publication, it was suggested as a unique genus, *Porodittia*, but without a formal description. This suggestion was later picked up by Kränzlin (1907) when formalising *Porodittia* but listing the name *Stemotria* Wettst. & Harms (Wettstein, 1898) as a later synonym. However, the mistake went unnoticed until after the reintroduction of the species as a member of *Calceolaria* (Andersson, 2006). Virtually all publications between 1907-2006 have used the name *Porodittia*, but to keep *Calceolaria* as a monophyletic genus it must be referred to as *C. triandra* (Cav.). For conveniance and recognition the genus is occasionally referred to as *Porodittia* in this thesis.

Fig. 1. Representatives of the two recognised genera in Calceolariaceae, (left) *Calceolaria tetragona* (photo by author), (right) *Jovellana violaceae*.

The two modern revisions of *Calceolaria* (Molau, 1988; Ehrhart, 2001) subdivide the genus into a parallel number of sometimes differing and overlapping morphological sections. Molau (1988) emphasised morphological characters such as leaf shape and anther dehiscence as characters reflecting “natural groups” and used these for taxonomic delimitations at the sectional level. These ideas were largely followed by Ehrhart (2001) for Chilan species.

1.2 Orogeny of the Andes

The uplift of the Andes has not only resulted in creation of one of the driest locations on the planet (the Atacama Desert), but also indirectly to the emergence of one of the planets wettest regions (the Neotropical Rainforests). Emergence of the Andes is consequently the responsible force for creation of an area of unprecedented organism diveristy.

The South American continent is located on the South American tectonic plate which, when moving west, meets and subdues the Nazca plate causing the crust of the South American Plate to be shortened and pushed upward (Fig. 2).

Elevation of the Andes is caused by two major forces. One is a subduction-uplift process traceable in the high level of volcanic and seismic activity throughout the continents west coast, and is believed to be responsible for approximately one fifth of the total height (Graham, 2009).The remaining four fifths are caused by shortening (compression) of the South American plate crust and is influenced by a variety of factors such as subduction angle, crustal thickness, magmatic activity, and erosion (Graham, 2009; Gregory-Wodzicki, 2000). Crustal shortening in combination with thinning of the crust can hence result in zones with dramatic uplifts. It has been speculated that crustal shortening only cannot account for the Andes high peaks, but that climatic changes due to the emergence of rain shadow on the Andes west slopes prevented sediments from reaching down into the subduction zone effectively leading to an increase in friction (Lamb and Davis, 2003). Two principle areas can be identified along the subduction zone where the subduction angle decreases from 30º to 5º - 10º (Jordan et al., 1983). Such areas occur at 2º - 15º S (Peru, Ecuador), and 28º - 33º S (central Chile), and are characterised by low levels of magmatic and volcanic activity in Holocene and late Miocene (Gregory-Wodzicki, 2000). These areas of low recent volcanic activity intersect the Andes and create three zones with elevated geological activity that can be divided into a southern (south of 33º S), a central (15º - 28º S), and a northern zone (north of 2º S). These regions together host 196 identified Holocene, and 5 Pleistocene volcanoes (Simkin and Siebert, 1994), together with an additional area in Patagonia with similar, yet unrelated, volcanic activity. As a result the Andes is the worlds longest uninterupted rampart of unsubmerged mountains streching from the southern tip of Patagonia all the way to the coast on the Caribbean, a distance of almost 9.000 km (Fig. 3). Despite the apparent continuity, the Andes consist of many successive parallel and transverse ranges with intervening valleys and depressions related by a immensly complex geological history. These sets of ridges can roughly be divided into three main areas, the southern Andes consisting of the Chilean and Argentinean ranges, the Central Andes consisting mainly of Peruvian ranges, and the Northern Andes consisting of Ecuadorean, Colombian, and Venezuelan ranges (Gansser, 1973). Related areas in Patagonia were not primarily formed by the Nazca Plate collision (Ramos et al., 2008), but by a complex interaction of collisions between the South American plate, the Nazca plate, the Antarctic plate, and to a minor degree the Scotia plate. As a result the Patagonian orogeny differs from the principal Andes in being more closely related to that of the Antarctic plate.

Two major processes have played a crucial role in shaping and maintaining the Andean mountain range. One is the tectonic and related forces responsible for the main uplift process, the other being the climatic effect of such an enormous

FIG. 2. Tectonic image of South America and adjacent plates. Purple lines indicate contineta/oceanic convergent boundaries, red lines continental rift boundary or oceanic spreading ridges, green lines continental/oceanic transform vaults, traingulated lines subduction zones, and numers with arrow direction and speed in mm/year with respect to Africa. Image used with courtesy of Eric Gaba.

emerging high altitude mountain range (Strecker et al., 2007), coupled with the formation of the Humboldt Current and the Antarctic Circumpolar Current when the South American continent separated from the Antarctic continent (Barker and Burrell, 1977; Scher and Martin, 2006). Both these processes have had a vast effect on global as well as regional climate (Hartley, 2003), and still do to present day. Without proper understanding of these processes, it is impossible to draw any conclusions on the overall biogeography of the Andes.

1.3 CLIMATE AND UPLIFT HISTORY OF THE ANDES.

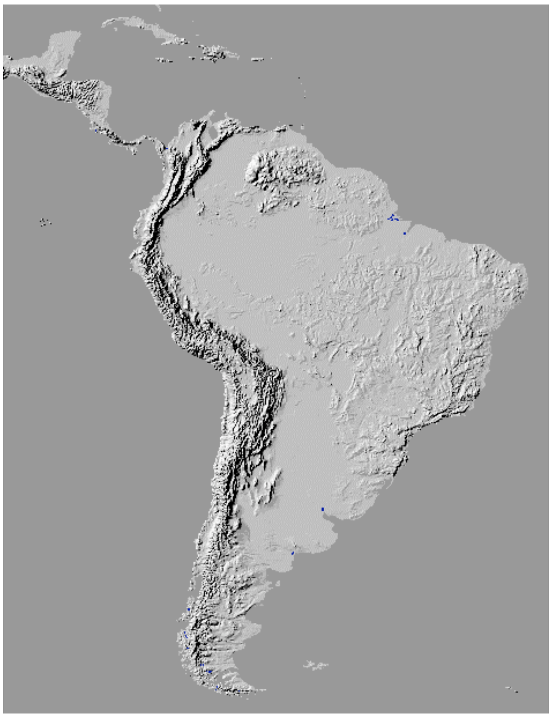

FIG. 3. Topological relief of the Andean mountain range.

The Andean uplift is believed to have begun in mid Jurassic (approximately 170 mya) when the South American plate, pushed west by the Atlantic ridge made contact with and began to subdue the Nazca plate (Gregory-Wodzicki, 2000). However, it was not until Cretaceous (145.5 – 65.6 Ma) the Andes began taking present form. Describing the uplift history of the Andes is a complex task as different parts of the mountain range has gone through different phases of uplift, subduction zone angles, erosion and crustal stress. Therefore, while the fine scale of the uplift process is too complex to describe accurately, a number of generally notable events, crucial for the orogenic history can be identified and dated.

Two major events have irreversibly and permanently defined the climate history of the Andes. The first is the development and opening of the Drake Passage, when the South American continent departed from the Antarctic continent approximately 49-17 Ma (Scher and Martin, 2006). This led to the formations of the Polar Current and the Humboldt Current (Fig. 4), isolating Antarctica while pushing cold, upwelling low-saline water towards the coast of Chile and Peru, having a major impact on the climate in the region (Toggweiler and Bjornsson, 2000). Prior to the formation of the Drake Passage the western border of South America hosted plenty of dinosaurs, roaming lowland swamps and lakes, sometimes inundated by ocenaic saline waters (Gayó et al., 2005). Approximately 40 Ma (Late Eocene) sediments show build up of sand and silt, indicating an increasingly more rapid uplift process had begun (Graham, 2009). At the same time, it is believed that the Nazca Plate subduction slowed down (Lamb, 2004), resulting in a build up of friction and consequently the emergence of highlands safe from saline inundation.

At about the same time, the Altiplano region in Bolivia and Peru was a river basin still located at sea level, caracterised by increased crustal thickness

(Horton et al., 2002). As the South American shield shifted westward by force from the Atlantic Ridge, it eventually undercut the emerging Cordillera Oriental leading to a massive crustal shortening and rapid uplift. Between 30 and 20 Ma the crust is believed to have shortened by 100 km, and once again by 100 km between 10 Ma and present day (Graham, 2009).

About 15 Ma (Mid Miocene) the Andes had reached approximately half (2.000 m) of its present (4.000 m) average height (Gregory-Wodzicki, 2000; Jordan et al., 1983; Kono et al., 1989), sufficiently high to cast rain shadow on the west coast (Hartley, 2003; Houston and Hartley, 2003). While the orogenic history and the underlying reasons to the long term intacity and lack of crustal shortening of the Altiplano is subject to much debate (Ghosh et al., 2006; Oncken et al., 2006; Sempere et al., 2006), recent attempts to date the uplift of the area has concluded that major uplift events occurred between 10,3 – 6,7 Ma with an approximate average rate of 1.03 mm per year (Benjamin et al., 1987; Ghosh et al., 2006; Sempere et al., 2006). As a consequence the Altiplano rose from sea level to its present day average altitude (3750 m) in less than 4 million years, during which it was more or less covered by lake systems reaching depths of up to 80 m (Graham, 2009). Remnants of these lakes exist today as the Lake Titicaca on the border between Bolivia and Peru.

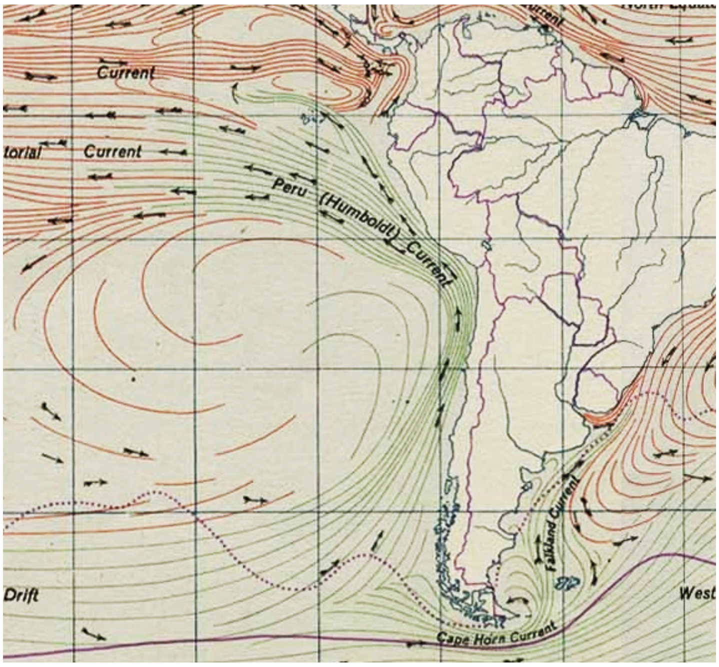

FIG. 4. Illustration of the Humboldt Current looping from Antarctic regions along the coast of South America and back into the Pacific. Warm currents are drawn with red lines, cold currents with green lines.

Evidence from paleobotanical and geological records indicate that the last sequential uplift of the central Andes took place from 10 – 6 Ma to present day (Ghosh et al., 2006; Gregory-Wodzicki, 2000), elevating the Andes from its then average height of approximately 2.000 m to present day 4.000 m. During this time the high altitude flora region known as the páramo emerged as one of the youngest of the Latin American ecosystems, and was the combined result of globally cooling cliamte and virgin high altitude areas (Simpson, 1975; Simpson and Todzia, 1990; Young et al., 2002).

Uplift was slightly delayed in the northern part of the Andes (5º S or less) and the mountains at this latitude is made up of three primary ridges, the Cordillera Occindetal running parallel to the Pacific Ocean, Cordillera Oriental extending east into Venezuela later branching in two enclosing the Maracaibo Lake, and the Cordillera Central in between them. Only the first is believed to be of volcanic origin, while the latter two is believed to be of non-vocanic origin (Millward et al., 1984). By late Cretaceous (~70 Ma) the Cordillera Occidental had begun to form along the Pacific coast, and by Late Miocene (~13 - 7 Ma) the northern range had reached approximately half of its present average altitude (Gregory-Wodzicki, 2000). The main uplift of the Cordillera Oriental is believed to have taken place between 6 - 3 Ma when reaching its present height (Clapperton, 1993).

By the time 15 Ma the emergence of relatively high altitude regions, coupled with local and global climate effects due to the rise of the Andes, suggest increasing difficulties for species to migrate from east to west and vice versa across the mountain range, creating a general vicariance shift, while the ability to transverse across the emerging mountain range was and still is largely dependant on group or species specific abilities and affinities.

Dating the orogeny of such a massive mountain range as the Andes is an incredibly difficult task given the complexity and co-dependant affinities and processes forming the many valleys and ridges. Methodology for dating geological events are unprecise and often rely on data leaving considerable margins of error. Geological studies are further complicated in periods of regional unrest, making long term field studies even more difficult.

1.4 RELATIONSHIP BETWEEN SOUTH AMERICA AND NEW ZEALAND

Breakup of the Gondwana super continent roughly began with the initial separation of Africa in Jura approximately 165 – 155 Ma (Jokat et al., 2003), and was located more or less where Antactica is today. India broke away during early Cretaceous (125 Ma) and began to move northward, while Australia began separating from Antarctica by late Cretaceous (approximately 80 Ma). While the tectonic history of New Zealand is complicated by the Alpine Fault and the islands being located on the edge of both the Australian and Pacific plates (Laird and Bradshaw, 2004; Norris et al., 1990), the main break away from Gondwana began by Cretaceous (85 Ma). Late Eocene marine transgression at the Oligocene-Eocene boundary (35 Ma) is believed to have fully or partly submerged the islands. South America was connected to west Antarctica at least as late as 30 Ma (Scher and Martin, 2006) before the full formation of the Antractic Circular Current. The latest occurring land connection between South America and Australia can be traced back to late Cretaceous, while the latest occurring land conection between South America and Antarctica was cut at the formation of the Antarctic Cicular Current.

1.5 ASSUMPTIONS ON THE MOLECULAR CLOCK

The idea of a molecular clock has been in existence since the early observations of Zuckerkandl et al. (1962). Implementations of the clock have varied from simplistic models of constant rates across lineages, to rates being correlated across lineages (Sanderson, 1997, 2002), and various relaxed models of rates being fully independent and uncorrelated to those of parental lineages (e.g. Drummond et al., 2006; Huelsenbeck et al., 2000). Success of clock models for date inference rely not only on choice of clock model, but to a large extent on the quality of the inferred phylogeny, choice, number, implementations, and reliability of calibrations, and taxon sampling (Ho and Phillips, 2009; Ho et al., 2008). The general idea of molecular clocks is by and large accepted as a natural part of modern systematic theory. The methodological implementation of the clock model remains an enigmatic issue of ongoing debate, as are the reliability and robustness of results (Bandelt, 2007; Emerson, 2007; Heads, 2005; Ho and Larson, 2006; Howell et al., 2008; Pulquério and Nichols, 2007).

1.6 GENE TREE – SPECIES TREE CONCEPT

Species in this study follow the taxonomy and species delimitations of Molau (1988), and Ehrhart (2000, 2005). The vast majority of species included in this study have been either collected or determined by Prof. Molau or Dr. Ehrhart, vouching for minimising the effect of possible misidentifications on phylogenetic inferences.

Ever since the emergence of molecular phylogenetics there has been a general assumption that gene(s) under study should reflect, and be a more or less accurate representation of, how individual species have evolved. The changes and evolutionary adaptations of the species to ever changing environments is coded for and stored in the genes, and since all genes shared in the same species under this view consequently should share the same history, any gene chosen for studying the phylogeny of a group should be a more or less resolved representation of that groups evolution through time. The level of resolution is reduced to a matter of amount and type of concatenated data (Hedtke et al., 2006; Rokas et al., 2003).

The definition of a species tree is the phylogeny representing the relationships among a group of species. A gene tree is the phylogeny for a set of gene sequences from the species. The species tree framework does not require genealogies to be in mutual agreement, expressing speciation events at the least possible time for the genealogy splits. For any hypothesis relating to the evolutionary history of the species, it is preferrable to relate such to the species phylogeny rather than individual genealogies, as the timing and nature of gene tree splits can differ from the actual speciation events.

Though ideas on situations where information from gene trees may differ from the presumed species tree (Fig. 4) were raised early (Pamilo and Nei, 1988) it was not until later integrated theoretical concepts for how to model this was proposed (Doyle, 1992; Maddison, 1997; Rosenberg, 2002) taking advantage of multi-species coalescent theory. Recently we have seen various implementions of these concepts in popular software for species tree estimates from multiple source gene data (Heled and Drummond, 2010; Liu and Pearl, 2007; Rannala and Yang, 2003; Yang and Rannala, 2010). Accumulated observations of differences between commonly used genes from the nucleus (i.e ITS and ETS), and the chloroplast, suggest different parts of an organism's set of genes may evolve independently in the host species, each gene describing its own evolution. Processes acting on genes resulting in discordance between the gene trees and the true species tree are incomplete lineage sorting and lateral transfer (e.g. hybridisation), and must hence be accounted for in order to infer the true species phylogeny.

Mutli-species coalecent theory state that individual genealogies will merge (share a most recent common ancestor) at the time of, or preceeding, speciation (Rosenberg, 2002). This is complicated by gene or whole genome duplications (i.e, polyploidy) of plants where a species can carry several copies of its genome, thus introducing several copies of genes that may or may not be homeologies and/or selected against or lost (paralogy or incomplete lineage sorting). It could however, be argued that data from the chloroplast is a more reliable approximation of the species tree as the effects of smaller population sizes for a haploid genome reduces the effect of lineage sorting on the phylogenetic inference. However, since such effects cannot be ruled out any result analysing chloroplast data only must be considered as an unconfirmed hypothesis of the true species relationships.

The introduction of sequence data for phylogenetic inference has revolutionised systematics and provide a foundation for evaluating taxonomy. Until recently most phylogenetic studies of plants have been based on various information in the plasmid (chloroplast), while nuclear information has been largely limited to nuclear ribosomal DNA (rDNA), consisting of highly conserved parts coding for the ribosomal units (18S, 5.8S, and 28S), which are separated by internal transcribed spacer regions 1 and 2 (commonly abbreviated ITS1 and ITS2). The rDNA is known to occur in multiple copies and is associated with extensive issues (Álvarez and Wendel, 2003; Buckler-IV et al., 1997; Nilsson et al., 2010), but has nevertheless remained popular in molecular systematics due to the availability of suitable and general primers located in the highly conserved coding regions. It has been estimated that over a five year period some 65% of all plant molecular studies included the ITS region (Álvarez and Wendel, 2003).

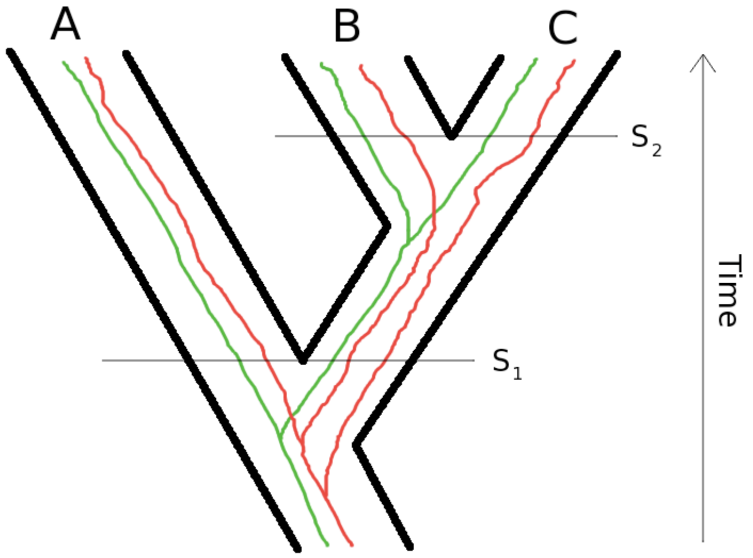

FIG. 4. Illustration of how individual genealogies (red and green) may differ in evolutionary history compared to the species tree (black outline) of three hypothetical taxa (A-C). S_1 and S_2 indicate timing of speciation events.

Sequence data from other nuclear regions has shown to be more difficult to amplify and sequence, largely because of the lack of universal primers for amplification. As the polyploidy of many plants postulate the presence of multiple alleles for many genes, and these genes can be subject to paralogy following gene duplication events, it is likely that universal primers for one gene will amplify more than one gene copy. Disentangling of paralogues and gene copies is known to be a time consuming process often requiring extensive cloning, but recent advancements in sequencing technology e.g. (Jarvie, 2005; Mardis, 2008) will make future use of information from the nucleus more readily available for systematic purposes. Since phylogenetic studies largely aim at drawing conclusions on the relationship of species, and the species relationships to its surroundings, the ultimate aim of systematics should be to strive for production of species trees.

1.7 BIOGEOGRAPHIC ASSUMTIONS

Biogeography as subject stem from the general observation that all species are not present everywhere at the same time, but are spatially separate and the spaces they occupy can change considerably over time depending on time scale (Brown and Lomolino, 1998).

Methodological development of analytical biogeography stepped forward as science when combined with the emergence of cladistic thought (Nelson and Platnick, 1981) and merged with plate tectonics theory (Wegener, 1966). A second leap was achieved when drawing on nucleotide sequence data for phylogenetic inference. The most widely used software implement event-based methods through parsimony optimisation in order to reconstruct ancestral areas (Ronquist, 1997). A common critique to this appraoch is the lack of consensus on how to define areas of interest (Brooks and Van Veller, 2003; Henderson, 1991), and lack of methodological implementation of time aspects on the phylogenies (Upchurch and Hunn, 2002). Despite these preceived shortcomings, large efforts have been put into prior definition of areas of biogeographic interest (Morrone, 2001a, 2001b; Posadas et al., 1997)

Advancing the field of biogeographical methodology further requires integration of probabilistic methods for ancestral state reconstructions, while allowing for use of molecular clock models. Such advancements have been achieved recently (Lemey et al., 2009; Lemey et al., 2010), solving the controversy of a priori definitions of biogeographic areas as analyses are based on specimen locations, while introducing separate concepts for analysis of continuous and discrete states. Continuous traits such as geographic distributions can now be analysed by means of Brownian motion in continuous space through the application of geographic diffusion rates on branches (Lemey et al., 2010). As implemented in BEAST, a software primarily intended for datedof phylogenies (Drummond and Rambaut, 2007) it allows for estimates of diffusion rates in three dimensions, and can potentially be adapted to simultaneous estimation of species trees from multiple genealogies in a gene tree - species tree concept (Lemey, pers. com.), though the applicability and potential for this is yet unexplored.

2. Objectives

Modern revisions of *Calceolaria* (Ehrhart, 2000, 2005; Molau, 1988) have speculated on the origin of the family, and evolutionary factors playing a role in diversification of the genus. The observed patterns of diversity suggest a close relationship between the origin and different parts of the Andes and radiation of *Calceolaria*, wheareas the area of origin and dispersal patterns remain unknown. Putative relationships between South American and New Zealand species of *Jovellana* suggest them being remnants of ancient continental breakup, though this hypothesis have remained untested. The presumed relationship of the monotypic *Porodittia* to *Calceolaria* and *Jovellana* will be evaluated in light of phylogenetic results.

Previously mentioned advancements in estimation of species trees from multiple source data, together with development of probabilistic models for reconstruction of discrete and contiuous ancestral trait states, allow an improved and more flexible approach to provide insights to estimation of ancestral areas. Such diversification events can only be understood in relation to detailed orogeny of the Andes and the tectonic history of the southern hemisphere.
The major aim of this thesis is to achieve a resolved phylogeny of the genera of Calceolariaceae and investigate the putative co-evolution of the family to the orogeny and uplift history of the Andean mountain range, and its historical connection to New Zealand/Australia.

3. Thesis Parts

3.1 PAPER I

On the phylogeny of the genus Calceolaria *(Calceolariaceae) as inferred from ITS and plastid* matK *sequences*

In Paper I the Calceolariaceae were, for the first time ever, phylogenetically investigated by use of the nuclear ITS region and partial chloroplast *mat*K data. Based on results from analysis of *mat*K data from a selection of Lamiales representatives the Calceolariaceae was concluded to share a sister relationship to the family Gesneriaceae, but with moderate bootstrap support. The Calceolariaceae was first suggested as a separate family by Olmstead et al. (2001), and a later study based on a wider taxon sampling and more sequence data (Oxelman et al., 2005) suggested the Calceolariaceae as a sister group to remaining representaitves of the Lamiales, but with low support and conflicting signals from the different genes included. Tank et al. (2006) reached the same conclusion with results showing the Calceolairaceae and Gesneriaceae sharing a polytomy with the remaining Lamiales. A recent study (Albach, 2009) indicated the Calceolariaceae being nested within the Gesneriaceae, but with low taxon sampling and support values. Despite the many attempts the position of the Calceolariaceae in Lamilaes must be considered uncertain.

For phylogenetic purposes sequences were retrieved from 85 species representing all but five morphologically defined sections sensu Molau (1988) and Ehrhart (2000). A single representative of *Jovellana* was included (*J. violacea*) together with a single representative of the Gesneriaceae (*Kohleria spicata*) chosen as outgroup for the study. Results confirmed the hypothesised sister relationship of *Jovellana* and *Calceolaria*, but the overall phylogenetic resulotion within *Calceolaria* was low. However, a selection of Chilean species were retrieved as sister to the remaining species suggesting southern South America could be part of the ancestral area of the family. Furthermore, the results indicated at most two radiation events for species with their main distribution north of the Huancabamba deflection. This confirmed the assumption of the Huancabamba region being an important barrier for further radiation to the north Molau (1988).

Sectional delimitations sensu Molau (1988) and Ehrhart (2000) were discussed in light of the phylogenetic inference. While there seem to be little support for a morphological signal in the phylogeny, at least one section was retrieved as monophyletic (sect. *Calceolaria*) mainly consisting of annual autogamous weeds widely distributed throughout the Andes and central America (Mexico). Further, the phylogenetic position of *Porodittia* was concluded to be nested deep within *Calceolaria*. In order to keep *Calceolaria* as a monophyletic genus the species was reintroduced into *Calceolaria* as *C. triandra* (Cav.). The deviant flower shape of *Porodittia* could consequently be presumed as an evolutionary fixed teratology, a view later supported by Mayr and Weber (2006).

3.2 PAPER II

Phylogeny and taxonomic revision of the genus Jovellana *Ruiz & Pav. (Calceolariaceae: Lamiales) using digital illustrations for taxonomic purposes*

Paper II provides a modern taxonomic revision of *Jovellana* based on phylognetic inference. The study was based on the taxonomy sensu Kränzlin (1907) and material for molecular studies were retrieved for four of them (*J.*

violacea, *J. punctata*, *J. repens*, and *J. sinclairii*). An additional selection of species representing five major clades of *Calceolaria* (Andersson, 2006) toghether with a single representative from Gesneriaceae (*Peltanthera floribunda*) was included. Sequence data for the ITS region together with three chloroplast loci (*trn*LF, *rps*16, and the *atp*B-*rbc*L spacer) were retrieved and analysed using Bayesian inference. Results confirm the monophyly of *Jovellana* and its placement as a strongly supported sister group to *Calceolaria*. In *Jovellana* the South American species *J. violacea* and *J. punctata* is revealed as the sister group to the New Zealand species *J. sinclairii* and *J. repens*.

In addition to the phylogenetic results the morphological characters important to taxonomy are discussed, and the leaf blade length/width ratio is proposed as a new supporting character for species delimitation. A taxonomic revision is given for the genus, and flower morphology and indumentum is concluded to be very variable throughout the genus depending on growth habit, and based on an evaluation of the morphology for species not included in the phylogenetic analysis, a synonymisation for *J. sturmii* and *J. albula* being included in a wider circumscribed *J. sincalirii* is suggested. As *J. sturmii* has been distinguished from *J. sinclairii* based on the density of indumentum and secondary corolla lobation, and *J. albula* is distinguished from *J. sinclairii* based on flower colour and secondary corolla lobation, a synonymisation is motivated (see below). Leaf blade ratios for all species except *J. violacea* (because of its deviant leaf size and few number of paired lobes compared to other *Jovellana* species), indicate partial overlap for all species. However, in conjuction with supportive characters such as ovary placement and length of upper corolla lip the character is useful for species delimitaiton purposes. Fully overlapping leaf blade ratios and the variable level of secondary corolla lobation suggest the previously recognised species *J. sturmii* is indistinguishable from individuals of *J. sinclairii*. Also, the apparent lack of collected material for *J. albula* and the characters used to delimit the species from individuals of *J. sinclairii* suggest a synonymisation of the species names.

Distribution maps based on information from voucher labels are generated for the recognised species. While the South American species are limited to central Chile with *J. punctata* being more widely distributed than *J. violacea*, the New Zealand species does not show much overlap in distribution. *Jovellana* sinclairii is mainly limited to an area on the northeast part of the north island with few ocurrences elsewhere, while *J. repens* show a much wider distribution mainly restricted to the west side of the north island. A similar pattern is evident on the south island with representatives of *J. repens* being restricted to the northwest part of the island, and only scattered collections of *J. sinclairii* on the east side.

3.3 Paper III

The disjunct distribution of Jovellana *Ruiz & Pav. (Family Calceolariaceae; Lamiales) explored by a dated species tree approach*

Paper III investigates the historical relationship between South American and Chilean species of *Jovellana* in a gene tree – species tree framework, through dating of the species tree. Two additional linkage groups were added to the loci used for phylogenetic inference in paper II together comprising four linkage groups, the chloroplast group (*trn*LF, *mat*K, *rps*16, and the *atp*B-*rbc*L spacer), the ITS region, the APETALA3 gene, and the Gcyc gene. All generated data were used in a dated gene tree – species tree analysis using secondary calibration from paper IV for the stem age of Calceolariaceae. The separate genealogies show signs of incomplete lineage sorting.

Posterior node age estimates for speciaiton events in *Jovellana* suggests the split between South American and New Zealand species occurred between 0.6–4.0 Ma (Fig. 5). The inferred age is not in correspondance with the assumed age of Gondwanan breakup between South America and Australia/Antarctica and the formation of the Drake Passage (28 Ma) (Barker and Burrell, 1977; Jokat et al., 2003; Scher and Martin, 2006), concluding the occurrence of *Jovellana* in New Zealand being the result of recent long distance dispersal.

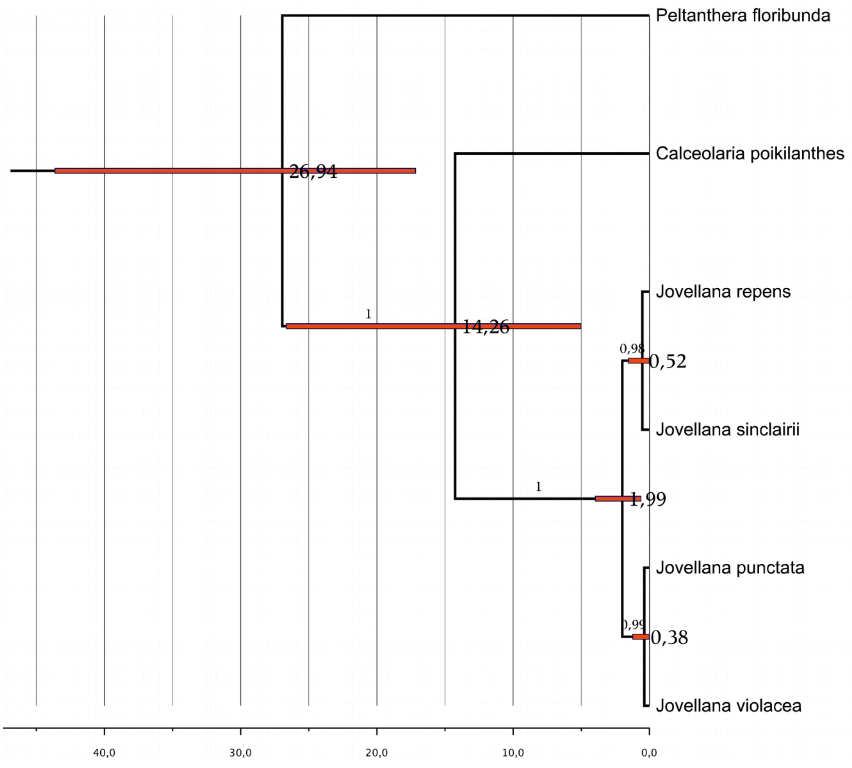

FIG. 5. Species tree of Jovellana as inferred from four linkage groups. Node ages are in Ma, numbers above branches indicate Bayesian posterior proba-bility.

Modes for such long distance dispersal have been hypothesised, birds often being mentioned as important vectors. However, the fruit and seed characteristics of Jovellana species show no adaptation to long distance dispersal (paper II). Fruits are dry capsules dispersing seeds passively by wind movement while the seeds are minute without surface modifications to allow increased adherence ability. As bird dispersal would be highly coincidental and unlikely given the fruits and seeds, ocean surface dispersals are more likely due to the proximity of *Jovellana* to the Pacific Ocean and inland rivers (Higgins et al., 2003; Jokiel, 1990). The unconsolidated geology of coastal Chile coupled with past and present seismic activity could be contributing factors for debris islands carrying *Jovellana* plants or seeds being dislocated into the ocean, fascilitating ocean surface dispersal.

3.4 PAPER IV

Phylogeny and biogeography of Calceolariaceae (Lamiales) inferred using novel continuous and discrete models for phylogeographic traits.

Paper IV aims at achieving a deeper understanding of the phylogeny and biogeographic affinities of Calceolariaceae through means of molecular dating and ancestral state reconstruction of continuous and discrete traits believed important to the evolutionary success of the family. Building on the study in paper I an additional number of taxa are sampled, with emphasis on the southern group in Chile with the aim of resolving the extensive polytomies for that group. Additionally, three chloroplast loci (*trn*L-F, *rps*16 and the *atp*B-*rbc*L spacer) were amplified for all included species along with the nuclear ITS region. In total, 136 species were sampled across the phylogeny.

Phylogenetic inference of the chloroplast data and ITS region revealed extensive topological incongruence, none of which is possible to relate to morphological sections as defined by Molau (1988) or Ehrhart (2000). The majority of Chilean species form a monophyletic group, showing extensive and rapid radiation, while a few representatives form a sister relationship to the remaining species. For species occurring in Bolivia and furhter north one group with main distribution areas north of the Huancabamba deflection form a monophyletic group. The position of *C. triandra* (formerly *Porodittia* (=*Stemotria*)) as nested within *Calceolaria* is confirmed.

In order to provide a timeframe for radiation events in the Calceolariaceae an extensive molecular dating of the stem and crown age of the family was done through an approach with two sets of data using separate but partly overlapping fossil calibrations. For more accurate estimates of the radiation events in

Calceolaria achieved dates from the fossil calibrated dated phylogenies were transferred to the data matrix for phylogenetic inference.

The date estimates for Calceolairaceae stem ages differed depending on data used, ranging from 43.3 Ma (*mat*K, median age) to 79.1 Ma (*rbc*L, median age). For Calceolairaceae crown age median values range between 20.0 Ma (*rbc*L) and 32.5 Ma (*mat*K) for fossil calibrated analyses, and 32.8 Ma (*rps*16-*trn*LF data) and 31.8 Ma (*atp*B-*rbc*L data) for analyses based on secondary calibration. Transferring node ages from the two fossil calibrated analyses yielding the most diverse node ages (*rbc*L and *mat*K data) to a Calceolariaceae matrix only for *trn*LF, *rps*16 and the *atp*B-*rbc*L spacer, posterior estimates for the *Calceolaria* crown age converge to virtually the same values (13.8 and 14.1 Ma, repsectively) with virtually identical 95% Height Posterior Density values (HPD).

In order to trace the radiation history of the Calceolariaceae to the uplift history of the Andes, each species was coded for geographic location and altitude based on the sampled specimen data, and ancestral areas and altitudes were reconstructed using a trivariate phylogeographic model. Results show a correlation between ancestral altitudes and the hypothesised uplift history of the Andes (Fig. 6), while a geographic trend from south to north can be identified. The results support the idea that the radiaiton history of the Calceolariaceae is connected to the uplift history of the Andes, and that ancestral representatives of the family have been associated with the continuously temporal maximum altitudes of the Andes. The ancestral area of the family is indicated to be in central Chile, on lowland hills or lower west slopes of the Andes.

To trace the impact of trait changes on the radiation history of the family, a number of such traits were identified and coded accordingly for each species (general growth habit, ploidy level, pollinator, pollinator reward, and pollinator mechanism), and optimised on the phylogeny using a phylogeographic model for discrete traits. The results show correlation between growth habit and geography, and ploidy level and geography. Important shifts in both are in correlation with important orogenic changes in the Andes, and occur at a time when the Andes reach sufficient height to produce a rain shadow on the east slopes and affecting the climate in the entire southern hemisphere (Graham, 2009; Gregory-Wodzicki, 2000; Jordan et al., 1983; Kono et al., 1989).

Traits relating to pollination does not show a clear correlation between geography and pollinators. Oil-collecting pollinators become more sprasely distributed towards the northern Andes, and is somewhat correlated to multiple losses of elaiophore (oil-producing pad of hairs in *Calceolaria* flowers). Pollinator mechanism for pollen presentation show a clear shift from a nototribic

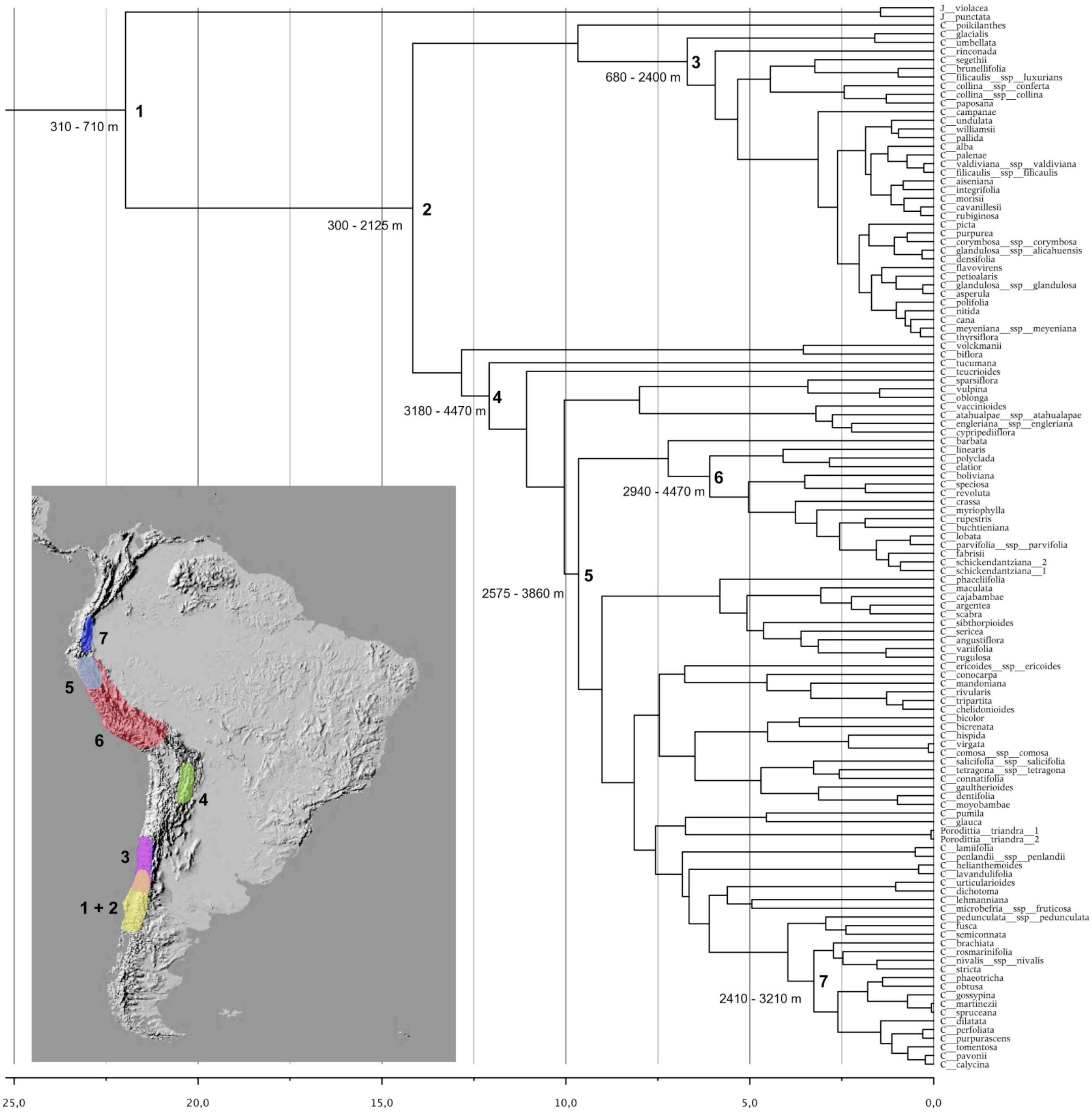

Fig. 6. A Bayesian chronogram of the Calceolariaceae with simultaneous estimates of ancestral areas and altitude with major areas identified at nodes (1–7) plotted on a map of South America. Areas correspond to the 95% HPD intervals for ancestral area estimates.

ancestral state to sternotribic flowers in Chilean representatives, with few scattered unrelated shifts occurring outside this group.

4. Discussion

This thesis have pointed out the difficulties in relating the evolution of the Calceolariaceae to the acquisition of morphological characters (paper I and IV) on which to base sectional delimitations. While the data is inconlusive regarding how to relate morphology to evolution of *Calceolaria*, the phylogenetic analyses still provide well resolved and supported topologies. In order to gain a deeper insight into the evolution of *Calceolaria*, an approach similar to the one used for *Jovellana* (paper III) is required where conclusions on species relationships are based on inference of a species tree and not being limited to extrapolation from single genealogies. The young age of the family coupled with the presumed radiation pattern of *Calceolaria* (paper IV) does not exclude extensive hybridisation being an important factor in the evolution of the genus. However, accounting for such processes in a genus as extensive as *Calceolaria* is well beyond the limits of this thesis, requiring a wide array of nuclear sequence information not available today.

Given the explanatory limitations of the chloroplast phylogeny (paper IV) certain evolutionary trends are distinguishable. Simultaneously estimating ancestral geography and altitude provides an image of *Calceolaria* as having a lowland origin in Southern South America with subsequent radiations on higher altitudes successively further north. Certain trends in acquisition of ecological traits important for pollination and growth habit seem to be correlated to key features in the uplift history of the Andes.

However, the robustness of the results can be questioned on basis of the hypothesised relationship between the phylogeny of a group and the connection to the areas in which it resides. As is evident from the reconstruction of ancestral areas (paper IV) certain internal nodes exhibit altitude estimates not in agreement with hypothesised altitudes in the region at the inferred time. This could be due to one of three issues: (1) A bias in altitude among sampled specimen forces an ancestral estimate not corresponding to factual altitudes present at that time. (2) Insufficient phylogenetic information to infer branch lengths long enough to achieve reasonable estimates of the geographic diffusion rates (Lemey et al., 2010). (3) Insufficient geological information from the area in question to provide reasonable hypotheses of ancestral altitudes at different times (Ghosh et al., 2006; Gregory-Wodzicki, 2000; Sempere et al., 2006).
In part, this issue could be overcome with use of heterogenous landscape models (Balkenhol et al., 2009; Currat et al., 2004) allowing priors to constrain ancestral altitudes for particular nodes at certain times, but remains for further research and development to provide solutions to. Until then, results based on present phylogeographic models should be considered as hypothetical.

In biogeography it has become increasingly understood that long distance dispersal has had a larger impact on regional as well as global patterns of plant diversity than previously believed (Higgins et al., 2003; Higgins and Richardson, 1999; Sanmartín and Ronquist, 2004). By drawing on genetic information from multiple linkage groups relating to both the nucleus and the chloroplast, a reasonable estimate of the species relationship can be made for

Jovellana. The species phylogeny can then be used for testing hypotheses on the divergent distribution of *Jovellana* across the Pacific, concluding such a pattern being the result of recent age long distance dispersal rather than remains of a past vicariance event. In order to advance the understanding of the biogeography in *Calceolaria* a similar approach to the one used for *Jovellana* can potentially solve both the genealogy incongruence issues between nuclear and chloroplast data, and provide a fundament for formulating proper biogeographic hypotheses on the radiaiton of species in the Andes.

One particular advantage of the used phylogeographic model in this thesis is not requiring a priori definition of biogeographic areas (Lemey et al., 2010). As phylogenetic inference is based on sequence data from one or a few specimen taken as representative for the entire species and its distribution area, assumptions of the relationship between the a priori defined biogeographic area is in fact based on single or a few specimen from each species. Ideally, instead of defining areas a priori they can be estimated a posteriori based on the sampled specimens. Robustness of analysis and ancestral area reconstruction is hence directly an effect of specimen sampling without making prior assumptions on the processes acting on species distribution patterns (vicariance vs. dispersal). This potentially also allows for phylo-geographic analyses of organism groups in a gene tree – species tree framework, allowing for simultaneous estimate of the species phylogeny and ancestral area reconstruction using genetic information from multiple sources.

5. Conclusions and future aims

Since the dawn of using nuclotide information in systematic contexts the array of analytical methods and new insights into phylogenetic relationships have increased for each year. Coupled with improved means for large scale sequencing systematics has become one of the fastest expanding research fields in recent years. At the same time there has been a lack of similar expansion and development in phylogenetic and biogeographic methods allowing for use of environmental information in the understanding of evolutionary processes.
Recent advancements in estimating species trees from independently evolving genealogies can, when integrating ecological niche preference information for species and full geographical data for included specimens, in conjuction provide a powerful tool for the future of biogeographic analyses.

As the species phylogeny and biogeography of the genus *Jovellana* can be considered answered in a general sense, much remains to be done in *Calceolaria*, such as:

- Providing a robust hypothesis of the phylogenetic position of the Calceolariaceae in Lamiales, preferrably using sequence information not limited to the chloroplast. As the family has been hypothesised as being closely related to the Gesneriaceae, the exact relationship between them remains uncertain.

- Analysing the phylogenetic relationships in *Calceolaria* in a species tree framework, providing a more complete picture on the morphological evolution within the genus.

- Further investigate the hypothesised relationship between the uplift history of the Andes and the radiation patterns in *Calceolaria*.

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