universität wien

DISSERTATION

Titel der Dissertation

Genetic diversity, speciation and evolutionary relationships in *Pozoa* (Apiaceae), *Nassauvia*, and the *Hypochaeris apargioides* complex (Asteraceae) in southern South America

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angestrebter akademischer Grad

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Wien, im Jänner 2010

Matrikelnummer:	0700918
Studienkennzahl lt. Studienblatt:	A091 438
Dissertationsgebiet lt. Studienblatt:	Botanik
Betreuer:	Univ.-Prof. Dr. Tod F. Stuessy

Acknowledgements

I express my sincere gratitude to the following people and institutions:

Prof. Dr. Tod F. Stuessy, who give me the opportunity to come to Vienna and work in this project. I appreciate his continued support concern with all my activities.

Prof. Dr. Rose Samuel, for their help during this time,

Prof. Dr. Josef Greimler, for his help in my arrival to Vienna, and with my work,

Dr. Karin Tremetsberger, for their incredible support and infinite patience to explain each of the analysis and show me the correct way to work with molecular data,

Dr. Werner Huber and Mag. Elisabeth Lindner, for giving me your unconditional friendship and constant support throughout my stay in Vienna,

Dr. Pedro Escobar, Dr. Carolin Anna Rebernig, Mag. Cordula Blöch, Mag. Anne-Caroline Cosendai, Mag. Khatere Emadzade, and Mag. Gudrun Kohl, colleagues and friends over the last 3 years, always ready to help,

Dr. Marcelo Baeza, and Dr. Patricio Peñailillo for their support from Chile,

Verena Klejna, Ing. Elfriede Grasserbauer, Mag. Michael Barfuss, Mag. Anton Russel, and Mag. Stefan Safer, for their assistance in laboratory activities and /or informatics help,

Mrs. Monika Paschinger, for her help in all logistic topics,

All my family in Chile,

FWF (Austrian Science Foundation) for financial support,

And in special to my wife Ms.Sc. Julia Cáceres Chamizo, and my daugther Miss Catalina López Cáceres, for all the support they have given me over the past three years, part of which we have had to remain separated by thousands of kilometer.

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Abstract

The processes involving colonization of new open areas, speciation, and adaptive radiation in plants of southern South American have been little investigated, and very few molecular methods have been applied. The aim of this thesis is to examine genetic diversity during populational divergence and speciation in flowering plants of this region, focusing on selected species of the genera *Nassauvia*, *Pozoa* and *Hypochaeris*. To achieve these objectives, morphometric analysis and Amplified Fragment Length Polymorphism (AFLP) have been employed, due to their known efficacy in revealing patterns of genetic variation within and among natural populations.

The first study deals with the genetics of colonizing species. The ecological gap created as a result of volcanic activity on Volcán Lonquimay on December 25, 1988 in south-central Chile, offers an excellent opportunity for studying the genetic diversity and structure of established and recently colonized populations of *Nassauvia lagascae* var. *lanata* (Asteraceae, Mutisieae) in areas on and around the Navidad cone. A reduced level of genetic divergence and genetic variation within colonizing populations has been found, reflecting a founder effect that has not yet been compensated by subsequent population growth and migration.

The second investigation focuses on modes of speciation. Progenitor-derivative speciation is a particular type of allopatric speciation, whereby an isolated peripheral population diverges to form a derivative species. The genus *Pozoa* (Apiaceae, Azorelloideae) is a good model to examine for this phenomenon, because it contains only two species, *Pozoa coriacea* and *Pozoa volcanica*. Sequences of chloroplast markers confirm monophyly of the genus, and molecular analysis suggests *P. volcanica* as newly derived from its progenitor *P. coriacea*. AFLP analysis reveals that the former harbors less genetic variation and lower levels of unique alleles, as well as having diverged into a distinct habitat.

The third study examines adaptive radiation in a species complex of continental distribution. Adaptive radiation refers to the formation of an evolutionary group that has undergone an extremely rapid diversification into a variety of ecological niches. One of the genera in South America where this process has occurred repeatedly is *Hypochaeris* (Asteraceae, Cichorioideae), which after arrival from northeastern Africa, has colonized a wide variety of ecological zones. Study of the *Hypochaeris apargioides* complex,

consisting of four closely related species, *H. apargioides*, *H. gayana*, *H. spathulata*, and *H. thrincioides,* suggests that the principal environmental conditions influencing distributions of species and morphological adaptations are salinity and elevation. The presence of numerous characters with intermediate stages, and the low levels of genetic cohesion within and among species, suggest an early stage of adaptive radiation.

Zusammenfassung

Prozesse wie die Kolonisierung neuer, vegetationsloser Gebiete, Artbildung und adaptive Radiation wurden bislang in Pflanzen des südlichen Südamerika relativ wenig erforscht und es wurden dabei kaum molekulare Methoden angewandt. Das Ziel dieser Doktorarbeit besteht darin, die genetische Diversität der Blütenpflanzen dieser Region während Populationsdivergenz und Artbildung zu untersuchen. Das Hauptaugenmerk wurde dabei auf ausgewählte Arten der Gattungen *Nassauvia*, *Pozoa* und *Hypochaeris* gelegt. Um dieses Vorhaben zu realisieren, wurden morphometrische Analysen und AFLP-Untersuchungen verwendet, die mit hoher Effizienz Variationsmuster innerhalb und zwischen natürlichen Populationen aufzeigen können.

Die erste Studie beschäftigt sich mit der Genetik kolonisierender Arten. Die ökologische Lücke, die als Folge der vulkanischen Aktivität des Vulkans Lonquimay im südlich-zentralen Chile am 25.12.1988 entstand, bietet eine hervorragende Gelegenheit, um die genetische Diversität und Struktur etablierter und neu kolonisierender Populationen von *Nassauvia lagascae* var. *lanata (*Asteraceae, Mutisieae) in den Gebieten um den Vulkankegel Navidad zu untersuchen. Es konnte bei den kolonisierenden Populationen ein geringerer Grad an genetischer Divergenz und Variation festgestellt werden. Dies spiegelt einen Gründereffekt wider, der noch nicht durch nachfolgendes Populationswachstum und Migration kompensiert wurde.

Die zweite Studie konzentriert sich auf Artbildungsmechanismen. Die Vorfahre-Abkömmling-Artbildung („progenitor-derivative speciation“) ist eine spezielle Form der allopatrischen Artbildung, wobei eine neue, abgeleitete Art durch Divergenz einer isolierten, peripheren Population der Vorfahren-Art entsteht. Die Gattung *Pozoa* (Apiaceae, Azorelloideae) besteht aus nur zwei Arten (*Pozoa coriacea* und *Pozoa volcanica*) und ist daher gut geeignet, um dieses Phänomen zu untersuchen. Durch die Sequenzanalyse von Chloroplastenmarkern wurde die Monophylie der Gattung bestätigt und die molekulare Analyse deutet darauf hin, dass sich *P. volcanica* neu aus ihrem Vorfahren *P. coriacea* entwickelt hat. Die AFLP-Ergebnisse zeigen im Fall von *P. volcanica* eine geringere genetische Variation und eine niedrigere Anzahl einzigartiger Allele. Außerdem zeigt *P. volcanica* Divergenz in ein unterschiedliches Habitat.

Die dritte Studie untersucht die adaptive Radiation in einem Artenkomplex mit kontinentaler Verbreitung. Unter adaptiver Radiation versteht man die Bildung einer evolutionären Gruppe, in der eine extrem schnelle Diversifikation in eine Vielzahl

ökologischer Nischen stattgefunden hat. Eine der Gattungen in Südamerika, bei der dieser Prozess wiederholt stattgefunden hat, ist *Hypochaeris* (Asteraceae, Cichorioideae). Die Gattung kolonisierte nach ihrer Einwanderung aus Nordwestafrika eine Vielfalt an ökologischen Zonen. Die Untersuchung des aus vier eng verwandten Arten (*H. apargioides*, *H. gayana*, *H. spathulata* und *H. thrincioides*) bestehenden *Hypochaeris apargioides*-Komplexes weist darauf hin, dass Salzgehalt und Höhe die hauptsächlichen Umwelteinflüsse sind, die die Verteilung der Arten und ihre morphologischen Anpassungen beeinflussen. Das Vorhandensein zahlreicher Merkmale mit intermediären Ausprägungen und der geringe Grad an genetischem Zusammenhalt sowohl innerhalb als auch zwischen den Arten deuten auf ein frühes Stadium adaptiver Radiation hin.

Co-Authorship Statement

Chapter one has been submitted as a manuscript to the *American Journal of Botany* co-authored with Dr. K. Tremetsberger (University of Natural Resources and Applied Life Science), Dr. T. Stuessy (University of Vienna), Dr. S. Gómez-González (University of Concepción), Ms.Sc. A. Jiménez (University of Concepción), and Dr. C. Baeza (University of Concepción). As the first author, I was in charge of analysis of the data set, literature review, and preparing the first version of the manuscript. The manuscript has been accepted for publication (*in press*)

Chapter two has been written as a manuscript co-authored with Dr. K. Tremetsberger (University of Natural Resources and Applied Life Science), Mag. G. Kohl (University of Vienna), and Dr. T. Stuessy (University of Vienna). My contribution to this manuscript included data collection, analyzing of the data, setting the research question, and preparing the first version of the manuscript. This chapter will be submitted to *Evolution* for possible publication.

Chapter three will be revised into a manuscript co-authored with Dr. K. Tremetsberger (University of Natural Resources and Applied Life Science), Dr. M.A. Ortiz (University of Sevilla), and T. Stuessy (University of Vienna). As the first author, I was in charge of data collection, analyzing of the data, literature review, selecting the measurements, and prepare the first version of the manuscript. This will be submitted for publication to the *New Phytologist*.

All co-authors contributed to the identification and design of the research project and have assisted in different ways in the preparation of these manuscripts. I will be in charge of submitting chapter two and three for publication (as I do with chapter one).

Introduction

The theory of biological evolution, or the changes affecting a group of organisms over many generations by means of natural selection (Futuyma, 2005), revolutionized the way we see and understand life. During the last century, new genetic and mathematical perspectives have generated theoretical and practical foundations for emergence of the modern synthesis of evolution, including concepts relating to the mechanisms of recombination, mutation, and genetic drift, and impact from the process of natural selection.

For evolution to occur, however, genetic variation is required that allows a population to increase chances of exploiting new resources to survive catastrophic events, maintain a high degree of reproductive performance, and to adjust to new and changing environments (Foster, 1991). Moreover, the diversity represented by individuals within different populations, when subjected to certain genetic, geographic, or reproductive events, allows formation of new species. In certain situations, if populations of an ancestral species diverge rapidly each into a different environment, adaptive radiation can occur (Schluter, 2000).

The three concepts of diversity, speciation and adaptive radiation, therefore, will be addressed in this thesis. To address these points, models have been used from three different genera of flowering plants: *Pozoa* of Apiaceae, and *Hypochaeris* and *Nassauvia* of Asteraceae. All are restricted to southern South America. Details are provided below.

The Apiaceae comprises approximately 400-450 genera and approximately 3500-3700 species, with a cosmopolitan distribution, although most common in temperate areas. The principal characters are an umbellate inflorescences, fruits with two mericarps, and minute epigyneous flowers (Downie *et al.*, 1998). Traditionally the family has been divided into four subfamilies, Apioideae, Saniculoideae, Azorelloideae, and Mackinlayoideae. The Azorelloideae subfamily includes 12 genera and approximately 150 species (Heywood *et al.*, 2007). The genus *Pozoa* consists of only two species, divergent morphologically and distributed in the Andes of Chile and Argentina (Mathias and Constance, 1962)

Asteraceae include approximately 24,000 species in all environments except in Antarctica. Diagnostic characters are floret organized on a receptacle surrounded by bracts, fused anthers, and achaenes usually with a pappus. Although the family is

monophyletic, the morphological variations within the group are considerable. Species are grouped into 12 subfamilies and 43 tribes (Funk *et al.*, 2009). In the context of this thesis, two subfamilies need our attention.

The first is subfamily Mutisioideae, formed by tribes Mutisieae, Onoseriidae, and Nassauvieae. In particulary, the tribes Nassauvieae grow primarily in southern South America (Andes and Patagonia), including a total of 25 genera and more than 300 species. The diagnostic characters are a bilabiate corolla, and the style papillae form a tuft restricted to the branch apex (Katinas *et al.*, 2009). The genus *Nassauvia* includes 37 species distributed in Argentina, Bolivia, and Chile. These taxa grow in different habitats, such as in moist localities in sandy soil near streams, or in xerophytic regions among rocks. Pollination is entomophilous, and dispersal of fruits apparently is hydrophilic apparently hydrophilic (Cabrera, 1982).

The second subfamily is Cichorioideae, consisting of seven tribes. In particular the tribe Cichorieae comprises approximately 90 genera and 1400 species, growing especially in the temperate zone. The morphology is characterized by homogamous capitula, ligulate flowers, and lactiferous canal (Kilian, 2009). In particularly, the genus *Hypochaeris* is distributed in Europe with only 15 species, but in South America these are about 45 (Tremetsberger *et al.*, 2006; Zuloaga *et al.*, 2008). Diagnostic features for the genus include presence of flowers protected by a palea, floret yellow or white, achaenes with or without a peak, and most of the species with a single or double pappus (Ortiz, 2008). The arrival of the genus to South America occurred presumably about 1.0-3.5 million years by transoceanic dispersal from Africa (Tremetsberger *et al.*, 2005). Its rapid diversification in South America in a short time is explained by the existence of process of adaptive radiation that have allowed colonization of many habitats on the continent, except for the tropics and very dry deserts. (Tremetsberger *et al.*, 2006).

This doctoral thesis is organized into three chapters, with focus on understanding genetic diversity during populational divergence and speciation in flowering plants of southern South America using the genera *Pozoa* (Apiaceae), *Nassauvia*, and *Hypochaeris* (Asteraceae). The methodologies to achieve these objectives include morphometric analysis, sequencing of noncoding chloroplast region, and specially Amplified Fragment Length Polymorphism (AFLP, Vos *et al.*, 1995). The latter methodology has proven efficacious in revealing patterns of genetic variation in natural populations and the structure of intra- and inter-specific taxa. The three chapters of the thesis have been

formatted for specific journals (Chapter One, *American Journal of Botany*; Chapter Two, *Evolution*; Chapter Three, *New Phytologist*). Details of these chapters are as follows.

Chapter One, **Patterns of genetic diversity in colonizing plant species: *Nassauvia lagascae* var. *lanata* (Asteraceae:Mutisieae) on volcan Lonquimay, Chile.**

The establishment of pioneer plants in a landscape gaps is the first step to colonizing new open area. Subsequently through succession, a new ecological balance is achieved among the organisms that live there. In particular, the colonization of landscape gaps created as a result of volcanic activity offers an opportunity for studying the genetic variability and structure of colonizing and surviving populations.

The objectives of this chapter are to: (1) determine the levels of genetic variation in established and colonizing population of *Nassauvia lagascae* var. *lanata* located in areas around the Navidad cone of Volcán Lonquimay, which exploded on 25 December 1988, and (2) infer the relationships among established and colonizing population in the Lonquimay and surrounding area. To achieve these goals the molecular technique of Amplified Fragment Length Polymorphism (AFLP; Vos *et al.*, 1995) was used, complemented with environmental and demographic characteristics of the population. A total of 240 individual belonging to 15 populations was analyzed.

Chapter Two, **Progenitor-derivative speciation in *Pozoa* (Apiaceae, Azorelloideae) of the Southern Andes.**

The speciation process is linked to the creation of geographical, ecological and reproductive barriers that allow the emergence of new genetic combination in different populations, and that through natural selection permit the appearance of a new species (Grant, 1981; Levin, 2003; Coyne and Orr, 2004; Bolnick and Fitzpatrick, 2007). Different modes of geographic speciation are known to have occurred among higher plants. The progenitor-derivative speciation is a particular type of allopatric speciation, whereby an isolated peripheral population diverges to form a derivative species (Jaramillo-Correa and Bousquet, 2003).

A very interesting case study is the genus *Pozoa* in the Andes Mountain in South America, which with a limited geographical distribution, similar morphology among the species, and adaptations to different environments, allows hypothesizing with regard to the process of speciation. This chapter tests the hypothesis of progenitor-derivative speciation within *Pozoa*.

The objectives of this chapter are to: (1) test if *Pozoa* is a monophyletic genus; (2) determine which species of *Pozoa* is ancestral to the other; and (3) investigate levels of genetic divergence and variation in the derived species in comparison to its progenitor. Sequences of non-coding region of chloroplast DNA and AFLPs were used to reach these aims. A total of 21 populations of *P. coriacea* and *P. volcanica* were analyzed throughout the entire range of distribution of both species.

Chapter Three, **Adaptive radiation in the *Hypochaeris apargioide*s complex (Asteraceae, Cichorioideae) in southern of South America.**

Adaptive radiation refers to the formation of evolutionary groups that exhibit an extremely rapidly diversification into a variety of ecological niches (Schluter, 2000). In this way an ancestral species can radiate into many groups through the development of evolutionary innovation, providing advantages for colonization and adaptation to particular environmental. To study this process in detail, especially at the molecular level, the *Hypochaeris apargioides* complex was used, which consist of four species restricted to southern South America. These are *H. apargioides, H. gayana, H. spathulata*, and *H. thrincioides,* distributed mainly in Chile (with some extension into Argentina) in different habitat from the high Andes cordillera to the coast of the Pacific Ocean.

The aims of this chapter are to: (1) examine pattern of morphological variation within each species of the complex using morphometric and greenhouse experiment; (2) examine pattern of genetic variation within and among population of each species using AFLP technique; and (3) relate observed variation to know environmental parameters occurring within the distributed area. A total of 34 populations in morphometric study, 4 populations in greenhouse experiment, and 47 populations in AFLP analyses were used.

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PATTERNS OF GENETIC DIVERSITY IN COLONIZING PLANT SPECIES: *NASSAUVIA LAGASCAE* VAR. *LANATA* (ASTERACEAE: MUTISIEAE) ON VOLCÁN LONQUIMAY, CHILE.[1]

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(Accepted in *American Journal of Botany*)

[1] Manuscript received _____; revision accepted_____.

The authors wish to thank the Corporación Nacional Forestal (CONAF) for permission to collect samples in Chilean National Parks, R. Hössinger (Vienna) and M. J. Parra (Concepción) for help with collecting, G. Kadlec (Vienna) for technical assistance, M. Á. Ortiz (Seville), C. A. Rebernig (Vienna), and A.-C. Cosendai (Vienna) for help with programs, and M. Mort (Lawrence, Kansas) and an anonymous reviewer for very helpful comments on a previous draft of the manuscript. This project was funded by the Austrian Science Fund (FWF; grant P18446 to TFS). AJ is financed by the Institute of Ecology and Biodiversity (ICM P05-002, PFB-23) and a doctoral grant (CONICYT). SGG is financed by the Institute of Ecology and Biodiversity (ICM P05-002) and a post-doctoral grant (FONDECYT-3090018).

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ABSTRACT

The effect of colonization on the distribution of genetic diversity within and among populations in relation to species characteristics remains an open empirical question. The objective of this study is to contrast genetic diversity within and among established and recently colonized populations of *Nassauvia lagascae* var. *lanata* on Volcán Lonquimay (Araucanía Region, Chile), which erupted on December 25, 1988, and relate it to biological characteristics of the populations. We analyzed a total of 240 individuals from 15 populations distributed along the Andes Cordillera using AFLP and obtained a total of 307 AFLP bands, of which 97.7% are polymorphic. Values of population differentiation (F_{ST}) are not significantly different among established and recently colonized populations, but recently colonized populations do have reduced levels of genetic divergence (as indicated by private and rare bands) and genetic variation (e.g., Shannon index). We conclude that a founder effect through limited numbers of founding propagules derived from nearby source populations has not yet been compensated for by subsequent population growth and migration. Low rates of secondary dispersal via running water, kin-structure within populations, and slow population growth seem to contribute to the slow recovery of genetic diversity.

Key words: AFLP; Andes; colonization; Compositae; dispersal ability; *Nassauvia*; population genetic parameters; volcanoes.

INTRODUCTION

Disturbances in the landscape created by volcanic activity offer an excellent opportunity to study the effect of such disturbances on the genetic structure and variability within and among populations. Extinction and re-colonization after local disturbances could result in sampling from the available gene pool (founder effect) or in additional gene flow. The relative contributions of these opposing forces in different plant systems and their consequences for the genetic composition of the species as a whole remain open empirical questions.

From a population genetic standpoint, the founder effect impacts allele frequencies and genetic diversity in recently colonized populations, whereby immigrant seeds carry only a small sample of alleles from the source population (Slatkin, 1977; Pannell and Charlesworth, 1999; Silvertown and Charlesworth, 2001). The founder event is associated with a decline in genetic diversity, because it is less likely that rare alleles are included in the colonizing individuals, thus favoring the most common alleles. It was early recognized that reduction in average heterozygosity depends on both the size of the bottleneck (or strength of the founder effect) and the rate of population growth (Nei et al., 1975). If population size increases rapidly after going through a bottleneck (or founder event), the reduction in average heterozygosity is rather small. Loss in the average number of alleles per locus, however, is profoundly affected by bottleneck size (Nei et al., 1975). Slatkin (1977) emphasizes the importance of the mode of establishment of new populations for genetic variability in colonizing populations. According to him, colonization after local extinction has two consequences. The first is an additional sampling process similar to genetic drift resulting from the sampling of the colonizing individuals from their source populations (founder effect). The second is an additional component to gene flow between the local populations, because the colonizing individuals originate from one or more of the local populations. The direction and the magnitude of the effect of colonization after local extinction are then dependent on the relative contributions of the two opposing forces (genetic drift versus gene flow). Wade and McCauley (1988) refine Slatkin's (1977) models and relate the effect of the extinction/colonization process on genetic variability to the source of propagules (from one or several local populations) and the relative number of propagules colonizing vacant habitats (compared to the number of migrants between extant populations). Whitlock and

McCauley (1990) add the importance of kin-structure and inbreeding within recently colonized populations as critical factors affecting genetic variability after extinction and colonization, whereby kin-structure and inbreeding lead to increased population differentiation.

With time, migration would reduce the differentiation between populations caused by colonization (Pannell and Dorken, 2006). Concomitantly, the immigration process after colonization determines the speed at which the population can recover the genetic variation lost during the founder effect (Ingvarsson, 1997). In summary, results from population genetic theory suggest that a variety of factors impact allele frequencies and genetic diversity in recently colonized populations. These include the relative number of founding propagules in comparison to migrants among extant populations, the probability of common origin of the founding propagules, kin-structure and inbreeding within the recently colonized populations, and rate of population growth and immigration after colonization.

Colonization of landscape gaps created as a result of volcanic activity offers an excellent opportunity for studying the genetic diversity and structure of colonizing and surviving populations. The number of studies on continental environments, however, is small, and most of them are concerned with the settlement of Mount St. Helens, USA (del Moral and Wood, 1993; del Moral, 1998; del Moral and Eckert, 2005; del Moral and Lacher, 2005; Yang et al., 2008). In this context, it is profitable to examine Volcán Lonquimay (located in the southern Andes of Chile at ~38° S), which experienced a major eruption on December 25th of 1988, causing the formation of a new side cone, "Navidad". The emission column during the activity of the volcano reached 9,000 m, with a total volume of lava emitted of around 180,000,000 m^3, which mainly covered old lava deposits. Acid rain, falling ash (to the southeast), and lava flow resulted in the destruction of the surrounding vegetation. Due to the intensity of the eruption, no diaspores are believed to have survived in the area covered with ash. In the years following the eruption, specialized colonizers arrived, including *Chaetanthera villosa*, *Hypochaeris tenuifolia*, *Nassauvia argentea* and *N. lagascae* var. *lanata* (all Asteraceae), *Loasa nana* (Loasaceae), and *Pozoa volcanica* (Apiaceae).

This study seeks to determine the levels of genetic diversity in established and recently colonized populations of *Nassauvia lagascae* var. *lanata* located in areas around

the Navidad cone of Volcán Lonquimay and also to infer the relationships among established and recently colonized populations in the Lonquimay and surrounding area. We selected the AFLP (Vos et al., 1995) fingerprinting technique because of its high efficacy to reveal patterns of genetic diversity in natural populations (e.g., Gaudeul et al., 2000; Nybom, 2004; Andrade et al., 2009). Furthermore, we relate the levels of genetic diversity in established vs. recently colonized populations with population size as well as environmental and biological characteristics of the populations (e.g., vegetation coverage, attributes of vegetative growth and reproduction). The results are discussed in comparison with previous results from *Hypochaeris tenuifolia* (Asteraceae) from the same study area (Tremetsberger et al., 2003). In contrast to *Hypochaeris*, *Nassauvia* has low dispersal ability (Castor, 2002). We therefore expect to find evidence for a founder effect in recently colonized populations of *Nassauvia lagascae* var. *lanata*.

MATERIAL AND METHODS

The species—*Nassauvia lagascae* (D.Don) F.Meigen var. *lanata* (Phil.) Skottsb. (Kongl. Svenska Vetensk. Acad. Handl. 56(5): 329. 1916), is a perennial, cushion-forming herb, with ascending or decumbent stems, a few centimeters high and densely covered with leaves up to the apex. Leaves are imbricate, oblanceolate-spatulate to obovate-espatulate, recurvate, and densely woolly on the lower side. Capitula are numerous, arranged in very dense globulate spikes at the tips of the branches. The involucre is cylindrical with woolly phyllaries. Flowers are white and smell subtly sweetish. Achenes (cypselas) are glabrous, with a pappus consisting of numerous linear plumose bristles (Cabrera, 1982), which detaches easily from the rest of the fruit. No experiments have been carried out to determine the breeding system of *Nassauvia* species, but the white, fragrant flowers suggest an outcrossing mode. The variety grows in the Andes from the Maule Region to the Araucanía Region in Chile and from the south of the Mendoza Province to the Santa Cruz Province in Argentina (~35-52°S; Cabrera, 1982).

Sampling—In the Lonquimay and surrounding area (Araucanía Region, Chile), *N. lagascae* var. *lanata* grows in an altitude of ~1500–2200 m. Thus, it has an island-like distribution on the volcanoes and mountain tops. Because the focus of this study is to compare genetic composition of established and recently colonized populations, we put the emphasis of our sampling in the Lonquimay and surrounding area. We sampled six established populations (three in the immediate vicinity of Volcán Lonquimay and three in the surrounding area [Sierra Nevada, Llaima, and Pino Hachado]), seven recently colonized populations (growing on the ash fields of the December 1988 eruption of the Navidad cone), and one population growing on ash from an older eruption of Volcán Lonquimay (Table 1, Fig. 1). Two populations further north (Chillán, Biobío Region, Chile, and Copahue, Neuquén Province, Argentina) and one population further south (Villarrica, Araucanía Region, Chile) were also sampled. Additional potential habitats of *N. lagascae* var. *lanata* in the farther adjacencies of Volcán Lonquimay, from which we do not have material, include Volcán Callaquí and the Nevados de Sollipulli. Leaves of 16 individuals per population were collected on silica gel. Individuals were chosen randomly throughout the area occupied by the populations. Vouchers of each population sampled are on deposit in the herbarium WU.

Established populations in the Lonquimay and surrounding area (pops. 3-7) grow on volcanic lava and ash as well as non-volcanic, siliceous debris and sand. The altoandine vegetation includes *Adesmia longipes* (Fabaceae), *Azorella* spp. (Apiaceae), *Cerastium arvense* (Caryophyllaceae), *Empetrum rubrum* (Empetraceae), *Ephedra andina* (Ephedraceae), *Gamocarpha alpina* (Calyceraceae), *Haplopappus* spp. (Asteraceae), *Loasa nana* (Loasaceae), *Mulinum spinosum* (Apiaceae), *Oreopolus glacialis* (Rubiaceae), *Oxalis adenophylla* (Oxalidaceae), *Poa* spp. (Poaceae), *Pozoa volcanica* (Apiaceae), and *Senecio* spp. (Asteraceae).

The recently colonized populations (pops. 8-13A) grow on volcanic ash. The soil consists of a >15 cm thick layer of volcanic ash homogeneously mixed with very little organic material. In two populations (pops. 11 and 13A), a stony brown soil is topped by 5 cm volcanic ash. The competitors include *Chaetanthera villosa* (Asteraceae), *Hypochaeris tenuifolia* (Asteraceae), *Nassauvia argentea* (Asteraceae), *Loasa nana*, *Oxalis adenophylla*, *Poa* sp., and *Pozoa volcanica*. Population 14 grows on ash from an older eruption of the volcano with *Euphorbia collina* (Euphorbiaceae), *Loasa nana*, *Phacelia secunda* (Hydrophyllaceae), and Poa sp.

The northern populations (pops. 1 and 2) were collected in stable scree and earth on volcanic and non-volcanic substrate with typical altoandine vegetation; the competitors include *Acaena* spp. (Rosaceae), *Adesmia* spp., *Draba gilliesi* (Brassicaceae), *Gamocarpha alpina*, *Nassauvia revoluta*, *Olsynium junceum* (Iridaceae), *Pozoa coriacea*, and *Senecio* spp.

Finally, the southern population (pop. 15) grows on stable scree and earth, in volcanic lava and ash. The competitors include *Adesmia emarginata*, *Gaultheria phillyreifolia* (Ericaceae), *Nassauvia revoluta*, *Poa* spp., and *Senecio* spp.

Population characteristics—For each population, we estimated the following parameters in order to relate them to inferences of genetic diversity: total number of individuals, area occupied (m^2), average diameter of plants, average height of plants, number of shoots per individual (median of 10 plants), proportion of reproductive individuals (with flowers or fruits), number of flowering shoots per reproductive

individual (median of 10 plants), as well as coverage and height of the herb layer. In all populations, we searched for seedlings. The Mann-Whitney *U* test was used to estimate the significance of differences in population characteristics between established and recently colonized populations in the Lonquimay and surrounding area using SPSS ver. 15.0 (© SPSS Inc.).

AFLP fingerprinting—We scored 240 individuals from 15 populations of *N. lagascae* var. *lanata* for three AFLP primer combinations (two populations [pops. 6A and 13A] have not been subjected to AFLP analysis). Genomic DNA was extracted from silica-gel dried leaf material following the CTAB method (Doyle and Doyle, 1987) with minor modifications (Tremetsberger et al., 2003). The AFLP protocol followed Vos et al. (1995) with modifications as indicated in Tremetsberger et al. (2003). The selective primer combinations chosen following a primer-trial are *Mse*I-CTAG/*Eco*RI-ACT (Fam), *Mse*I-CACC/*Eco*RI-ACG (Hex), and *Mse*I-CATA/*Eco*RI-ACC (Ned). The software Genographer ver. 1.6.0 (Benham, 2001) was used for scoring of AFLP bands. Presences and absences of bands in the size range of 100–500 bp were scored in all individuals in a single file after normalizing on total signal. Criteria for selecting AFLP bands were visual clarity, straightforward interpretability, and similar fluorescence intensity across individuals. Cutoff levels were adjusted for each selected band and automatic scores were visually checked and modified if necessary.

Estimation of divergence of populations and within-population genetic variation—The number of different AFLP phenotypes present in a population was counted with Arlequin ver. 3.1 (Excoffier et al., 2006). Divergence of populations was estimated via the occurrence of private bands, i.e. those bands confined to only one population, and rare bands. The number of private bands in each population was counted using FAMD ver. 1.108 (Schlüter and Harris, 2006). The Rarity Index or DW (frequency-down-weighted marker values) was first applied by Schönswetter & Tribsch (2005) for AFLP data, but is equivalent to range-down-weighted species values in historical biogeographical research (Crisp et al. 2001). It was calculated with the R-script AFLPdat (Ehrich 2006; last modified 23 January 2008) in R ver. 2.6.0 (© The R Foundation for Statistical Computing; available from http://www.r-project.org/). For each individual, each AFLP band was divided by the total number of occurrences of this band in the data

set. These relative values were then summed to the rarity index for this particular individual. Population values were estimated as the average of the individual values. The presence of private and rare bands is characteristic of populations with a long *in situ* history, most probably going back to the last glaciation (Schönswetter & Tribsch 2005; Ehrich et al. 2008).

Within-population genetic variation was assessed for each population by the total number of AFLP bands, percentage of polymorphic bands (by dividing the number of polymorphic bands by the total number of bands in the dataset), and Shannon diversity index $H_{Sh} = -\Sigma(p_i \times \ln(p_i))$, where p_i is the frequency of the i^{th} band in the respective population based on all AFLP bands recorded using FAMD ver. 1.108 (Schlüter and Harris, 2006). The Pearson correlation was used to test correlation among different estimates of genetic variation using SPSS ver. 15.0 (© SPSS Inc.). The Mann-Whitney *U* test was used to estimate the significance of differences of divergence of populations and within-population genetic variation between established and recently colonized populations in the Lonquimay and surrounding area using SPSS.

Estimation of population differentiation—Genetic differentiation among local populations was assessed by analysis of molecular variance (AMOVA) using Arlequin ver. 3.1 (Excoffier et al., 2006), where total genetic diversity was partitioned into components among two hierarchical levels, among populations (F_{ST}) and among individuals within populations. An alternative Bayesian approach (Holsinger et al., 2002) was used to obtain an independent estimate of F_{ST} in established and recently colonized populations. This method allows estimation of F_{ST} from dominant markers without assuming Hardy-Weinberg proportions in populations. The original data matrix was imported into Hickory ver. 1.1 (Holsinger and Lewis, 2003-2007) and used for a full model, $f = 0$ model, theta = 0 model, and *f*-free model run with default parameters (i.e., the hickory block omitted). The *f*-free model, which estimates theta without estimating *f* (thus incorporating all the uncertainty in the prior of *f*), is available for dominant marker data, because estimates of *f* derived from dominant marker data may be unreliable. The deviance information criterion (DIC; Spiegelhalter et al., 2002) was used to estimate how well a particular model fits the data and to choose between models.

Population structure—In order to examine the population structure of *Nassauvia lagascae* var. *lanata* we performed Bayesian clustering using BAPS ver. 5.1 (Corander et al. 2003, 2004; Corander and Marttinen, 2006), which uses stochastic optimization to find the optimal partition. Simulation were run from $K = 2$ to $K = 16$ with five replicates for each number of clusters (K). Admixture clustering based on results of mixture clustering was performed with the following settings: minimal size of clusters at five individuals, 100 iterations to estimate the admixture coefficients for the individuals, 200 simulated reference individuals from each population, and 20 iterations of each reference individual.

To construct a phenogram representing genetic distances among populations, population-pairwise F_{ST} values were generated using Arlequin ver. 3.1 (Excoffier et al., 2006). The F_{ST} values were used to construct a neighbor-joining (NJ) tree in PAUP* ver. 4.0b10 (Swofford, 2002). Support for each node was tested with 500 bootstrap replicates of the NJ method in conjunction with Nei and Li's (1979) genetic distances on the original presence/absence matrix in PAUP*.

RESULTS

AFLP—The total number of AFLP bands found in all individuals and all populations is 307, of which 300 (97.7%) are polymorphic. The primer combination *Mse*I-CTAG/*Eco*RI-ACT (Fam) yielded 104 bands in the range of 100–486 bp, *Mse*I-CACC/*Eco*RI-ACG (Hex) yielded 96 bands in the range of 104–474 bp, and *Mse*I-CATA/*Eco*RI-ACC (Ned) yielded 107 bands in the range of 100–440 bp. All individuals have unique AFLP phenotypes.

Divergence of populations and within-population genetic variation—The number of private bands and the Rarity Index were used to estimate divergence of populations. In the Lonquimay and surrounding area, the established populations (pops. 3-7) have significantly higher values for these indices than the recently colonized populations (pops. 8-13; Table 2, Fig. 2A). Population 14 on ash from an older eruption has a low value for the Rarity Index, similar to the recently colonized populations. The northern populations (pops. 1 and 2) and the southern population (pop. 15) have comparably high values (similar to those found in the established populations of the Lonquimay and surrounding area).

The three estimates of genetic variation, total number of bands, percentage of polymorphic bands, and Shannon diversity, are all correlated. For example, the Pearson correlation between Shannon diversity and total number of bands is $r = 0.967$ ($N = 15$, sig. [2-tailed] = 0.000) and between this index (Shannon) and percentage of polymorphic bands $r = 0.974$ ($N = 15$, sig. [2-tailed] = 0.000). The estimates of genetic variation vary among populations (Table 2, Fig. 2B). In the Lonquimay and surrounding area, the established populations (pops. 3-7) have on average higher values for all three estimates of genetic variation than the recently colonized populations (pops. 8-13), although the differences are not significant. Population 14 on ash from an older eruption has low values for estimates of genetic variation, similar to the recently colonized populations. The northern populations (pops. 1 and 2) have comparably low values and the southern population (pop. 15) has intermediate values.

Among-population genetic diversity and geographical structure—Analysis of molecular variance (AMOVA) attributes 15.5% variance (d. f. = 14) among the 15

populations and 84.5% variance (d. f. = 225) among individuals within populations. The variance among the established populations in the immediate vicinity of Volcán Lonquimay (*N* = 3; pops. 3-5) is 8.6% (d. f. = 2; 95% C.I. = 6.4-10.7%); among the recently colonized populations (*N* = 6; pops. 8-13), it is 7.9% (d. f. = 5; 95% C.I. = 6.2-9.6%).

In a Bayesian analysis of the genetic variance among populations, the best approximation yielding the lowest DIC value was with the full model. For the 15 populations and using the full model (DIC value = 8936.3), the value of theta-II (corresponding to theta-B in previous versions of Hickory) is 0.125 (95% credible interval = 0.115-0.136). Among the established populations in the immediate vicinity of Volcán Lonquimay (*N* = 3; pops. 3-5) and using the full model (DIC = 2038.5), the value of theta-II is 0.073 (95% credible interval = 0.053-0.096). Among the recently colonized populations (*N* = 6; pops. 8-13) and using the full model (DIC = 3262.6), the value of theta-II is 0.061 (95% credible interval = 0.040-0.076). DIC values obtained with the *f*-free model, which estimates theta without estimating *f*, are not much higher than those obtained with the full model and values for theta-II estimated by the *f*-free model are also very similar to those estimated by the full model (data not shown). The values for genetic differentiation among established populations and among recently colonized populations obtained by AMOVA and Hickory analyses are very similar. Thus, established and recently colonized populations in the immediate vicinity of Volcán Lonquimay have very similar levels of population differentiation.

A neighbor-joining clustering based on pairwise F_{ST} values among populations (Fig. 3) reveals the strongest separation between the two northern populations (pops. 1 and 2) and the other populations (all populations in the Lonquimay and surrounding area as well as the southern population; pops. 3-15). These results are consistent with those obtained by Bayesian clustering (Fig. 3), which assigns the individuals of the two northern populations (pops. 1 and 2) to one group (blue). Individuals of the populations in the Lonquimay and surrounding area (pops. 3-14) are intermixed in two groups (green and red). The southern population (pop. 15) is in a separate group (yellow), but some individuals in the Lonquimay and surrounding area are also assigned to this yellow group.

Population characteristics—When comparing population characteristics of the six established and seven recently colonized populations in the Lonquimay and surrounding area, recently colonized populations (pops. 8-13A) are smaller in size and occupy a smaller area than the established populations (pops. 3-7), though these differences are not statistically significant (Table 3). Population 14 on ash from an older eruption occupies a large area, similar to established populations. The two northern populations (pops. 1 and 2) are comparatively small in size and area, whereas the southern population (pop. 15) is very large.

In the vegetative growth category, the recently colonized populations (pops. 8-13A) are clearly more vigorous than established populations (pops. 3-7) as shown by the parameters diameter of plants, height of plants, and number of shoots per individual (Table 3; differences are statistically significant at the 0.05 level). Population 14 on ash from an older eruption is in the range of established populations in terms of diameter. The northern populations (pops. 1 and 2) and the southern population (pop. 15) are similar to established populations of the Lonquimay and surrounding area with respect to vegetative vigor.

In the reproduction category, the proportion of reproductive individuals in the populations and the number of flowering shoots per reproductive individuals are not significantly different among established (pops. 3-7) and recently colonized populations (pops. 8-13A; Table 3). Population 14, the northern populations (pops. 1 and 2) and the southern population (pop. 15) also have similar values.

Regarding the occurrence of seedlings in the populations, in five out of the eight recently colonized populations on Volcán Lonquimay (pops. 8-12), some seedlings were observed close to their presumed mother plants; no seedlings were observed for the other two recently colonized populations (pop. 13 and 13A) and for population 14, but populations 13 and 13A had some one-year old plants. Similar observations were made for five of the six established populations sampled here (observations were not made for pop. 3). This feature varied from many seedlings (pop. 4) to no seedlings (pop. 7) with populations 5, 6, and 6A having some seedlings close to their presumed mother plants. In the northern populations (pops. 1 and 2) and the southern population (pop. 15), no seedlings were observed.

In the general vegetation category, the coverage of the herb layer is significantly less in the recently colonized populations (pops. 8-13A) than in the established populations (pops. 3-7; Table 3). Population 14 has a low coverage like recently colonized populations. In the northern populations (pops. 1 and 2) and the southern population (pop. 15), the coverage is similar to that of established populations. To the contrary, the height of the herb layer is similar in established and recently colonized populations of the Lonquimay and surrounding area as well as in all other populations.

DISCUSSION

Effect of colonization on genetic diversity in Nassauvia lagascae var. lanata— We consider two aspects of the effect of colonization on genetic diversity, first the within-population component and second the among-population component (F_{ST}). A significant reduction of the number of private bands and Rarity Index in recently colonized populations in comparison to established populations suggests that there indeed was a founder effect, because rare alleles have not been transmitted by founding propagules (see Nei et al., 1975; we have to keep in mind, however, that only 16 individuals have been sampled per population and that inability to detect private and rare bands could also result from sampling error). Recently colonized populations also have reduced levels of within-population variation (as measured by the total number of bands, percentage of polymorphic bands, and Shannon diversity index) in comparison to established populations, although this reduction is not statistically significant.

Genetic differentiation (F_{ST}) among recently colonized populations, however, is not higher than among established populations in the immediate vicinity of Volcán Lonquimay (pops. 3-5), as would be expected with a founder effect. However, the number of generations needed to counterbalance a founder effect is unknown (see Pannell and Dorken, 2006), so that, alternatively, an eventual reduction of F_{ST} brought about by an initial founder effect could have been already abolished through population growth and immigration in subsequent years. If the newly exposed areas were colonized repeatedly from the surrounding populations, this would reduce F_{ST}. Thus, our results indicate that 14 years after creation of the vacant site a founder effect is still recognizable within populations as a significant reduction of rare alleles and a moderate reduction of within-population variation, but no longer at the among-population level.

Genetic diversity in relation to biological characteristics of the populations— Our estimates of the total number of individuals in the populations indicate that recently colonized populations are still rather small in comparison to established populations. Similarly, the area occupied by recently colonized populations is still smaller than that of established populations.

Plants of *N. lagascae* var. *lanata* in the recently colonized populations are significantly larger in their overall size than in the established populations (Table 3) indicating that the species is well adapted to the volcanic environment. We were not able to detect any significant differences, however, in reproductive features of plants in recently colonized versus established populations. The time from seedling establishment to first flowering and the periodicity of flowering in *N. lagascae* var. *lanata* are unknown. The plants are not expected to flower in their first year, as we have observed juvenile, probably one year-old plants, which were not yet in their reproductive stage. However, the majority of plants in the recently colonized populations seem to have reached their reproductive stage.

Nassauvia lagascae var. *lanata* also seems to be poorly adapted to dispersal over long distances. The great majority of fruits and seedlings remain in the immediate vicinity of the presumed mother plants (personal observation). The presence in this species of a pappus with deciduous bristles, plus a fruit with a waxy covering and lack of structures that facilitate dispersal, suggest that wind is not a relevant factor in dispersion. Diaspores appear to be dispersed via hydrochory involving water from rain or melting snow. Thus, secondary dispersal would have a greater importance, as also found in *Azorella madrepora*, *Madia sativa*, *Nassauvia pinnigera*, and *Pozoa coriacea*, all of which are species of the altoandine habitat (Castor, 2002). Considering the secondary dispersal of *N. lagascae* var. *lanata* via running water, immigration into newly available areas should come from survivor populations growing near the edges of the affected areas. Dispersal over longer distances (i.e., from different volcanoes and mountains), possibly through zoochory, is expected to be very rare. In coincidence with this expectation, the neighbor-joining tree reveals genetic affinities of the recently colonized populations with population 14 on ash from an older eruption and established populations in the immediate vicinity of Volcán Lonquimay (pops. 3-5) rather than with populations further away (pops. 6 and 7).

Our results show that the time elapsed from the creation of the new vacant habitat by the volcanic eruption to the date of sampling was not sufficient to restore genetic diversity within recently colonized populations. Population 14 growing on ash from an older eruption has also not recovered within-population genetic diversity yet. Reasons for

this might include slow population growth, kin-structure within populations, and infrequent long-distance dispersal events.

Broader geographical patterns in the southern Andes—In order to interpret groupings of populations of the Bayesian clustering and neighbor-joining tree on a broader geographical scale, it is necessary to consider the Pleistocene history of the region. During the last glacial maximum (c. 18–20 ka BP), the southern Andes were covered by a very large, continuous ice sheet, which reached the Araucanía region at its northern end (e.g., Singer et al., 2004; Rabassa 2008). North of the continuous ice sheet, local glaciers of decreasing size were developed. Groupings of populations of *N. lagascae* var. *lanata* revealed by Bayesian clustering and the neighbor-joining tree reflect this situation and parallel results obtained for other herbaceous species growing in the same area. For example, in *Hypochaeris tenuifolia*, the northern populations (including Chillán and Volcán Antuco) are distinct from a large group including all populations from the Lonquimay and surrounding area as well as populations further south (Volcán Villarrica and Volcán Lanín; Tremetsberger et al., 2003). This suggests that Pleistocene refugia of the altoandine vegetation isolated by glacial tongues were located north of the Araucanía region in the southern Andes, resulting in genetic distinctness of the refugial populations (e.g., in Chillán, Copahue, and Antuco). From the Araucanía region southwards, the Andes were re-colonized in the Holocene from adjacent refugia, resulting in a rather homogeneous genetic composition of populations.

Differences in patterns of colonization in plant species—Two early colonizers of volcanic ash fields in the southern Andes, *Nassauvia lagascae* var. *lanata* and *Hypochaeris tenuifolia*, both belonging to Asteraceae, have been studied. In *H. tenuifolia*, genetic diversity within and among established and recently colonized populations has also been investigated by means of AFLP on the volcanic ash fields resulting from the December 1988 eruption of the Navidad cone (Tremetsberger et al., 2003). The results indicated that population differentiation was lower among recently colonized populations (collected ten years after the eruption) than among other populations immediately and distantly outside the zone of disturbance. The genetic diversity within the recently colonized populations was not significantly different from that of the established populations. Closest genetic similarity occurred between recently colonized and nearby

established populations as well as populations in adjacent southern regions (Araucanía Region).

Thus, the effect of colonization on the distribution of genetic diversity within and among populations in the two perennial and probably outcrossing plants *N. lagascae* var. *lanata* and *H. tenuifolia* is different and these differences should relate to biological characteristics of the species. One major difference refers to dispersal capabilities, especially the ability to disperse over long distances. In *N. lagascae* var. *lanata*, whose offspring frequently remain in the close vicinity of the presumed mother plant and with long distance dispersals being probably rare events, immigrants to the newly available areas are probably few and recruited from the immediately surrounding areas. Population growth is also slow, enabling only a gradual development of within-population diversity. In contrast, *H. tenuifolia* possesses a well-developed and permanent pappus, which allows the primary dispersal of the fruit by wind far away from the parental plant (Andersen, 1993), probably including occasional among-site dispersal in the island-like altoandine habitats of the southern Andes. Immigrants of *H. tenuifolia* to the newly available areas are probably many and recruited from different source populations (Tremetsberger et al., 2003). Dispersal within populations should also be much more pronounced in *H. tenuifolia*, counteracting pronounced kin-structuring in populations. In addition, *H. tenuifolia* has a strong capacity to propagate vegetatively via underground stolons. Population growth in *H. tenuifolia* is fast, with the recently colonized populations having already exceeded the established populations in number of individuals only ten years after the eruption. In summary, therefore, there is evidence for an additional component to gene flow brought about through colonization in *H. tenuifolia*, but not in *N. lagascae* var. *lanata*, where we find evidence for a founder effect. In other words, the time scale for recovery of population sizes and within-population genetic diversity is fast in *H. tenuifolia* and slow in *N. lagascae* var. *lanata*.

Evidence for a founder effect such as inferred for *N. lagascae* var. *lanata* has also been found in other species, such as in *Lupinus lepidus* (Fabaceae; Bishop and Dyer, unpublished data) and in the two dioecious plants *Silene alba* (Caryophyllaceae; McCauley et al., 1995) and *S. dioica* (Giles and Goudet, 1997). Bishop and Dyer (unpublished data) examined the population genetic consequences of colonization in *Lupinus lepidus* var. *lobbii* on areas newly covered with ash of Mount St. Helens (USA)

after its eruption on 18 May 1980 based on size polymorphism of PCR products in two loci. They found a very strong founder effect with an increase of F_{ST} from 0.02 in surviving populations to 0.26 in newly founded populations (maximum four years old). Newly founded populations of *S. alba* (maximum four years old) showed only a small increase in F_{ST} (0.20) in comparison to older populations (0.13; McCauley et al., 1995). Similarly, *S. dioica* also showed a considerable increase of F_{ST} in younger populations (age < 30 years, size < 4,000 individuals, F_{ST} = 0.06) in comparison to intermediate populations, which had the highest genetic and demographic equilibrium in this system (30 years < age > 280 years, size > 4,000 individuals, F_{ST} = 0.03; Giles and Goudet, 1997). All of these species have low dispersal abilities similar to *N. lagascae* var. *lanata* (e.g., see Ingvarsson and Giles, 1999, for *S. dioica*). In a single less than 10 years old island population of *S. dioica* in the Skeppsvik Archipelago, Umeå, Sweden, Ingvarsson and Giles (1999) present evidence for kin-structured colonization resulting in as high or higher levels of genetic differentiation in the colonizing population in comparison to what is observed over larger scales in the archipelago. Similarly, Jacquemyn et al. (2009) investigated the genetic diversity in subsequent generations of a founding population of *Primula elatior* (Primulaceae) to understand the processes that affect them after the settlement. They found that genetic diversity increased substantially from the first generation to the second and third generations. *Primula elatior* is a species with limited seed dispersal similar to *N. lagascae* var. *lanata*. The authors found that seedling survival often occurs at particular micro-sites that are not heavily affected by competition leading to a highly clustered distribution pattern within the population. We hypothesize that kin-structure is also an important factor for slow increase of genetic diversity after colonization in *N. lagascae* var. *lanata*.

Contrasting results (i.e., an additional component to gene flow resulting from the colonization process) have been found in other colonizing species in addition to *Hypochaeris tenuifolia*, such as *Spartina alterniflora* (Poaceae; Travis et al., 2002) and *Vaccinium membranaceum* (Ericaceae; Yang et al., 2008). In *S. alterniflora* growing in restored versus natural wetlands, the genetic diversity in the restored population was as high as in the natural marsh populations (Travis et al., 2002). In the animal-dispersed *V. membranaceum* growing on volcanic deposits of Mount St Helens (Washington, USA), genetic diversity in the newly founded population 24 years post-eruption was higher than in most of the source regions, suggesting a lack of a strong founder effect (Yang et al.,

2008). Similar to *H. tenuifolia*, high gene flow among sources and long-distance dispersal were inferred to be important processes shaping the genetic diversity in the young *V. membranaceum* population.

In conclusion, this study demonstrates the importance of combining genetic, ecological, and demographic investigations in populations to understand better how colonization shapes the genetic structure of populations. It is clear that the effect of colonization can be in opposite directions (i.e., additional gene flow versus founder effect) in different species depending on the species' biological characteristics. Most importantly, the time frame needed for populations to reach equilibrium after colonization can be very different and is poorly understood. It would be advisable for future studies to perform population surveys in several subsequent time intervals on the same populations after colonization in order to trace the development of genetic diversity within and among populations in relation to population characteristics over time.

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TABLE 1. Collection data of populations of *Nassauvia lagascae* var. *lanata* in Chile used for the AFLP study. Vouchers are on deposit at WU. Populations 6A and 13A have not been subjected to AFLP analysis.

Region	Population	Collection number	Latitude	Longitude	Elevation (m)
North	1: Chillán	*KT et al. 1018*	36°54'08" S	71°23'46" W	2190
	2: Copahue	*KT et al. 1034*	37°49'53" S	71°06'44" W	2120
Volcán Lonquimay and surrounding area					
Established populations					
	3: Cerros de Lanco	*KT et al. 1066*	38°20'54" S	71°25'47" W	1835
	4: Tolhuaca	*KT et al. 1087*	38°21'02" S	71°36'13" W	1830
	5: Colorado	*KT et al. 17*	38°24'40" S	71°34'34" W	1900
	6: Sierra Nevada	*KT et al. 64*	38°36'54" S	71°35'45" W	1940
	6A: Llaima	*KT et al. 110*	38°41'26" S	71°46'27" W	1960
	7: Pino Hachado	*KT et al. 1041*	38°39'30" S	70°53'50" W	1900
Recently colonized populations (eruption of cone Navidad, December 1988)					
	8: Lonquimay	*KT et al. 1050*	38°21'50" S	71°31'16" W	2000
	9: Lonquimay	*KT et al. 1049*	38°21'50" S	71°31'37" W	1910
	10: Lonquimay	*KT et al. 1071*	38°22'05" S	71°31'48" W	1950
	11: Lonquimay	*KT et al. 1067*	38°22'12" S	71°32'18" W	1950
	12: Lonquimay	*KT et al. 97*	38°22'49" S	71°33'13" W	1930
	13: Lonquimay	*KT et al. 1047*	38°23'17" S	71°32'21" W	1975
	13A: Lonquimay	*KT et al. 32*	38°23'09" S	71°32'22" W	1960
Population growing on ash from an older eruption of Volcán Lonquimay					
	14: Lonquimay	*KT et al. 11*	38°24'32" S	71°34'06" W	1670
South	15: Villarrica	*KT et al. 1079*	39°23'57" S	71°57'46" W	1520

TABLE 2. Estimates of divergence of populations and within-population genetic diversity based on AFLP analysis from 16 individuals in each of 15 populations of *Nassauvia lagascae* var. *lanata*. The Mann-Whitney *U* test was used to assess the significance of differences between established and recently colonized populations in the Lonquimay and surrounding area. Significant differences are seen in number of private bands and Rarity Index.

Region	Population	Estimates of divergence		Estimates of diversity		
		Number of private bands	Rarity Index	Total number of bands	Percentage of polymorphic bands	Shannon diversity index
North	1: Chillán	13	2.0	114	32.9	26.3
	2: Copahue	5	1.1	96	26.1	20.5
	Mean (±SD)	**9.0 (±5.7)**	**1.6 (±0.6)**	**105.0 (±12.7)**	**29.5 (±4.8)**	**23.4 (±4.1)**
Volcán Lonquimay and surrounding area						
Established populations						
	3: Cerros de Lanco	2	1.1	123	35.5	27.9
	4: Tolhuaca	8	1.9	163	49.8	38.1
	5: Colorado	9	2.0	168	53.4	41.9
	6: Sierra Nevada	2	1.1	127	39.4	32.6
	7: Pino Hachado	4	1.3	129	40.1	29.1
	Mean (±SD)	**5.0 (±3.3)**	**1.5 (±0.4)**	**142.0 (±21.6)**	**43.6 (±7.6)**	**33.9 (±6.0)**
Recently colonized populations (eruption of cone Navidad, December 1988)						
	8: Lonquimay	0	0.8	111	31.6	22.4
	9: Lonquimay	1	0.7	112	32.9	23.6
	10: Lonquimay	2	1.1	138	40.7	30.3
	11: Lonquimay	2	1.2	135	40.4	32.9
	12: Lonquimay	0	0.8	118	36.2	26.7
	13: Lonquimay	1	1.0	135	42.0	31.9
	Mean (±SD)	**1.0 (±0.9)**	**0.9 (±0.2)**	**124.8 (±12.5)**	**37.3 (±4.4)**	**28.0 (±4.4)**
Population growing on ash from an older eruption of Volcán Lonquimay						
	14: Lonquimay	2	0.7	106	30.9	24.2
South	15: Villarrica	9	1.9	128	38.1	30.5
Mann-Whitney *U* test						
	Z	-2.441	-2.373	-1.098	-0.913	-1.461
	(2-tailed significance)	(0.015)	(0.018)	(0.272)	(0.361)	(0.144)

TABLE 3. Population characteristics of *Nassauvia lagascae* var. *lanata*. The Mann-Whitney *U* test was used to assess the significance of differences between established and recently colonized populations in the Lonquimay and surrounding area. Significant differences at the 0.05 level are seen in the three categories of vegetative growth (diameter, height, and number of shoots of plants) and coverage of herb layer; n. d. = no data.

		Size of population		Vegetative growth			Reproduction		General vegetation	
Region	**Population**	**Total number of individuals**	**Area (m²)**	**Average diameter of plants (cm)**	**Average height of plants (cm)**	**Number of shoots per individual**	**Proportion of reproductive individuals**	**Number of flowering shoots per reproductive individual**	**Coverage of herb layer (%)**	**Height of herb layer (cm)**
North	1	40	1,500	6.0	2.5	8.5	0.7	3.0	10.0	6.8
	2	300	2,500	7.0	2.0	9.0	0.9	5.0	5.0	2.6
	Mean (±SD)	**170 (±184)**	**2,000 (±707)**	**6.5 (±0.7)**	**2.3 (±0.4)**	**8.8 (±0.4)**	**0.8 (±0.2)**	**4.0 (±1.4)**	**7.5 (±3.5)**	**4.7 (±3.0)**
Volcán Lonquimay and surrounding area										
Established populations										
	3	50	1,000	n. d.	n. d.	10.0	0.9	3.0	4.0	3.0
	4	3,000	6,000	3.0	1.0	8.0	0.3	3.0	2.0	4.4
	5	100	5,000	7.0	1.8	14.5	0.8	1.0	25.0	1.9
	6	500	10,000	5.0	2.0	8.5	0.5	3.0	1.0	6.5
	6A	1,000	3,000	9.0	2.5	23.0	0.8	2.0	3.0	10.5
	7	1,000	30,000	6.0	3.0	10.0	0.7	3.0	10.0	4.0
	Mean (±SD)	**942 (±1,090)**	**9,167 (±10,647)**	**6.0 (±2.2)**	**2.1 (±0.8)**	**12.3 (±5.7)**	**0.7 (±0.2)**	**2.5 (±0.8)**	**7.5 (±9.1)**	**5.1 (±3.1)**
Recently colonized populations (eruption of cone Navidad, December 1988)										
	8	50	1,000	11.0	5.0	21.5	0.9	5.5	0.5	4.0
	9	250	5,000	10.0	4.0	24.5	0.9	8.0	0.2	3.5
	10	150	1,500	13.0	3.0	48.0	0.5	6.5	0.8	4.0
	11	50	1,250	6.0	2.0	9.0	0.5	2.0	0.5	4.3
	12	100	3,000	12.0	4.5	35.0	0.8	2.5	0.5	7.8
	13	540	1,800	10.0	4.0	31.0	0.8	3.0	1.5	5.5
	13A	300	1,500	10.0	4.0	43.0	0.8	3.5	1.5	5.5
	Mean (±SD)	**206 (±176)**	**2,150 (±1,411)**	**10.3 (±2.2)**	**3.8 (±1.0)**	**30.3 (±13.3)**	**0.7 (±0.2)**	**4.4 (±2.3)**	**0.8 (±0.5)**	**4.9 (±1.5)**
Population growing on ash from an older eruption of Volcán Lonquimay										
	14	300	7,500	6.0	3.0	17.0	0.5	3.0	0.5	5.1
South	15	100,000	100,000	5.0	2.0	12.0	0.5	1.0	10.0	7.9
Mann-Whitney *U* test										
Z		-1.368	-1.796	-2.458	-2.380	-2.289	-0.436	-1.545	-2.733	-0.288
(2-tailed significance)		(0.171)	(0.073)	(0.014)	(0.017)	(0.022)	(0.663)	(0.122)	(0.006)	(0.774)

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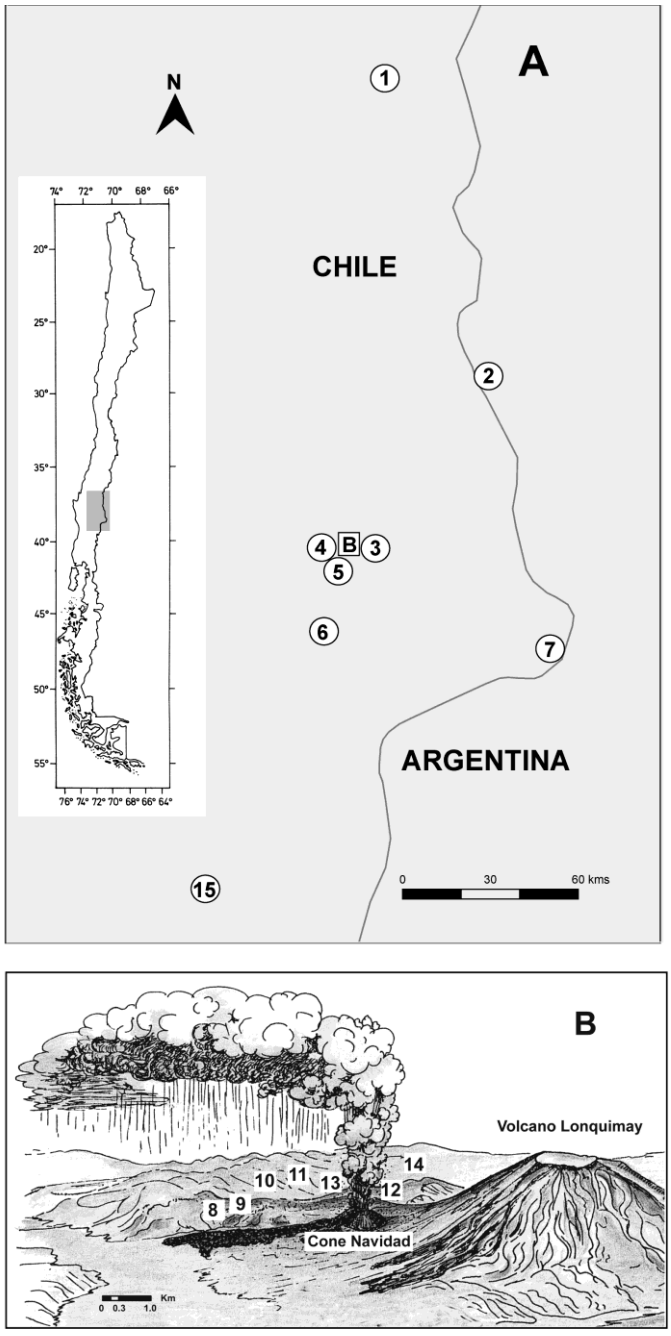

Figure 1. Map of populations of *Nassauvia lagascae* var. *lanata* sampled in the Andes Cordillera. (A) Established populations (pops. 1-7 and 15). (B) The volcanic explosion site (modified from González-Ferrán [1994]) with colonizing populations (pops. 8-14).

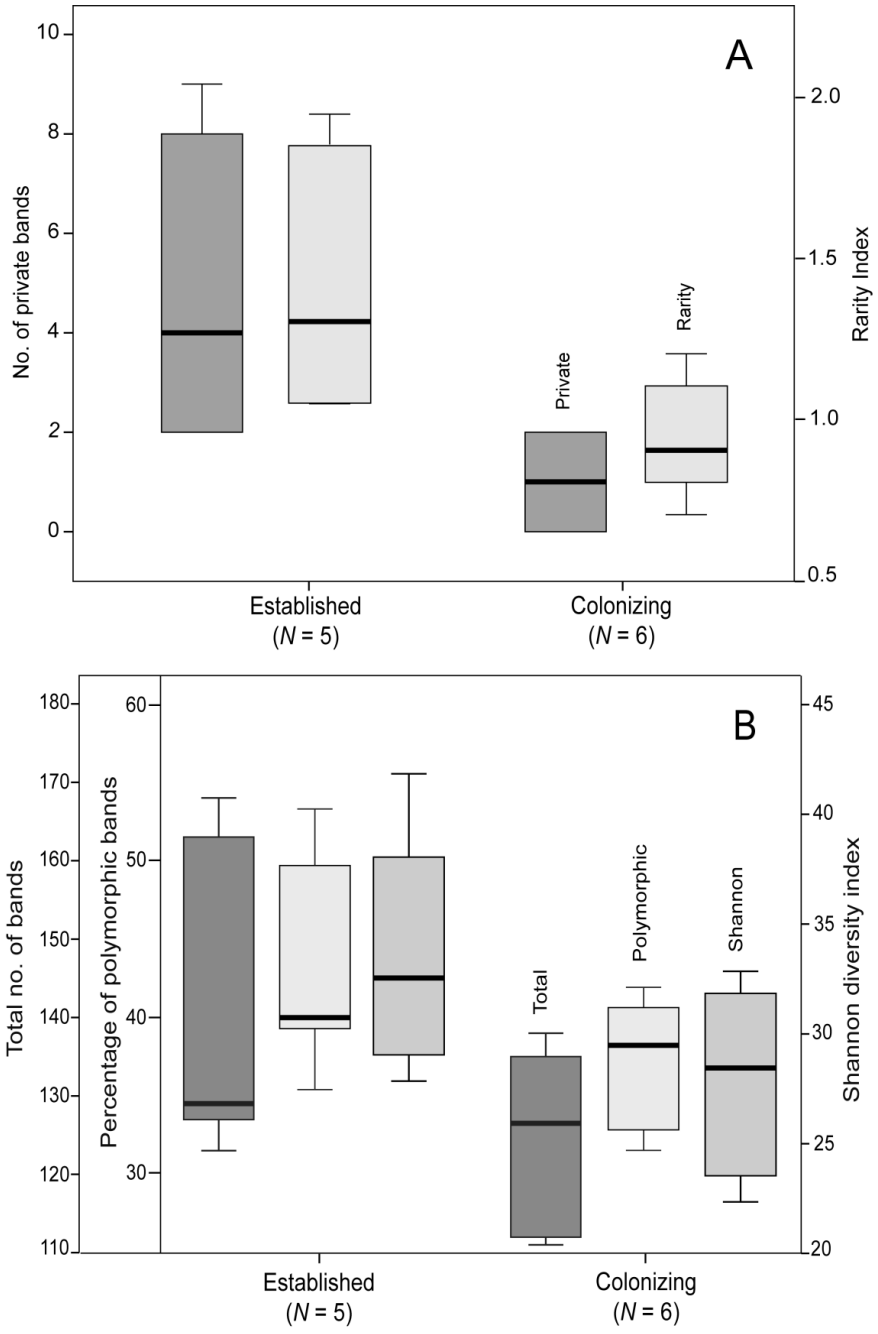

Figure 2. Boxplots showing the median, 25% and 75% quartiles (box), and non-outlier range (whiskers) of (A) number of private bands and Rarity Index and (B) estimates of genetic variation based on AFLP data for the five established and seven recently colonized populations of *Nassauvia lagascae* var. *lanata* of the Lonquimay and surrounding area.

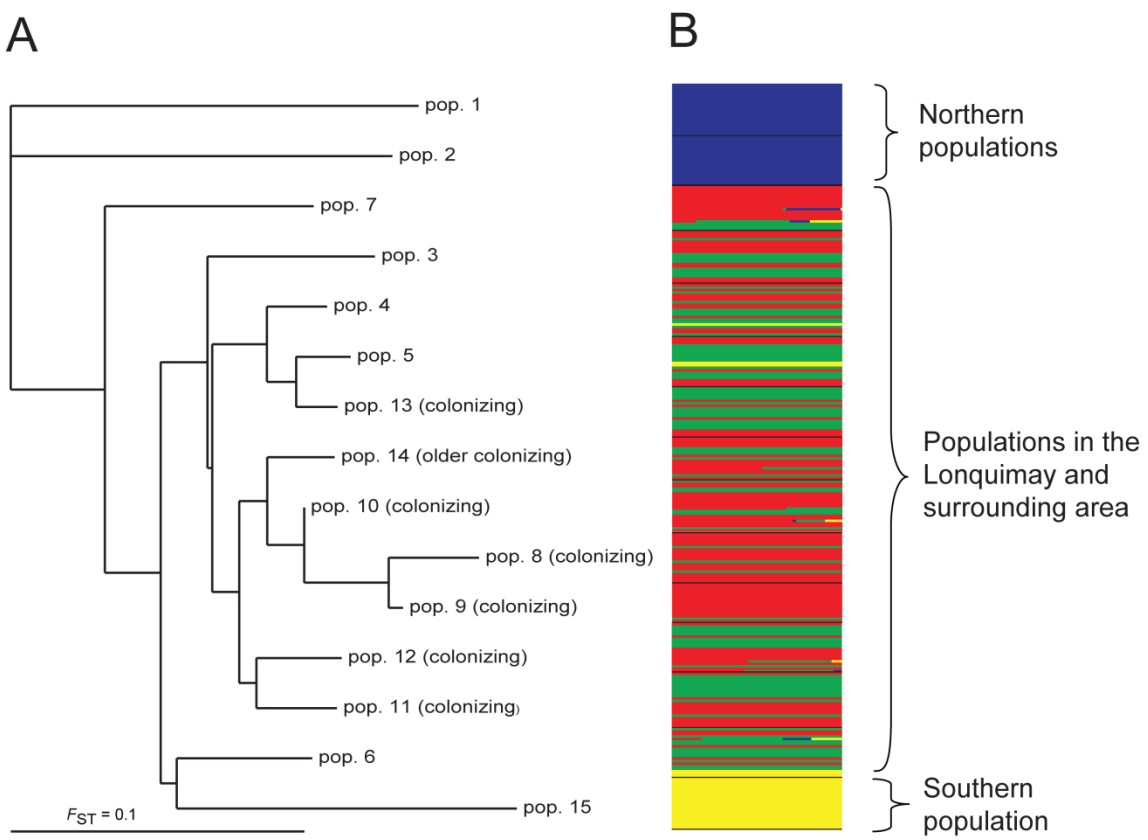

Figure 3. Geographical structure among populations of *Nassauvia lagascae* var. *lanata*. (A) Neighbor-joining clustering based on pairwise F_{ST} values among populations; bootstrap support is <50% for all bifurcations (obtained by subjecting the genetic [Nei and Li, 1979] distance matrix among individual AFLP phenotypes to a neighbor-joining analysis and running 500 replicates). (B) Population structure inferred by Bayesian clustering (K = 4).

Progenitor-derivative speciation in *Pozoa* (Apiaceae, Azorelloideae) of the southern Andes

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Abstract

Studies examining patterns and processes of speciation are scarce in South America in comparison to North America and Europe . One of the least well documented processes in any region has been progenitor-derivative speciation, whereby a more widespread species gives rise to a restricted isolate, often on the periphery of its range. The genus *Pozoa* (Apiaceae, Azorelloideae) consists of only two diploid (n = 10) outcrossing species in the southern Andes, the widespread *P. coriacea* and the very restricted *P. volcanica*. This paper tests the hypothesis that the latter species originated from the former through local geographic and ecological isolation. DNA sequences from *Pozoa* and the related South American genera *Asteriscium*, *Eremocharis*, and *Gymnophyton* from non-coding regions of the chloroplast genome, *ndhF-rpl32* and *rpl32-trnL*, plus incorporation of previously reported *rpl16* and *trnD-trnT* sequences, reveal *Pozoa* to be monophyletic. AFLP analyses using three selective primer combinations of 105 individuals in 21 populations throughout the entire range of distribution of the genus yielded a total of 406 bands, of which 405 were polymorphic. SplitsTree network analysis using AFLP data shows *P. coriacea* to be more similar genetically to outgroup genera. At the populational level, both species are monophyletic. Analysis of genetic variation among populations as well as divergence and genetic diversity of the species show highest values in *P. coriacea* and clear reductions in *P. volcanica*. All facts support that *Pozoa* represents an example of progenitor-derivative speciation in the Andes of southern South America.

KEY WORDS: AFLPs; Andes mountains; DNA sequencing; genetic diversity; geographical origin; South America; Umbelliferae.

Introduction

The speciation process relates to formation of spatial or geographic barriers and/or ecological and reproductive isolating mechanisms that allow the emergence of new and different gene combinations in separate populations. These novel gene combinations generate divergence between populations that eventually results in the recognition of new species (Grant, 1981; Coyne, 1992; Gavrilets, 2003; Levin 2003). The use of information from geographical, ecological, reproductive, and chromosomal studies has allowed a much more detailed theoretical framework to be developed over the past century to explain speciation in plants and animals. In the past two decades, the advent of molecular methods (sequencing of DNA, AFLP analyses, microsatellites, single nucleotide polymorphisms) has allowed a much deeper knowledge of the genetic patterns involved in the speciation process (Coyne and Orr, 2004).

Different modes of geographic speciation are known to have occurred among higher plants. Allopatric speciation takes place when a geographical boundary reduces gene flow between ancestral populations and leads to the formation of reproductive barriers (Grant, 1981; Lomolino et al., 2006). This concept includes vicariance and peripatric speciation. In the former, reproductive isolation evolves after the geographical range of a species divides into two or more large, isolated populations. In peripatric speciation, reproductive isolation evolves after an isolated habitat is colonized by a few individuals, or a small population becomes geographically isolated (Coyne and Orr, 2004). In this case, populations become spatially separated but share a common border (Tauber and Tauber, 1989). Sympatric speciation involves evolution of reproductive isolation within the average dispersal distance of a single individual (Mayr, 1963; Bolnick and Fitzpatrick, 2007), and where the initial restriction of gene flow is caused by biological features of the organisms (Futuyma and Mayer, 1980).

A particular type of allopatric speciation, whereby an isolated peripheral population diverges to form a derivative species, is called progenitor-derivative speciation. The derivatives species diverges from the ancestral condition but the progenitor species remains almost unchanged. This is different from typical geographic allopatric speciation, whereby two populations diverge simultaneously in numerous characters, and the ancestor disappears in the process (Gottlieb, 1973; Jaramillo-Correa and Bousquet, 2003). Thus, the identification of

differences between related taxa in the process of speciation should be somewhat easier in the progenitor-derivative model because it is relatively recent than the allopatric model with gradual divergence (Gottlieb, 2003). The necessary conditions to produce this kind of speciation are a high degree of genetic similarity between the species involved, a reduced distribution range of the derivative, and a low level of genetic diversity in the derivative taxon, especially few unique alleles (Perron et al., 2000; Jaramillo-Correa and Bousquet, 2003).

Only a few cases of progenitor-derivative speciation in plants have so far been documented, and these are all in the Northern Hemisphere: *Stephanomeria malheurensis* from *Stephanomeria exigua* ssp. *coronaria* (Compositae, Gottlieb, 1973); *Clarkia lingulata* from *C. biloba* (Onagraceae, Gottlieb, 1974); *Coreopsis nuecensis* from its progenitor species *C. nuecensoides* (Compositae, Crawford and Smith, 1982); *Lasthenia maritima* from its progenitor *L. minor* (Compositae, Crawford et al. 1985); *Layia discoidea* originating from *L. glandulosa* (Compositae, Gottlieb et al, 1985; Baldwin, 2005); *Camassia scilloides* from *C. angusta* (Asparagaceae, Ranker and Schnabel, 1986), and *Picea rubens* from *P. mariana* (Pinaceae, Jaramillo-Correa and Bousquet, 2003).

In South America, numerous tectonic changes together with elevation of the Andes mountain chain have stimulated allopatric speciation in different plant groups. However, only a handful of papers have examined speciation by means of species-level phylogenies using molecular methods such as in *Perezia* (Compositae, Simpson 1973), *Nothofagus* (Nothofagaceae, Manos, 1997), *Fragaria* (Rosaceae, Ontivero et al., 2000), *Malesherbia* (Malesherbiaceae, Gengler-Nowak, 2002, 2003), *Hypochaeris* (Compositae, Samuel et al., 2003; Stuessy et al., 2003; Tremetsberger et al., 2005, 2006), *Chaetanthera* (Compositae, Hershkovitz et al., 2006), and *Tristerix* (Loranthaceae, Amico et al., 2007). These studies have suggested avenues for synthesis of molecular, ecological, reproductive and biogeographic aspects, all of which are beginning to provide new understanding of evolutionary processes in the Andes of South America.

A very interesting example of possible progenitor-derivative speciation in South America occurs in the genus *Pozoa* (Apiaceae, Azorelloideae), endemic to the Andes of Chile and Argentina (Figs, 1, 2). This genus is represented by only two species (Mathias and Constance, 1962). *Pozoa coriacea* Lag. is widespread at elevations between 1000 and 4000 m and distributed along the southern Andes in Chile from Coquimbo south to the Region de

La Araucanía and from Province of San Juan south to Rio Negro on the Argentinian side. *Pozoa volcanica* Math. & Constance is very restricted in distribution, growing between 1200 and 2400 m only in the Lonquimay region and surrounding area in southern Chile, plus adjacent Province of Mendoza and Neuquen in Argentina (Mathias and Constance, 1962; Martínez, 2008). The restricted geographic distribution of *P. volcanica* it is in the center of the range of *P. coriacea*, the very similar morphology of the two species, the presence of occasional intermediate forms, and the existence of only two species within this morphologically very distinct genus, suggests the hypothesis that *P. volcanica* arose through a process of progenitor-derivative speciation from *P. coriacea*.

This paper tests the hypothesis of progenitor-derivative speciation within *Pozoa* with the following specific objectives: (1) confirming that *Pozoa* is a monophyletic genus; (2) determining which species of *Pozoa* is ancestral to the other; and (3) investigating levels of genetic divergence and variation in the derived species in comparison to its progenitor. To complete these objectives, we have selected DNA sequencing from the chloroplast genome and AFLP analysis (Vos et al., 1995). The latter are particularly efficacious for revealing patterns of genetic variation in natural populations (Gaudel et al., 2000; Nybom, 2004; Andrade et al., 2009) and for revealing genetic structure of intra- and inter-specific taxa (Wooten and Tolley-Jordan, 2009). These sensitive AFLP markers have to our knowledge so far not been applied to examination of progenitor-derivative speciation.

Materials and Methods

The species. *Pozoa coriacea* Lag. (Fig. 1A; common name "Anislao" or "Asta de cabra") is an outcrossing perennial herb with a massive, underground stem divided into many slender lateral divisions, and with spreading-ascending to recurved terminal flowering stalks (peduncles). Leaves are ovate to orbicular-reniform or obovate, with usually 3-15 shallow teeth. Umbels have 20-35 flowers, some staminate. Flowers are usually purplish or purple. Fruits are oblong-ovate to cuneate-oblong, with the mature carpels slightly compressed (Matthias and Constance, 1962). The chromosome number is $2n = 20$ (Bell and Constance, 1957; Rahn, 1960).

Pozoa volcanica Math. & Constance (Fig. 1B) is an outcrossing perennial herb, also with a massive and undivided underground stem, but with a short and enlarged terminal peduncle. Leaves are ovate-orbicular to reniform, with usually 13-30 triangular teeth. Umbels have 25-45 flowers, some staminate. Flowers are usually greenish-yellow. The fruits are oblong-ovate, the mature carpels being strongly compressed. The chromosome number is also $2n = 20$ (Bell and Constance, 1957; Rahn, 1960).

Sampling. Twenty-one populations of *Pozoa* were collected throughout the entire range of the two species (Fig. 2, Table 1), extending from Portillo in the north of Chile to La Hoya in the south of Argentina for *P. coriacea* (11 populations), and within the Lonquimay region in southern Chile and adjacent Mamuil Malal in Argentina for *P. volcanica* (10 populations). Leaves of five individuals from each population were collected in silica gel. Vouchers of each population sampled are on deposit in the herbarium of the University of Vienna (WU).

The populations of *P. coriacea* grow in different substrates (Fig. 1C), such as stable volcanic soil, black or clay soil, red gravel, sand, and between rocks. The number of individuals in each population ranges from 50 to 500. Genera of the high Andean vegetation that accompany *P. coriacea* include: *Mulinum* (Apiaceae); *Araucaria* (Araucariaceae); *Baccharis*, *Chuquiraga*, *Hypochaeris*, *Mutisia*, *Nassauvia* (all Compositae); *Berberis* (Berberidaceae); *Empetrum* (Empetraceae); *Adesmia* and *Lathyrus* (Fabaceae); *Nothofagus* (Fagaceae); *Polygonum* (Polygonaceae); *Acaena* (Rosaceae); *Nertera* (Rubiaceae); *Calceolaria* (Scrophulariaceae); and *Tropaeolum* (Tropaeolaceae).

Pozoa volcanica grows in new volcanic ash (Fig. 1D), with porous rock and pebbles, and occasionally black or brown soil. The number of individuals of each population ranges from 50 to 500. Accompanying vegetation includes the genera: *Baccharis*, *Hypochaeris*, *Nassauvia*, *Senecio* (Asteraceae); *Adesmia* and *Trifolium* (Fabaceae); *Loasa* (Loasaceae); *Chusquea* (Poaceae); *Polygonum* and *Rumex* (Polygonaceae); and *Acaena* (Rosaceae).

Sequences. Genomic DNA was extracted from individuals in 14 populations belonging to seven species in the four genera *Asteriscium*, *Eremocharis*, *Gymnophyton* and *Pozoa* (Table 1) from silica-gel dried leaf material following the CTAB method (Doyle and Doyle, 1987) with minor modifications (Tremetsberger et al., 2003). Sequences employed were two noncoding chloroplast regions, *ndhF-rpl32* and *rpl32-trnL*, corresponding to the intergenic spacer and located in the small single-copy region. Amplifications were made using the following primers: *ndhF* (5′-GAA AGG TAT KAT CCA YGM ATA TT-3′) and *rpl32*-R (5′-CCA ATA TCC CTT YYT TTT CCA A-3′) for *ndhF-rpl23*, and *rpl32*-F (5′-CAG TTC CAA AAA AAC GTA CTT C-3′)-*trnL*$^{(UAG)}$ (5′-CTG CTT CCT AAG AGC AGC GT-3′) for *rpl32-trnL* (Shaw et al., 2007).

PCR reactions were carried out using 0.4 mM of each primer and ReddyMix PCR Master Mix (ABgene, Vienna, Austria) including 2.5 mM $MgCl_2$ (according to manufacturer's instructions). Amplifications were performed in a GeneAmp PCR System 9700 (Applied Biosystems) with initial 5 min at 80 °C followed by 36 cycles each of 30 s denaturation at 95 °C, 30 s annealing at 50 °C, elongation phase of 4 min at 65 °C, followed by final elongation phase of 5 min at 65 °C. PCR products were purified using 0.5 µL exonuclease I, *Escherichia coli*, and 1 µL Shrimp. Alkaline Phosphatase (Fermentas) for 45 min at 37°C followed by enzyme inactivation for 15 min at 85 °C. Cycle sequencing was performed for the forward and reverse strand with 0.7 µL BigDye Terminator v3.1 Ready Reaction Mix (Applied Biosystems), 1 µL forward or reverse primer, and 6.8 µL PCR product in the following conditions: 1 min at 96° C followed by 35 cycles of 10 s at 96 °C, 5 s at 50°C, and 4 min at 60 °C. Sequencing reactions were analyzed on a capillary sequencer (3730 DNA Analyzer; Applied Biosystems).

The sequences were assembled and aligned using Seqman II (DNASTAR) and Clustal X (Thompson et al., 1997), followed by manual adjustments using the program BioEdit version 7.0.9.0 (Hall, 1999). Indels were treated as binary characters following the

"simple indel coding method" (Simmons and Ochoterena, 2000) using the program SeqState version 1.36 (Müller, 2005). A heuristic search for most parsimonious (MP) trees was performed with PAUP* version 4.0b8 (Swofford, 2002). The analyses involved 1000 replicates with stepwise random taxon addition, tree bisection–reconnection (TBR) and branch swapping saving no more than 10 trees per replicate. All characters were equally weighted and treated as unordered (Fitch, 1971). Clade support was estimated using non-parametric bootstrapping (Felsenstein, 1985) with 10,000 bootstrap replicates each with 10 random sequence addition replicates holding maximally 10 trees per replicate, TBR branch swapping, and MulTrees on.

AFLP fingerprinting. We scored a total of 105 individuals and two individuals of outgroup for three AFLP primer combinations. Genomic DNA was extracted from silica-gel dried leaf material following the CTAB method (Doyle and Doyle, 1987) with minor modifications (Tremetsberger et al, 2003). The AFLP protocol followed Vos et al. (1995) with modifications as indicated in Tremetsberger et al. (2003). The selective primer combinations chosen following primer-trials are *Mse*I-CTGA/*Eco*RI-ACT (Fam), *Mse*I-CTT/*Eco*RI-ACT (Vic), and *Mse*I-CAC/*Eco*RI-ACC (Ned).

Presence and absence of bands in all individuals were scored with GeneMarker ver. 1.85 by Soft Genetics. For each primer combination were selected: raw data analysis; local southern size call algorithm; smooth peak saturation; base line subtraction; pull-up correction; and spike removal. We used the range 150 to 510 for all primer combinations. The peak detection threshold was an intensity of relative fluorescent units over 50, with the percentage of relative minimum intensity of allele peaks at 5 and with the same value for local region percentage. The maximum relative fluorescent units threshold of peak height for peak detection was 30000. Size calibration was manually modified in those samples with values below 80%. The electropherograms were standardized using the automatic panel editor, generating a new panel for each color. A binary matrix was generated for each primer combination (Wooten and Tolley-Jordan, 2009).

Estimation of genetic diversity. The number of different AFLP phenotypes present in a population was counted with Arlequin ver. 3.1 (Excoffier et al., 2006). The number of private bands in each population and species was calculated using FAMD ver. 1.108 (Schlüter and Harris, 2006), and the Rarity Index, calculated by using the R-script AFLPdat

(Ehrich, 2006). For each individual, each AFLP marker is divided by the total number of occurrences of this marker in the dataset. These relative values are then summed to the Rarity Index for this particular individual. Population values are estimated as the average of the individual values, and species values are estimated as the average of the population values.

Genetic diversity was assessed for each population and species by using the total number of AFLP bands, percentage of polymorphic bands (by dividing the number of polymorphic bands by the total number of bands in the dataset), and Shannon Diversity index $H_{Sh} = -\Sigma(p_i \times \ln(p_i))$, where p_i is the frequency of the i^{th} band in the respective population based on all AFLP bands recorded using FAMD ver. 1.108 (Schlüter and Harris, 2006). The Pearson correlation was used to test correlation among different genetic diversity estimates using SPSS ver. 15.0 (© SPSS Inc.). The Mann-Whitney *U* test was used to estimate the significance of differences of divergence and genetic diversity of populations between species using SPSS.

Estimation of genetic differentiation. Genetic differentiation among species was assessed by analysis of molecular variance (AMOVA) using Arlequin ver. 3.1 (Excoffier et al., 2006), where total genetic diversity was partitioned into components among two hierarchical levels, among populations (F_{ST}) and among individuals within populations. An alternative Bayesian approach (Holsinger et al., 2002) was used to obtain an independent estimate of F_{ST} for each population. This method allows estimation of F_{ST} from dominant markers without assuming Hardy-Weinberg proportions in populations. The original data matrix was imported into Hickory ver. 1.1 (Holsinger and Lewis, 2003-2007) and used for a full model, $f = 0$ model, theta = 0 model, and *f*-free model run with default parameters (i.e., the hickory block omitted). The *f*-free model, which estimates theta without estimating *f* (thus incorporating all the uncertainty in the prior of *f*), is available for dominant marker data, because estimates of *f* derived from dominant marker data may be unreliable. The deviance information criterion (DIC; Spiegelhalter et al., 2002) was used to estimate how well a particular model fits the data and to choose between models.

Results

Sequence relationships. The Bayesian 50% majority rule consensus tree for the region *ndhF-rpl32* among *Asteriscium*, *Eremocharis*, *Gynmophyton*, and *Pozoa* (Fig. 3A) shows that *Pozoa* is most closely related to *Gymnophyton* (BS = 100), and that *Pozoa volcanica* appears to be derived from *P. coriacea*, but with low support. Both populations of *P. volcanica* form a single holophyletic clade, and the two populations of *P. coriacea* occur in a paraphyletic clade. The same analysis using the *rpl32-trnL* region (Fig. 3B) reveals the two species of *Pozoa* in one clade (BS = 100), and this connects nearest to the clade of *Asteriscium* (BS = 99). The results of Nicolas and Plunkett (2009), using chloroplastic *rpl16* and *trnD-trnT* (Fig. 3C), reveal *Pozoa* as holophyletic, connecting preferentially with *Asteriscium* and *Gymnophyton*. The four chloroplast sequences taken together, therefore, show *Pozoa* as a monophyletic genus.

AFLP relationships. *Fragment patterns*.--The total number of AFLP bands found in all individuals and all populations of both species of *Pozoa* are 406, of which 405 (99.7%) are polymorphic. *Pozoa coriacea* presents a total of 355 bands, of which 354 are polymorphic, whereas *P. volcanica* has a total of 253 bands with 246 being polymorphic. The number of fragments for all individuals and by species (*P. coriacea*/*P. volcanica*) are 142 (130/84) for primer *Mse*I-CTGA/*Eco*RI-ACT, 165 (147/97) for *Mse*I-CTT/*Eco*RI-ACT, and 99 (78/72) for *Mse*I-CAC/*Eco*RI-ACC. All individuals had unique AFLP phenotypes.

Genetic diversity and divergence of populations.--The genetic diversity estimates, i.e., total number of bands, and Shannon Diversity index, are higher in *P. coriacea* than in *P. volcanica* (Fig. 4), although the differences are not significant according to the Mann-Whitney U test (Table 2). The percentage of polymorphic bands is higher in *P. volcanica* than in *P. coriacea*. A significant correlation was observed between these indices in both species; the Pearson correlation between the Shannon Diversity index and Total number of bands is $r = 0.899$ (N = 21, sig. [2 tailed] = 0.000), and between Percentage of polymorphic bands and Total number of bands is $r = 0.675$ (N = 21, sig. [2-tailed] = 0.001). The correlation between Percentage of polymorphic bands and Shannon Diversity index is $r = 0.620$ (N = 21, sig. [2-tailed] = 0.003). It is noteworthy that values of the mean for Shannon Diversity among species are very similar between both species. Among the genetic divergence estimates, the number of private bands is significantly higher in populations of

Pozoa coriacea than in *P. volcanica* (Mann-Whitney U test, Table 2, Fig. 4). The Rarity Index is also higher in *P. coriacea*, with both indices being positively correlated, with Pearson correlation $r = 0.809$ (N = 21, sig. [2-tailed] = 0.000). Comparing values of estimates of divergence and diversity in each species, *Pozoa coriacea* has considerably higher values than *P. volcanica* for all measures (Table 3).

Genetic diversity between species.--Analysis of molecular variance (AMOVA) attributes 20.20% variance (d.f .= 1) between species and 79.80% variance (d.f. = 103) within populations of each species. The same analysis, but for each species, shows 54.48% variance among populations in *Pozoa coriacea* (d.f. = 10) and 45.52% variance (d.f. = 44) within populations (95% C.I. = 51.4-57.4%). *Pozoa volcanica* presents a 25.55% variance among populations (d.f. = 9) and 74.45% (d.f. = 40) variance within populations (95% C.I. = 22.1-28.9 %).

The genetic variance among species using a Bayesian analysis shows the lowest DIC value with the f=0 model (DIC value = 3242.31), where the theta-II value is 0.240 (95% credible interval = 0.213-0.239). Among populations of *P. coriacea* (N = 11; pop. 1-11) using the full model (DIC value = 4576), the value of theta-II is 0.475 (95% credible interval = 0.453-0.498); in *P. volcanica* (N = 10; pop. 12-21; DIC value = 3714.19) the theta-II value is 0.2172 (95% credible interval = 0.191-0.243).

Neighbor net analysis with Splits Tree using the whole AFLP dataset shows two groups clearly defined that correspond to *Pozoa coriacea* and *P. volcanica* (Fig. 5), plus connections to selected outgroups (i.e., *Asteriscium* and *Gymnophyton*). Of the two species of *Pozoa*, the outgroup genera attach most closely to *P. coriacea* (Fig. 5). Within *P. coriacea* there are two groups that correspond to a general geographic trend. The first group includes populations 1-7 of the central-south part of the range, and the second covers populations 8 to 11 distributed in the southern zone. Population 11 of *P. coriacea* (La Hoya) appears to be closely related to populations 18 and 19 of *P. volcanica*, which may suggest an original geographic origin of *P. volcanica* from populations in this southern region of *P. coriacea*. The populations of *P. volcanica* do not show a clear geographic pattern (Fig. 5).

Discussion

Monophyly of *Pozoa*. Before addressing the specific question of progenitor-derivative speciation in *Pozoa*, it is necessary to confirm that the genus is monophyletic. Although the morphology of *Pozoa* is unified and distinct (Fig. 1A,B), it is important to reject any consideration of biphylesis involving related genera.

Previous morphological and anatomical studies have suggested which genera of Apiaceae might be the closest relatives of *Pozoa*. Henwood and Hart (2001) completed a cladistic analysis using morphological and anatomical data with focus on Australian Hydrocotyloideae, but also including genera from other continents. In this study, *Pozoa* was generically distinct in possessing fused carpophores (free in the other genera), but it grouped nearest to *Asteriscium* due to shared non-inflexed petal apices. This subgroup joined next to *Eremocharis*, *Domeykoa*, and *Gymnophyton*, constituting the "*Pozoa* clade". Liu (2004), using 16 morphological and anatomical characters also in cladistic analyses, obtained a consensus tree that showed *Pozoa* generically distinct by a concave dorsal fruit surface but nearest to *Asteriscium* and *Gymnophyton*.

Previous molecular studies have also suggested relationship of *Pozoa* with *Asteriscium* and *Gymnophyton* of southern South America. Nicolas and Plunkett (2009), using plastid sequences of *rpl16* intron and *trnD-trnT* regions, examined affinities among 40 genera of subfamily Hydrocotyloideae. In this analysis with a combined dataset (Fig. 3C), *Pozoa* appears sister to *Asteriscium* and *Gymnophyton* (labeled as the *Gymnophyton* subclade) with bootstrap support 100% and posterior probability of 1.0.

In view of the importance of confirming monophyly in *Pozoa*, and following the suggestions of affinities revealed from previous studies, our own sequencing efforts focused, first, on examining relationships among *Pozoa*, *Asteriscium*, *Domeykoa*, *Eremocharis*, and *Gymnophyton*. Primer trials recommended employment of the chloroplast markers *ndhF-rpl32* and *rpl32-trnL*. Results of the former (Fig. 3A) showed the closest relative to be *Gymnophyton* (100% BS), and of the latter (Fig. 3B) to be *Asteriscium* (99%). The studies of Nicolas and Plunkett (2009; Fig. 3C), using *rpl16* and *trnD-trnT* also showed a strong tie of *Pozoa* (100% BS) to the genera *Asteriscium* and *Gymnophyton*. These two genera, therefore, were selected as outgroups for more detailed AFLP population level analyses. Second, all molecular data also point to *Pozoa* being monophyletic. The previous studies (Fig. 3C) of

Nicolas and Plunkett (2009) placed *P. coriacea* and *P. volcanica* together (over 95 % BS), as do our own results (Figs. 3A, B). AFLP analyses (Fig. 5) further support monophyly of *Pozoa*. Neighbor net analysis using Splits Tree of the many populations of both species of *Pozoa*, and including representatives of *Asteriscium* and *Gymnophyton*, show substantial degrees of divergence of these genera in attachment to populations of *Pozoa coriacea*. All data, therefore, support *Pozoa* as being monophyletic.

Ancestry of the species of *Pozoa*. In context of monophyly of *Pozoa*, the next consideration is specific ancestry of the two included species. There are three likely alternatives: (1) origin of both species from a common, now extinct, ancestor; (2) derivation of *P. coriacea* from *P. volcanica*; or (3) origin of *P. volcanica* from *P. coriacea*. Choosing among these alternatives involves examining data from geography, ecology, and patterns of genetic variation. The DNA sequences as a whole are not very informative on this question, with the exception that with *ndhF-rpl32* (Fig. 3A) the monophyletic *P. volcanica* appears most derived of the generic complex analyses.

The geography (Fig. 2) and ecology of *P. coriacea* and *P. volcanica* (Fig. 1B, D) suggest strongly that the latter was derived from the former. The distributional range of *P. coriacea* is broad, ranging along the Andean mountain chain. *Pozoa volcanica*, on the other hand, is very restricted to only the volcanic region near Volcán Lonquimay in southern Chile. Complex alternative hypotheses can be formulated, obviously, to suggest that *P. volcanica* might have been the original progenitor that survived refugially during Pleistocene glaciation, followed by derivative speciation into *P. coriacea* and subsequent extensive range expansion north and south. The broader level of genetic variation in *P. coriacea*, however, in contrast to that in *P. volcanica*, argues against this possibility (see below). The range of ecological tolerance of *P. coriacea* is also much broader than that of *P. volcanica*. The former is found in numerous habitats in and around *Nothofagus* and *Araucaria* forests, in varying types of substrate, including organic soils. *Pozoa volcanica*, on the other hand, is restricted to open sites in the active volcanic region centering around Volcán Lonquimay. In fact, the impetus for the present project came from noting that *P. volcanica* was one of the early colonizers into the fresh bare volcanic ash in the explosion zone of the Navidad cone of Lonquimay, which recently erupted in 1988 (González-Ferrán, 1994). The more open and uniform habitat in which *P. volcanica* occurs, therefore, argues for this species being a

populational derivative into a unique ecological zone from *P. coriacea* rather than the reverse.

Populational genetic data from AFLP analyses (Fig. 5) also argue for *P. volcanica* being derived from out of *P. coriacea*. First, Splits Tree analysis places the outgroup representatives of *Asteriscium* and *Gymnophyton* within populations of *P. coriacea* and not in *P. volcanica*. Second, and more compelling, is that the degree of genetic variation among populations of *P. coriacea* is much greater than that of *P. volcanica* (see also Fig. 4). The total number of bands, number of private bands, and the Rarity Index all support a reduced genetic profile in *P. volcanica*. This is what would be expected to occur with a founder effect origin of a derivative peripheral population system from a more genetically (and ecologically) diverse progenitor.

Levels of genetic variation in progenitor and derivative species. The genetic characteristics that a species must have to establish progenitor-derivative origins are (from Crawford et al., 1985): (1) high genetic similarity between the two species; (2) less genetic variation in the derivative species; (3) absence of alleles present in the progenitor, often in low frequencies, and (4) few or no unique alleles in the derivative species.

AFLP data from *Pozoa coriacea* and *P. volcanica* indicate a low F_{ST} value between the species (F_{ST} = 0.2019), and hence a high degree of genetic similarity due to a high proportion of similar alleles between them. In number of private bands, the value for *P. coriacea* is three times higher than that in *P. volcanica* (Table 3). This is concordant with the idea of reduced genetic variability via recent origin of the taxon in the context of a founding effect (Purps and Kadereit, 1998).

This same trend of loss of genetic variation in the derivative species has been documented in other species pairs. Perron et al (2000) and Jaramillo-Correa (2003), investigating black spruce (*Picea mariana*) and red spruce (*Picea rubens*), showed that the genetic diversity of the derivative species was a subset of that observed in the progenitor. Gottlieb (1974) found a reduced allelic diversity in the derivative *Clarkia lingulata* in comparison to *C. biloba* when analyzed for electrophoretic variation specified by eight loci. Crawford and Smith (1982), using allozymes, showed a decrease in genetic variation in the derivative species *Coreopsis nuecensis* in relation to the progenitor species *C. nuecensoides*. The same general trend has been documented in (progenitor species given first): *Lasthenia*

minor and *L. maritima* (Crawford et al., 1985), *Camassia scilloides* and *C. angusta* (Ranker and Schnabel, 1986), *Erythronium albidum* and *E. propullans* (Pleasants and Wendel, 1989), and *Senecio viscosus* and *S. nebrodensis* (Kadereit et al., 1995; Purps and Kadereit, 1998). The causes of decline in genetic diversity in the derivative taxon are several, such as origin from a small number of individuals (bottleneck), and limited gene flow from parental populations.

Conclusions

The conclusion, therefore, is that *Pozoa volcanica* represents a species newly derived from its progenitor *P. coriacea*. The volcanic activity in and around Volcán Lonquimay has provided an opportunity for establishment of peripheral populations from *P. coriacea* through dispersal into new open habitats, and subsequent divergence in isolation. The lower level of unique alleles in *P. volcanica* is consistent with the hypothesis of a founder effect. Biogeographically, it is likely that the origin of *P. volcanica* occurred after Pleistocene glaciation. Local glaciers along the Andean chain (Ortiz-Jaureguizar and Cladera, 2006), which resulted in a cooler climate, had the effect of shifting the vegetation to lower elevations, with the flora rebounding upwards only after the glaciers receded (Simpson, 1983). This may have coincided with volcanic activity, so frequent along the Chilean cordillera (González-Ferrán, 1994), which provided even more new ecological opportunities. These events may have been responsible for stimulating speciation within *Pozoa* as well as within other genera that inhabit the southern Andean mountain chain.

Acknowledgements

We are grateful to J. Cáceres-Chamizo, C. López Cáceres, I. Yañez, P. Arias, A. Jiménez, S. Gómez-González, M. J. Parra, and R. Hössinger for help with collecting population samples; Departamento de Botánica, Universidad de Concepción, Chile, for logistic support; Corporación Nacional Forestal (CONAF) for permission to collect samples in Chilean National Parks, and Administración de Parques Nacionales, Argentina, for permission to make collections in Argentina. This project was supported by the Fonds zur Förderung der Wissenschaftlichen Forschung, Austria (FWF grant P18446 to TFS).

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Table 1. Collection data for populations of *Pozoa* and generic relatives for sequencing (S) and AFLP (A) studies. Vouchers are on deposit at WU. PL = Patricio López; KT = Karin Tremetsberger.

Species	Analysis	Population	Collection number	Latitude	Longitude	Elevation (m)
Pozoa coriacea Lag.	A, S	1: Chile, Portillo	*PL et al. 2605*	32°50'09" S	70°07'43" W	2880
	A	2: Chile, Valle Nevado	*PL et al. 2531*	33°20'01" S	70°14'49" W	3100
	A	3: Chile, Embalse El Yeso	*PL et al. 2601*	33°37'05" S	69°58'04" W	2580
	A	4: Chile, Laguna Teno	*PL et al. 2600*	35°09'48" S	70°32'17" W	2540
	A	5: Chile, Baños de Colina	*PL et al. 2604*	35°50'01" S	69°59'25" W	2438
	A	6: Chile, Laguna del Maule	*PL et al. 2593*	36°00'54" S	70°30'20" W	2360
	A, S	7: Chile, Chillán, Shangri-La	*PL et al. 2548*	36°52'34" S	71°27'51" W	1550
	A	8: Argentina, Volcán Copahue	*KT et al. 1033*	37°49'53" S	71°06'44" W	2120
	A	9: Chile, Volcán Callaquí	*KT et al. 1026*	37°54'42" S	71°24'00" W	1675
	A	10: Chile, Termas de Rio Blanco	*PL et al. 2564*	38°34'34" S	71°37'38" W	1035
	A, S	11: Argentina, La Hoya	*PL et al. 2686*	42°49'58" S	71°15'26" W	1660
Pozoa volcanica Math. & Const.	A	12: Chile, Cerros de Lanco	*KT et al. 135*	38°20'55" S	71°25'52" W	1820
	A	13: Chile, Cerro Colorado	*KT et al. 16*	38°24'40" S	71°34'34" W	1880
	A	14: Chile, Cordillera Las Raíces	*KT et al. 1*	38°26'29" S	71°28'48" W	1800
	A	15: Chile, Sierra Nevada	*KT et al. 61*	38°37'02" S	71°35'50" W	1730
	A	16: Chile, Pino Hachado B	*PL et al. 2559*	38°39'20" S	70°55'11" W	1770
	A	17: Chile, Pino Hachado A	*KT et al. 130*	38°39'40" S	70°53'53" W	1900
	A, S	18: Argentina, Pino Hachado C	*PL et al. 2677*	38°40'03" S	70°50'46" W	1558

Table 1. Continued.

Species	Analysis	Population	Collection number	Latitude	Longitude	Elevation (m)
	A, S	19: Chile, Conguillío	*PL et al. 2565*	38°40'59" S	71°48'01" W	1440
	A	20: Chile, Volcán Llaima	*KT et al. 106*	38°41'26" S	71°47'06" W	1725
	A, S	21: Argentina, Mamuil Malal	*PL et al. 2680*	39°36'26" S	71°21'50" W	980
	S	22: Chile, Volcán Lanin	*PL et al. 2578*	39°35’47” S	71°30’20” W	1380
Asteriscium chilense Cham. & Schlecht.	S	23: Chile, Puerto Oscuro	*PL et al. 2517*	31°21’39” S	71°35’21” W	230
	S	24: Chile, Tulahuen	*PL et al. 2608*	31°03’40” S	70°39’40” W	2307
Asteriscium vidali Phil.	A, S	25: Chile, Huasco, Cerro Negro	*PL et al. 2508*	28°29'28" S	71°14'07" W	340
Eremocharis fruticosa Phil.	S	26: Chile, Quebrada Peralito	*PL et al. 2505*	25°01’50” S	70°26’15” W	710
Gymnophyton foliosum Phil.	S	27: Chile, Taltal, Quebrada San Ramón	*PL et al. 2504*	25°23’05” S	70°26’42” W	40
Gymnophyton isatidicarpum (Presl ex DC.) Math. & Const.	A, S	28: Chile, Vicuña	*PL et al. 2509*	30°08'42" S	70°30'17" W	1300
	S	29: Chile, Pirque, La Puntilla	*PL et al. 2524*	33°37’03” S	70°31’18” W	710

Table 2. Estimates of divergence and diversity based on AFLP analysis from five individuals in each of 21 populations of *Pozoa coriacea* and *P. volcanica*. The Mann-Whitney *U* test was used to assess the significance of difference between the two species.

		Estimates of divergence		**Estimates of diversity**		
Species	Population	Number of private bands	Rarity Index	Total number of bands	Percentage of polymorphic bands	Shannon Diversity index
Pozoa coriacea	1	5	4.70	93	14.53	17.13
	2	5	5.16	120	19.21	23.42
	3	3	5.51	128	20.44	25.53
	4	3	5.01	128	18.23	24.86
	5	5	5.47	120	20.93	22.56
	6	13	7.63	152	26.35	33.32
	7	4	4.89	135	22.17	26.11
	8	4	2.89	81	14.78	18.69
	9	4	2.25	68	12.56	15.69
	10	1	2.16	78	12.56	14.58
	11	19	7.46	129	14.78	29.99
	Mean (±SD)	6.00 (±5.25)	4.83 (±1.83)	112 (±27.33)	17.87 (±4.41)	22.90 (±5.94)
Pozoa volcanica	12	2	2.66	100	24.13	23.13
	13	4	2.96	100	18.23	22.82
	14	1	2.40	95	17.49	20.69
	15	1	1.95	83	16.50	17.92
	16	1	3.32	122	21.18	28.55
	17	2	3.73	128	22.66	27.08
	18	4	3.54	100	19.95	24.39
	19	4	3.26	106	13.79	26.28
	20	1	2.30	90	21.18	18.85
	21	0	1.74	76	15.27	19.44
	Mean (±SD)	2.00 (±1.49)	2.79 (±0.68)	100.00 (±15.97)	19.04 (±**3**.34)	22.92 (±3.67)
	Mann-Whitney *U* test ***Z*** (2-tailed significance)	 -2.695 (0.007)	 -2.394 (0.017)	 -1.059 (0.289)	 -0.881 (0.387)	 -0.211 (0.863)

Table 3. Estimates of divergence and diversity based on AFLP analysis of *Pozoa coriacea* and *P. volcanica*.

Species	Number of private bands	Rarity Index	Total number of bands	Percentage of polymorphic bands	Shannon Diversity index
Pozoa coriacea	153	4.83	355	87.19	75.86
Pozoa volcanica	51	2.79	253	60.59	47.08

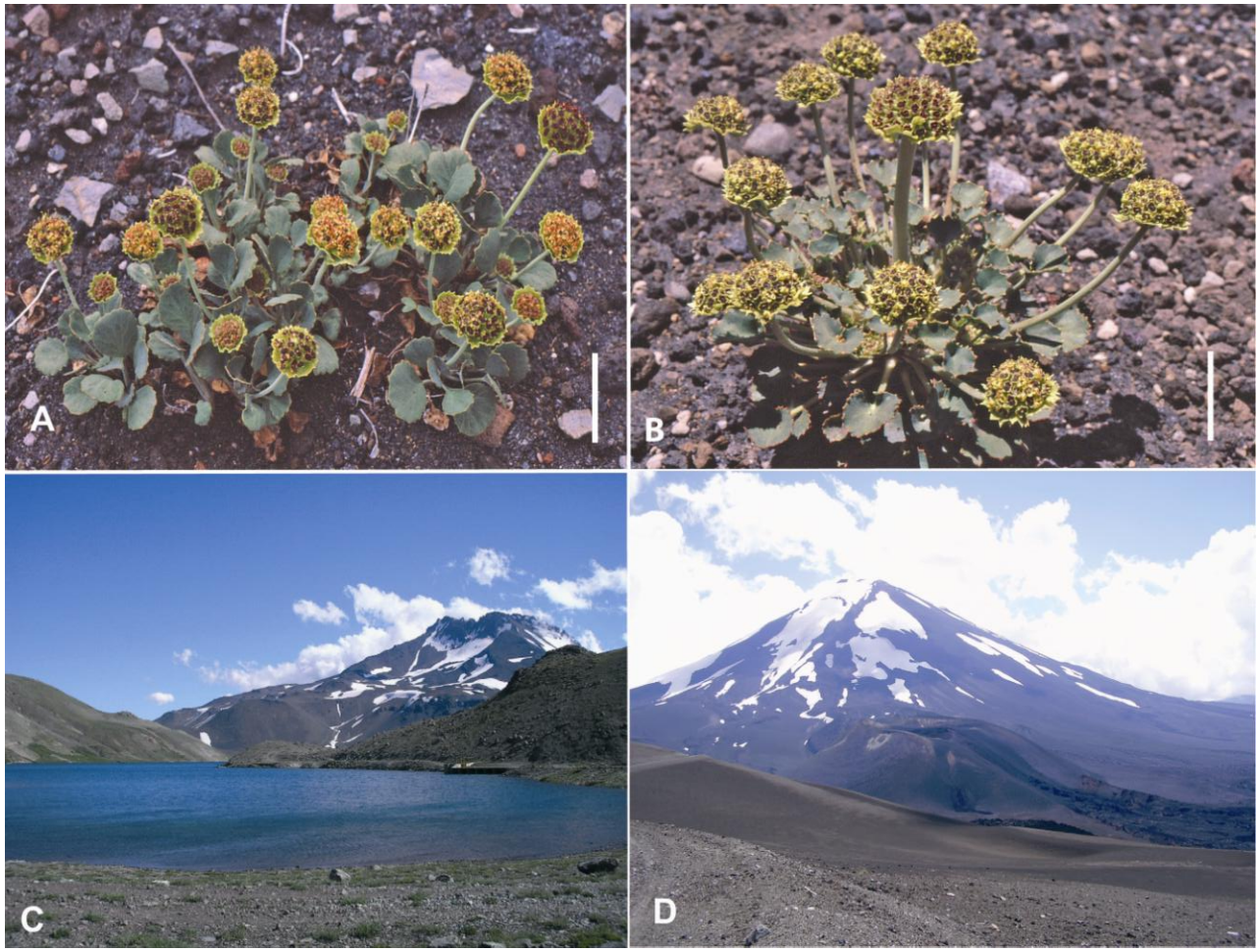

Figure 1. The genus *Pozoa* (A, B) and typical habitats (C, D, respectively) in southern South America. A, *P. coriacea*; B, *P. volcanica*; scale bar = 3 cm, C, Chile, Region del Maule, Laguna Teno; D, Chile, Region de la Araucanía, cone Navidad, Volcán Lonquimay.

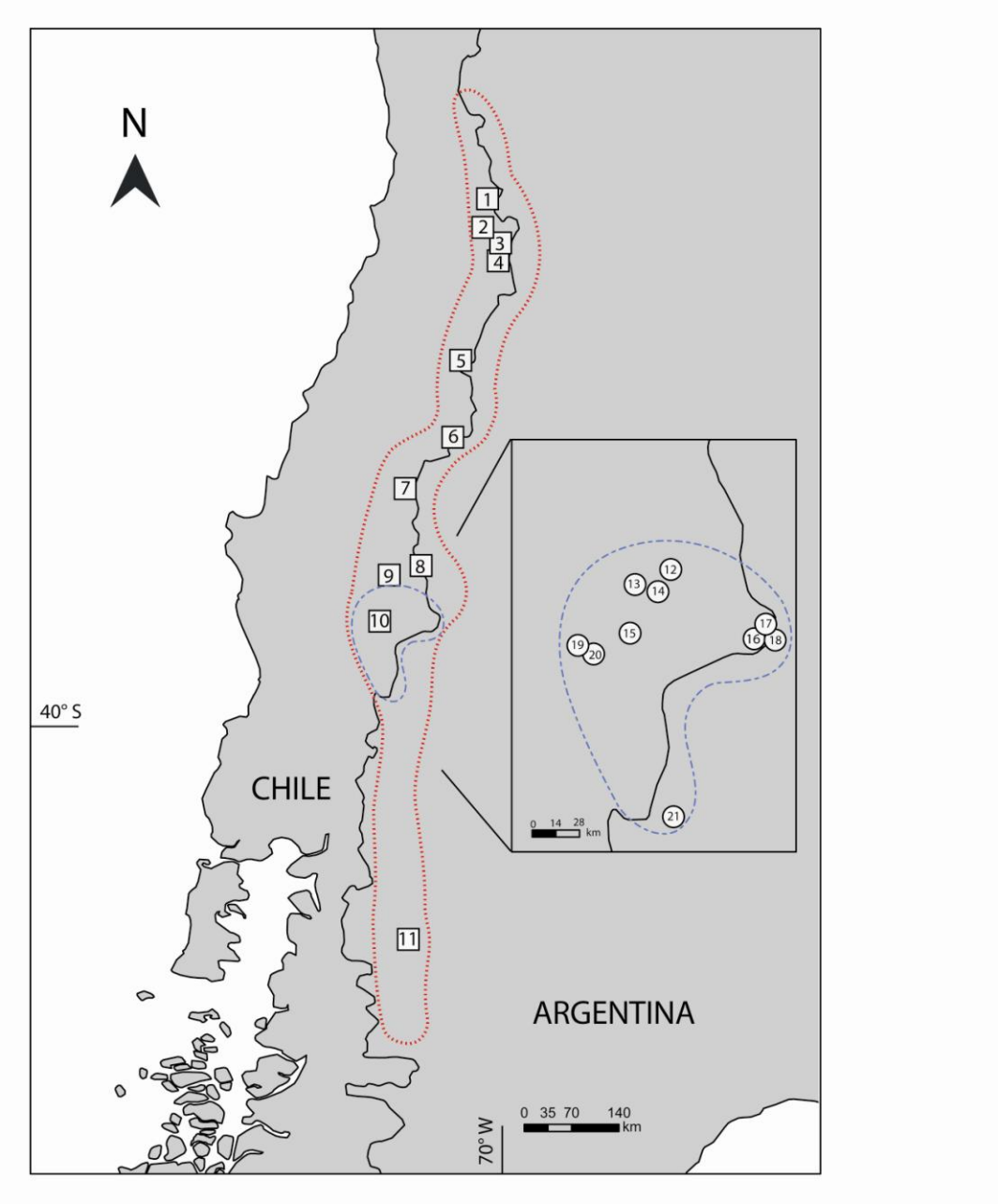

Figure 2. Distribution of sampled populations of *Pozoa coriacea* (squares) and *P. volcanica* (circles) in southern South America. Generalized distributions of *P. coriacea* and *P. volcanica* are shown by the dotted and dashed lines, respectively.

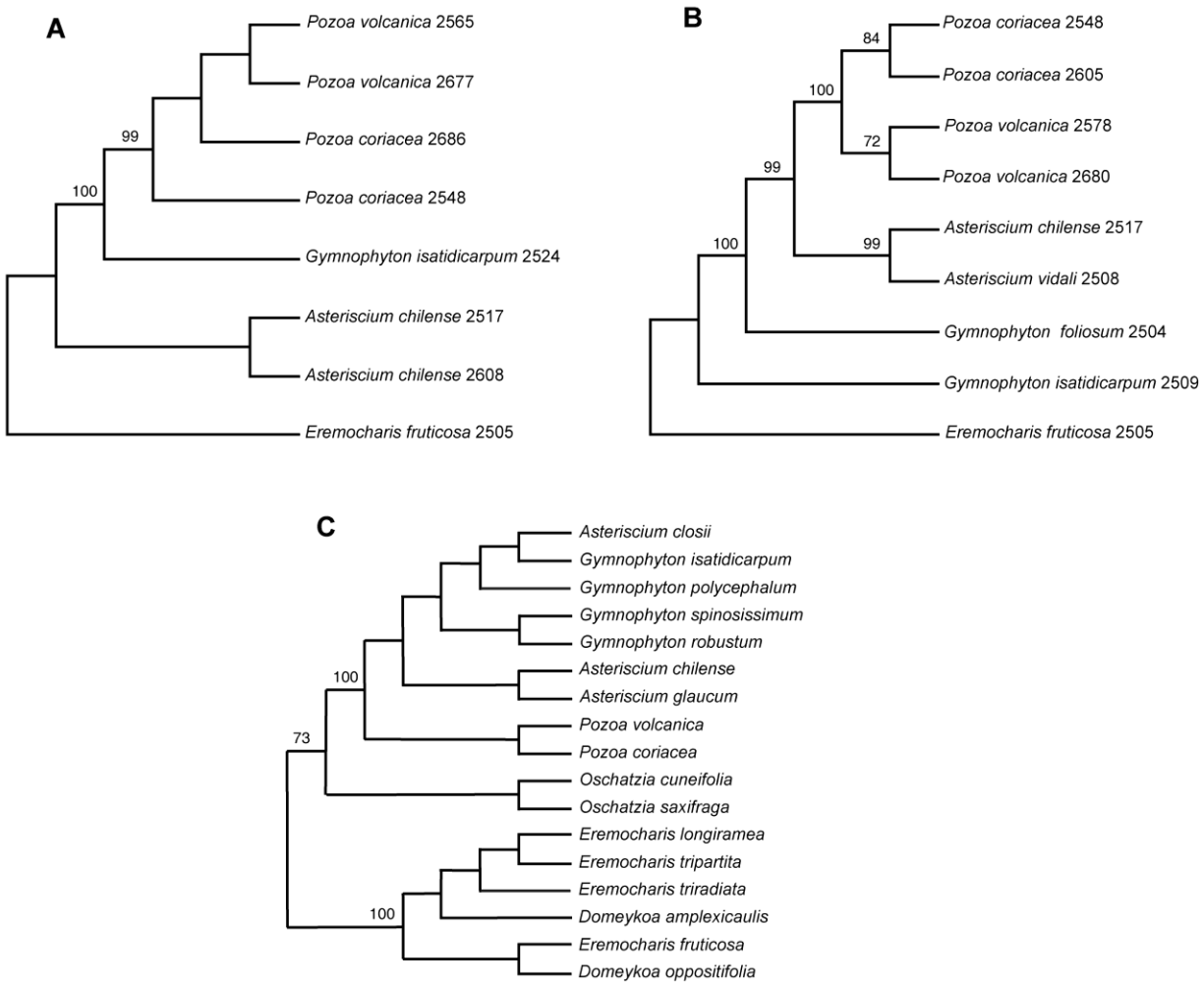

Figure 3. Phylogenetic relationships using consensus trees based on parsimony analyses among the genera *Asteriscium*, *Eremocharis*, *Gymnophyton*, and *Pozoa* based on chloroplast DNA markers: (A) *ndhF-rpl32*, for 50% majority rule consensus tree; (B) *rpl32-trnL*, for 50% majority rule consensus tree; and (C) *rpl16* combined with *trnD-trnT*, with maximum likelihood and Bayesian inference (from Nicolas and Plunkett, 2009).

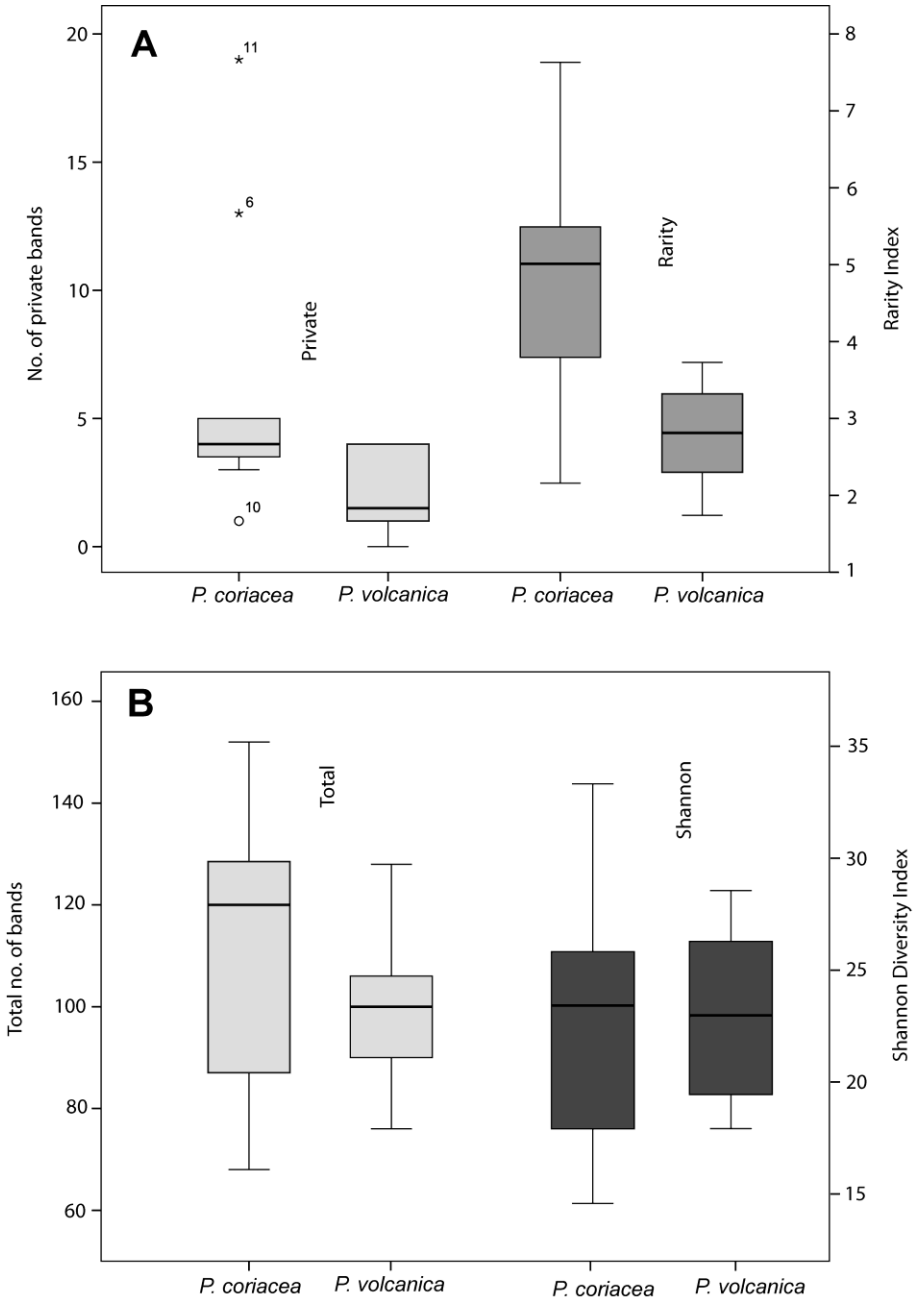

Figure 4. Boxplots of AFLP data showing the median, 25% and 75% quartile (box), and non-outlier range in *Pozoa coriacea* and *P. volcanica* of (A) number of private bands and Rarity Index and (B) total numbers of bands and Shannon Diversity. Population numbers with asterisks represent outliers.

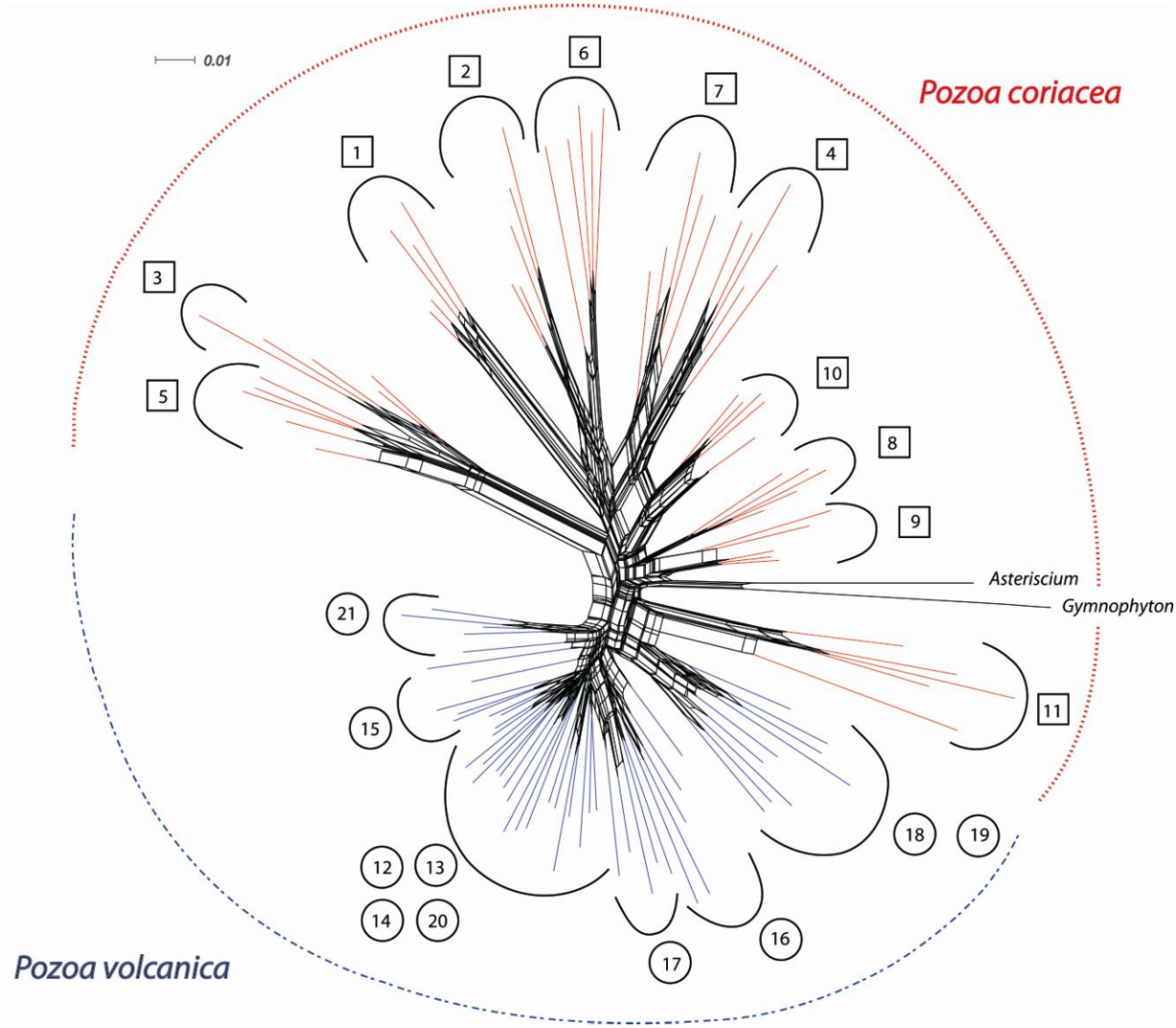

Figure 5. SplitsTree Neighbor net analysis of AFLP data showing genetic variation within and among populations of *Pozoa coriacea* (squares; dotted line) and *P. volcanica* (circles; dashed line).

Adaptive radiation in the *Hypochaeris apargioides* complex (Asteraceae, Cichorioideae) of southern South America

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Summary

Adaptive radiation, which involves rapid speciation into different environments, is a common evolutionary phenomenon in oceanic archipelagos. Lineages that have evolved in this fashion are less evident in continental areas, although examples do exist in regions of high environmental heterogeneity. The genus *Hypochaeris* (Asteraceae, Cichoroideae) contains approximately 45 species in South America that have evolved during the past one million years. Dispersal of propagules to new regions followed by speciation at the diploid level into different ecological zones, has resulted in adaptively radiated groups. One such group, the *Hypochaeris apargioides* complex, consists of four closely related species, *H. apargioides*, *H. gayana*, *H. spathulata*, and *H. thrincioides*, all of which are distributed in central-south Chile and adjacent Argentina.

Morphometric and molecular (AFLP) data were used to help reveal the processes involved in the evolution of the complex. A total of 60 populations was sampled: 34 were analyzed morphometrically and 47 were examined for genetic variation and divergence using AFLP methodology. Four populations were used for greenhouse experiments to help reveal the environmental vs. genetic control of morphological features in the plants.

Morphometric analysis shows that two species, *H. spathulata* and *H. gayana*, are clearly separated phenotypically from the others, but that *H. apargioides* and *H. thrincioides* are more similar to each other. Greenhouse-grown plants show slight deviation in characters of leaves from those found in wild populations, revealing that these species largely maintain their distinctness in a common garden environment. AFLP analyses corroborate morphological distinctions plus showing that *H. spathulata* consists of two populational systems, perhaps reflecting two distinct origins into coastal habitats.

The principal environmental conditions influencing morphology and distribution of species in the *H. apargioides* complex appear to be salinity and elevation in *H. spathulata* and *H. gayana*, respectively, and ambient temperature in *H. thrincioides*. The overall pattern in the evolution in the complex is one of subtle morphological divergence in response to environmental selection, perhaps reflecting initial stages of adaptive radiation. The low level of molecular divergence among species also suggests rapid speciation.

Key Words: Adaptive radiation, AFLP, Asteraceae, genetic divergence, Pleistocene glaciations, greenhouse, *Hypochaeris apargioides* complex, morphological trends, morphometry.

Introduction

The concept of adaptive radiation, originally proposed by Osborn (1902), refers to rapidly evolving lineages from a common ancestor that diverge into well-defined ecological zones. This contrasts with non-adaptive radiation, whereby rapid differentiation of species occurs but without corresponding adjustment to differences in the environment (Kozak *et al*., 2006; Gillespie, 2009). Conditions that encourage adaptive radiation are the emergence of key innovations and the appearance of new environments (Pellmyr & Krenn, 2002; Gillespie, 2009; Guzmán *et al*., 2009) that allow successive generations to form new species adapted to different ecological niches (Gavrillets & Losos, 2009).

Classical examples of adaptive radiation include groups of island or island-like habitats, such as *Anolis* lizards in the Caribbean, the silverswords alliance in Hawaii, Darwin's finches in the Galápagos Islands, and the cichlids of the East African Great Lakes (Schluter, 2000; Meimberg *et al*., 2006; Baldwin, 2007; Givnish *et al*., 2008; Gavrilets & Losos, 2009). In the last decade, papers on adaptive radiation have also considered the process in continental environments from theoretical (Barriers *et al*., 2001; Seehausen, 2004; Gavrilets & Vose, 2005; Shaffer & Thomson, 2007; Whitfield and Lockhart, 2007) as well as empirical perspectives (Dunbar-Co *et al.*, 2008; Agrawal *et al*., 2009; Gavrilets & Losos, 2009; Guzmán *et al.*, 2009; Hodges & Derieg, 2009; Organ *et al*., 2009). Few studies have focused on groups of South America, however, despite the wealth of habitats in this continent, especially in the Andean region. One recent example comes from the genus *Lupinus* (Leguminosae; Hughes & Eastwood, 2006), which does show a remarkable diversification into different ecological Andean zones in a short period of geological time.

Another plant genus that has also undergone considerable adaptive radiation in South America is *Hypochaeris* (Compositae, Lactuceae). This genus has an intercontinental disjunct distribution between Europe and South America (Samuel *et al.*, 2003), with 15 species in the northern hemisphere and c. 45 in South America (Tremetsberger *et al*., 2006). Molecular sequence data have shown this to be a monophyletic group (Cerbah *et al.*, 1998; Samuel *et al.*, 2003). Diversification of *Hypochaeris* in South America has occurred during the past one million years, including the Pleistocene, after a transatlantic dispersal from NW Africa (Tremetsberger *et al*., 2005). All data (morphological, chromosome counts, chloroplast sequences, and AFLP

analyses) suggest that the group radiated explosively in South America after arrival from African ancestors (Tremetsberger *et al*., 2005). After arrival, the genus colonized and speciated into many ecological zones of South America, except tropical and extreme arid regions, growing in habitats from the coast to over 4000 m into the Andean cordillera (Samuel *et al.*, 2003). Radiation into divergent habitats has resulted in evolution of many diverse morphological features, including leaves, flowering heads, shape and vesture of the phyllaries, color of the florets, and changes in reproductive biology (Tremetsberger *et al*., 2006).

Previous AFLP analyses among the species of *Hypochaeris* of South America have revealed six major phylogenetic groupings (Tremetsberger *et al*., 2006). One of these groups, the *Hypochaeris apargioides* complex, consists of four outcrossing species (Fig. 1), *H. apargioides*, *H. gayana*, *H. spathulata*, and *H. thrincioides*. These species have different morphologies (Fig. 1) and occur in very different habitats (Fig. 2) in southern South America between the Andes of Argentina and the Chilean coastline. *Hypochaeris apargioides* is broadly distributed from Trehuaco (Chile) to Bariloche (Argentina), including Andean habitats and the central Chilean valley. *Hypochaeris gayana* is very restricted, growing only in the Cordillera de Nahuelbuta in Chile. *Hypochaeris thrincioides* is distributed from La Serena to Temuco, Chile, concentrated in the central valley between the Andes and the coast, and *H. spathulata* is confined to the coastal region (in the salty spray zone) of Chile from Boyeruca to Cucao. Previous studies in the outcrossing *Hypochaeris apargioides* complex have revealed chromosome numbers of $2n = 8$ (Baeza *et al*., 2000, 2001, 2004, 2007; Weis-Schneeweiss *et al.*, 2003), with polyploidy occurring only rarely (Baeza *et al*., 2006).

To document and understand processes of adaptive differentiation within the radiating *H. apargioides* complex in southern South America, our objectives in this paper are to: (1) examine patterns of morphological variation within each species of the complex using morphometric and greenhouse experiments; (2) examine patterns of corresponding genetic variation within and among populations of each species using AFLP techniques (Vos *et al.*, 1995); and (3) relate observed variation to known environmental parameters occurring within the distributional area.

Materials and Methods

Sampling. A total of sixty populations was collected throughout the distributional ranges of the species (Fig. 3, Table 1). The sampling of *H. apargioides* (22 populations) extended from Trehuaco (pop. 1) in the north of Chile to Cerro Buitrera (pop. 22) in the South of Argentina. All the samples of *H. gayana* (5 populations) come from Chile in the Nahuelbuta National Park (pops. 23-27). *Hypochaeris spathulata* (13 populations) is represented from Llico (pop. 28) in central Chile to Cucao (pop. 40) in the South. Finally, *H. thrincioides* (20 populations) was collected from La Serena (pop. 41) in northern Chile to Cerro Ñielol (pop. 60) in the South. Vouchers are on deposit in the herbarium of the University of Vienna (WU).

Morphometric analyses. Prior to detailed morphometric analyses, an exploratory principal component study was done, which involved examination of 170 characters (113 vegetative and 57 reproductive), from 10 individuals in 3 populations of each species. This allowed a first approximation of the most discriminatory characters within the complex. Results from this preliminary study identified 26 characters (13 vegetative, 13 reproductive) that explained the major proportion of variation within of each the components. These are: first, number of rosettes; second, features of the leaves, total number, succulence, perimeter, total area, total length, maximum width including lobes, width without lobes, vesture, and total numbers of lobes; third, features of the phyllaries, total number, number of rows, perimeter, area, length, maximum width, shape, apex, vesture; fourth, remaining features, length and vesture of peduncle, number, length and width of capitulum, and number and color of florets.

Using the selected characters, morphometric analysis was executed with five individuals per population from a total of 34 populations (170 individuals) within the complex (Table 1). A scan of the leaf and phyllaries for each individual was necessary to allow calculation of area and perimeter using the program ImageJ v. 1.38 (9 July 2007, National Institutes of Health). To identify the characters that are diagnostic for each species and that are likely under major environmental influence, principal component analysis was performed using SPSS ver. 15.0 (© SPSS Inc.).

Greenhouse experiments. To understand the degree to which the environment can cause plasticity in morphological features, in contrast to variation that is genetically controlled, plants of *Hypochaeris apargioides* (pops. 21, 22), *H. spathulata* (pop. 35), and *H. thrincioides* (pop. 56)

were cultivated in the greenhouse of the University of Seville, Spain. Seeds from open pollinated heads were collected in the field and germinated in the greenhouse. The experimental conditions were a photoperiod of 16 h light/ 8 h darkness, temperature 18-22°C, and watering every 4 h. Morphometric analysis was subsequently performed in the same way as for the broader populational analyses, plus qualitative observation of leaves and phyllaries. The Mann-Whitney *U* test using SPSS ver. 15.0 (© SPSS Inc.) was used to estimate the significance of differences between species grown in the greenhouse and those collected originally in the field.

AFLP analyses. The total numbers of individuals scored were 225 for six AFLP primer combinations. Genomic DNA was extracted from silica-gel dried leaf material following the CTAB method (Doyle and Doyle, 1987) with minor modifications (Tremetsberger *et al.*, 2003). The selective primer combinations chosen following primer-trials are *Mse*I-CAG/*Eco*RI-ACT (Fam), *Mse*I-CTC/*Eco*RI-ACG (Vic), and *Mse*I-CAG/*Eco*RI-AGC (Ned); *Mse*I-CTGA/*Eco*RI-ACT (Fam), *Mse*I-CTTC/*Eco*RI-ACG (Vic), and *Mse*I-CTCG/*Eco*RI-ATC (Ned).

Presence and absence of bands in all individuals were scored with GeneMarker ver. 1.85 by Soft Genetics. For each primer combination were selected: raw data analysis; local southern size call algorithm; smooth peak saturation; base line subtraction; pull-up correction; and spike removal. We used the range 110 to 510 for all primer combinations. The peak detection threshold was an intensity of relative fluorescent units over 150, with the percentage of relative minimum intensity of allele peaks at 5 and with the same value for local region percentage. The maximum relative fluorescent units threshold of peak height for peak detection was 30000. Size calibration was manually modified in those samples with values below 80%. The electropherograms were standardized using the automatic panel editor, generating a new panel for each color. A binary matrix was generated for each primer combination (Wooten & Tolley-Jordan, 2009). The reproducibility of the AFLP is 90 %.

Estimation of genetic diversity and differentiation. The number of different AFLP phenotypes present in a population was counted with Arlequin ver. 3.1 (Excoffier *et al.*, 2006). The number of private bands in each population and species was calculated using FAMD ver. 1.108 (Schlüter & Harris, 2006), and the Rarity Index, calculated by using the R-script AFLPdat (Ehrich, 2006).

Genetic diversity was assessed for each population and species by using the total number of AFLP bands, percentage of polymorphic bands (by dividing the number of polymorphic bands by the total number of bands in the dataset), and Shannon Diversity index $H_{Sh} = -\Sigma(p_i \times \ln(p_i))$, where p_i is the frequency of the i^{th} band in the respective population based on all AFLP bands recorded using FAMD ver. 1.108 (Schlüter & Harris, 2006). The Pearson correlation was used to test correlation among different genetic diversity estimates using SPSS ver. 15.0 (© SPSS Inc.). One way ANOVA test was used to estimate the significance of differences of divergence and genetic diversity between species using SPSS.

Genetic differentiation among species was assessed by analysis of molecular variance (AMOVA) using Arlequin ver. 3.1 (Excoffier *et al.*, 2006), where total genetic diversity was partitioned into components among two hierarchical levels, among populations (F_{ST}) and among individuals within populations. An alternative Bayesian approach (Holsinger *et al.*, 2002) was used to obtain an independent estimate of F_{ST} for each population. This method allows estimation of F_{ST} from dominant markers without assuming Hardy-Weinberg proportions in populations. The original data matrix was imported into Hickory ver. 1.1 (Holsinger & Lewis, 2003-2007) and used for a full model, $f = 0$ model, theta = 0 model, and *f*-free model run with default parameters (i.e., the hickory block omitted). The *f*-free model, which estimates theta without estimating *f* (thus incorporating all the uncertainty in the prior of *f*), is available for dominant marker data, because estimates of *f* derived from dominant marker data may be unreliable. The deviance information criterion (DIC; Spiegelhalter *et al.*, 2002) was used to estimate how well a particular model fits the data and to choose between models.

Results

Morphometric-greenhouse analyses. Principal components (PCoA) of individuals in populations of the *H. apargioides* complex (Fig. 4), revealed that the first three components explained 79.5% of the variation (axis one explains 62.5% of the variance, axis two 14. 0 %, and axis three 3.0%), allowing the formation of three groups, the first of which consists of *Hypochaeris spathulata*, the second of *H. gayana*, and the third a mixture of *H. apargioides* and *H. thrincioides* (Fig. 4). Characters that contribute a high percentage for group formation are area of phyllaries in component one, succulence of leaves in component two, and color of flowers in component three.

To verify whether variations observed in the different characters have a genetic basis, we morphometrically compared populations grown in the greenhouse with those from the field. Greenhouse-grown populations in a controlled environment reveal higher mean values (Figs. 5, 6). The Mann Whitney *U*-test showed significant differences in total area of the leaf (Z = -2.148, 2-tailed sig. = 0.032), and width of the leaf without lobes (Z = -2.152, 2-tailed sig. = 0.030) in *H. apargioides*. The characters in *H. spathulata* with significant differences were succulence of leaves (Z = -3.317, 2-tailed sig. = 0.030), total area of leaves (Z = -2.148, 2-tailed sig. = 0.032), and total length of leaves (Z = -2.148, 2-tailed sig. = 0.030). In *H. thrincioides* there were no significant differences. The same comparisons for reproductive features shows generally fewer differences. In *H. apargioides* only the total number of phyllaries (Z = -2.420, 2-tailed sig. = 0.016) gives a significant difference. In *H. thrincioides* the characters showing significant difference are total number of phyllaries (Z = -2.571, 2-tailed sig. = 0.010), length of phyllaries (Z = -2.380, 2-tailed sig. = 0.017) and shape of phyllaries (Z = -3.536, 2-tailed sig. = 0.000). *Hypochaeris spathulata* shows no significant differences between field and greenhouse plants.

AFLP analyses. *Fragment patterns.* The total number of AFLP fragments found in all individuals and all populations of the four species are 2127, with 100 % polymorphism. All individuals have unique AFLP phenotypes. *Hypochaeris apargioides* presents a total of 1661 fragments (100 % polymorphic), *H. gayana* has 750 fragments, of which 743 are polymorphic (99.06 %), *H. spathulata* has a total of 999 fragments (100 % polymorphic), and *H. thincioides* presents 1566 fragments (100 % polymorphic). The number of fragments for all individuals and by species (*H. apargioides*/*H. gayana*/*H. spathulata*/*H. thrincioides*) for primer *Mse*I-

CAG/*Eco*RI-ACT are 440 (364/153/176/357), for *Mse*I-CTC/*Eco*RI-ACG are 362 (244/158/185/301), for *Mse*I-CAG/*Eco*RI-AGC are 326 (239/178/169/225), in *Mse*I-CTGA/*Eco*RI-ACT are 352 (290/59/144/251), for *Mse*I-CTTC/*Eco*RI-ACG 362 (304/124/180/237), and in *Mse*I-CTCG/*Eco*RI-ATC are 285 (220/78/145/195).

Genetic diversity and divergence of populations. The three estimates of genetic diversity, i.e., total number of bands (TNB), percentage of polymorphic bands (PPB), and Shannon Diversity Index (SDI), are highest in *H. apargioides* and lowest in *H. spathulata*, the highest percentage of variation being found in *H. apargioides* and the lowest in *H. gayana* (Table 2, Fig. 7). These three indices are positively correlated, the Pearson correlation between TNB and PPB is $r = 0.995$ (N =47, sig. [2-tailed] = 0.000), among TNB and SDI is $r = 0.996$ (N =47, sig. [2-tailed] = 0.000), and between PPB and SDI is $r = 0.998$ (N =47, sig. [2-tailed] = 0.000). One way ANOVA analyses show in all indices significant differences among species (Table 2). Bonferroni correction identifies that significant differences occurred between the species *H. apargioides* and *H. spathulata*.

Among the genetic divergence estimates, the value of the number of private bands and Rarity Index are highest in *H. apargioides* and lowest in *H. spathulata* (Table 2, Fig. 7). Both indices are positively correlated, the Pearson correlation being $r = 0.812$ (N =47, sig. [2-tailed] = 0.000). One way ANOVA only shows significant differences between species in values of the Rarity Index (Table 2). Bonferroni correction for multiple comparisons shows that this difference was significant between *H. apargioides* and *H. spathulata*.

Genetic estimates among species. Analysis of molecular variance (AMOVA) attributes 7.10 % variance (d.f. = 3) among species and 92.90 % (d.f. = 221) within populations of each species. The result of the same analysis, but for each species, shows in *H. apargioides* a 23.11 % variance among populations (d.f. = 14) and 76.89 % variance (d.f. = 56) within populations (95% C.I. = 21.8-24.4 %). In *H. gayana*, variance among populations was 15.03 % (d.f. = 4) and 84.97 % (d.f. = 19) within populations (95% C.I. = 12.8-17.3 %). *Hypochaeris spathulata* shows a value of 26.94 % (d.f. = 8) and 73.06 % (d.f. = 35) (95% C.I. = 24.7-29.1 %) among and within populations, respectively. Finally, in *H. thrincioides* the value of variance between populations was 26.82 (d.f. = 17) and 73.18 % (d.f. = 68) (95% C.I. = 25.3-28.3 %) within populations.

Genetic variance among species using a Bayesian analysis shows the lowest DIC value with the full model (DIC value = 26448.1), where the theta-II value was 0.056 (95 % credible interval = 0.048-0.055). The results among populations of each species using the full model were in *H. gayana* (N = 5; DIC value = 6742) with a theta-II value of 0.142 (95 % credible interval = 0.121-0.163); in *H. spathulata* (N = 9; DIC value = 12124) the value of theta-II was 0.243 (95 % credible interval = 0.227-0.259); the value in *H. thrincioides* was (N = 18; DIC value = 30425) theta-II = 0.192 (95 % credible interval = 0.183-0.201); in *H. apargioides* (N = 15; DIC value = 30722) the value of theta-II was 0.169 (95 % credible interval = 0.161-0.779).

Neighbor net analysis with Splits Tree using the whole AFLP dataset reveals a series of closely knit populations (Fig. 8). *Hypochaeris apargioides* contain two principal groups, the first including populations in the southern limit of the species (Argentina, pops. 15, 18, and 19), and the other populations distributed in Chile (except pops. 3 and 6, the latter near to *H. gayana).* All populations of *H. gayana* cluster are distinct together, not intermixing with *H. apargioides*. *Hypochaeris spathulata* presents two groups (pops. 31-34 and pops. 28-30, 37, 38), perhaps suggesting a double origin for this species. Populations of *H. thrincioides* are gathered into four groups, some of them connected to *H. apargiodes* and *H. spathulata*. In general, populations of the *Hypochaeris apargioides* complex show no clear geographical pattern.

Discussion

Diversification of morphology during exploitation of new habitats and adaptations to ecological niches is a major feature of adaptive radiation (Schluter, 1993, 1996). Different approaches have been used for understanding the bases and dynamics of this process, including theoretical and empirical studies (Gavrilets and Losos, 2009). We seek correlated of adaptive radiation in the *Hypochaeris apargioides* complex, therefore, using morphological and molecular data and interpretations.

Morphological variation. Analyses of morphological variations in the *H. apargioides* complex show that characters of species restricted to more divergent environments (i.e., *H. gayana* and *H. spathulata*), are more morphologically distinct than those occurring over a wider range and occupying a broader spectrum of habitats (i.e., *H. apargioides* and *H. thrincioides*). Principal Component analyses clearly reflect this trend (Fig. 4), with three distinct groups corresponding to *H. spathulata*, *H. gayana,* and a third intermixed group with *H. apargioides* and *H. thrincioides*. A very important aspect of analysis of adaptive radiation is confirmation that the morphological variation observed in different species of the complex have a genetic basis. In the specific case of the leaves, numerous studies have addressed the issue of whether morphological variations reflect only the limits within a particular phenotype (Givnish, 1987; Winn, 1999; Fleming, 2003; McLellan, 2003, Meade & Parnell, 2003), or have a genetic basis (Kessler & Sinha, 2004; Tsukaya, 2006). In the *Hypochaeris apargioides* complex, it is common to find leaves in the same population with different shapes, sizes, margins, absence or presence of setose or lanuginose hairs, or a mixture of them. It is therefore very important to determine if the morphological variations observed in the field remain when plants are grown under uniform greenhouse conditions.

Plants grown in the greenhouse have higher values for leaf characters than those collected in natural environments (Fig. 5). This can be explained by optimum conditions of humidity, light, and temperature, which allowed a greater increase in leaf size, but still maintaining its original shape. With respect to hairiness of the leaves (qualitative observations), in *H. apargioides* the greenhouse-grown plantn show similar variations to those found in the natural environment. Some plants are completely glabrous, whereas others have differing densities of setose hairs. The same effect was observed in the leaves of *H. thrincioides*. When analyzing

degrees of thickness of leaves in individuals of *H. spathulata* in the greenhouse, these tend to be less fleshy in comparison to those collected in the wild. Taking into account that the habitat of *H. spathulata* includes sandy and rocky areas very close to the coast, under constant influence of the salty spray, and thus a drying atmosphere, the thickness of the leaves may be an adaptation to salinity. This thickness may also provide greater resistance to mechanical damage due to wind (Rozema *et al.*, 1985). In the case of the phyllaries, variations observed in the field were similar among individuals of different populations, and vary mainly in the presence or absence of setose or/and lanuginous hairs. In the greenhouse (Fig. 6), phyllaries of *H. thrincioides* in some plants appear glabrous and in others with variable vesture similar to those grown in the field. In *H. spathulata* the presence of phyllaries with a dense cover of hairs was common, similar to plants in the field. In this case, this character may have a function of protecting the young capitula, which can be an adaptation to coastal habitats (Voronkova *et al.*, 2008).

Based on results of the greenhouse trials, characters involving succulence of leaves and vesture of phyllaries, do appear to be under genetic control. This would be significant for supporting adaptations to different environments. While degree of succulence of the leaves shows some morphological plasticity, this may simply reflect an initial stage of the process of adaptive radiation (Ramsey *et al.*, 2008). Overlapping characters in *H. apargioides* and *H. thrincioides* reinforces the idea of early adaptive radiation (Whang *et al*., 2002).

Genetic divergence and diversity. *Among species.* AFLP data from the *H. apargioides* complex indicate a low F_{ST} value between species (F_{ST} = 0.0710), and hence a high degree of genetic similarity due to a high proportion of alleles similar between them. This high degree of genetic similarity has also been documented in other groups having undergone adaptive radiation, such as in *Achillea* (Ramsey *et al.,* 2008) and *Dabautia* (Remington & Robichaux, 2007). The number of private bands in *H. apargioides* is ten times higher than in *H. gayana*, and six times higher than in *H. spathulata*, which suggest that *H. apargioides* may be the oldest species in the group (along with *H. thrincioides*). The youngest species of the complex may be *H. gayana*.

Speciation. *Phylogenetic relationships.* Molecular data (number of the private bands and Rarity index; Fig. 7) support *H. apargioides* as the most ancient species within the complex. With this ancestry, we might hypothesize that *H. apargioides* served as the ancestor of *H.*

gayana through peripatric speciation, the latter being geographically restricted to the Cordillera de Nahuelbuta. The presence of some individuals of *H. thrincioides* growing sympatrically with *H. spathulata*, plus alleged presence of hybrids between the two (Tremetsberger *et al.*, 2006) and the AFLP data (Fig. 8), also allow suggestion of multiple origins of *H. spathulata* from *H. thrincioides* through parapatric speciation (Futuyma, 1998). Finally, one might also speculate on the origin of *H. thrincioides* from *H. apargioides*, as they maintain an geographic overlap in the southwest and northeast, respectively.

Environmental correlations. Hypochaeris apargioides shows no clear correlation with environmental factors, although there is a tendency for it to occupy the climatic "Temperate" zone (Amigo & Ramírez, 1998; Luebert & Pliscoff, 2006), with a small projection into the "Mediterranean" zone. Annual average precipitation and temperature vary significantly between different bioclimatic zones, as do also edaphic factors (e.g., semi-coastal zone, central valley and Andes Mountains).

The restricted distribution of *H. gayana* in the Cordillera de Nahuelbuta (Nahuelbuta National Park) is correlated with altitude 1200-1400 m and soil characteristics, derived from granitic rocks, with sandy-clay texture, being moderately acid, and with normal levels of organic matter in the soil (Carrasco & Millán, 1990; Schmidt *et al.*, 1991). Climatic influences derive from annual precipitation between 2,000-2,700 mm, with a dry season of four months, and an average annual temperature between 10 °C - 13 °C (Contreras & Perret, 1984; Santibañez & Uribe, 1993). Luebert & Pliscoff (2006) characterize the vegetation of the Nahuelbuta mountains as a temperate coastal resin forest of *Araucaria araucana*, mixed with *Nothofagus dombeyi, N. pumilio* and *N. antarctica*. An other interesting characteristic in this species is the presence of orange florets, which might be related to a specific pollinator, but no data are available to test this hypothesis.

The distribution of *H. spathulata* shows a clear environmental correlation. This species grows along the coastal fringe, on sandy beaches or rocky cliffs and sand, and always under the constant influence of the salt spray of the sea. These soils are characterized by low water retention capacity, as well as lack of nutrients (Bernabé, 2004, Medina *et al*., 2008). It is known that decrease in soil fertility or increase in irradiation can cause an increase in thickness of plant leaves (Sobrado & Medina, 1980; Medina 1984; Givnish 1987). Salt spray can produce an

increase in leaf water content (Griffiths & Orians, 2003), and this effect appears to have occurred in the leaf morphology of *H. spathulata*.

Finally, the distribution of *H. thrincioides* is related to the Mediterranean climatic zone, which is characterized by a summer of at least two months (Amigo & Ramírez, 1998). The distribution of this species coincides well with a map of an average annual 13 °C isotherm (Romero, 1985) and with vegetation that includes elements of thorny, sclerofilous, and deciduous forests, this latter formed by *Nothofagus macrocarpa*, *N. glauca,* or *N. obliqua*, and low shrubs (Luebert & Pliscoff, 2006).

Biogeography. *Pleistocene glaciations.* The current distributions of species in the *Hypochaeris apargioides* complex can be explained, at least partially, by examining patterns of Pleistocene glaciation. Heuser (2003) indicated that Chile was completely covered with ice until latitude 42°S, then gradually restricted to the Andes as it moved northward, reaching Cordillera de Vallenar at 28°S. We assume that populations of *H. spathulata* were not affected by Pleistocene glaciation in the Chiloe area, nor on the coast northward. In the case of *H. apargioides,* we offer three possibilate.

The first hypothesis would be the presence of original populations along the coastal zone that were not affected by glaciations. Once the ice retreated, populations colonized the new areas, finally the resulting in the present distribution. The second hypothesis is displacement during glacial cycle toward the coastal zone of populations in the Andean region and subsequent recolonization of the Andes. The third option would be existence of refugia in the area of Laguna del Laja (in the Andes), which were not affected by Pleistocene glaciation, similar to what happened at Paso Pino Hachado (Lonquimay area; Heusser, 2003). *Hypochaeris gayana* survived glaciations in the refuge of Cordillera de Nahuelbuta, an area not markedly affected by Pleistocene event (Paula & Leonardo, 2006). Finally, *H. thrincioides,* being distributed further north into the Mediterranean climate, was probably little affected by Pleistocene glaciations.

Biogeographic hypothesis. Comparing measures of population antiquity and divergence (i.e., Number of private bands and Rarity Index), the species with the highest values is *H. apargioides*, suggesting that this could be the oldest species of the complex, followed by *H. thrincioides, H. gayana* and *H. spathulata* in that order. In *H. apargioides*, the populations with the highest value of genetic divergence are those located in the Andes Mountains (pops. 7, 8).

These populations correspond to the highest values of genetic diversity within populations, suggesting possible origin in this zone and subsequent colonization southward into areas of lower elevation (central Valley of Chile; second hypothesis above).

The absence of strong phylogeographic patterns in the molecular analyses of the *Hypochaeris apargioides* complex is not because of insufficient sampling of the genome, as noted by Vijverberg *et al.* (2000). We have used six primer combinations, with a total of 2127 fragments scored. Givnish (1997) notes that lack of divergence between species in an adaptively radiating complex may point to a recent radiation or that only few genes control the features of ecological adaptation. The like-star shape of the AFLP genetic network (Fig. 8) presents similarities with those found in the genus *Achillea* (Ramsey *et al*., 2008), which has also been suggested to undergo incipient adaptive radiation.

AFLP analyses do not show ecotypic separations within each species of the complex, giving results similar to those found in the incipient adaptive radiation of *Microseris* (Vijverberg *et al.*, 2000). When populations of *H. thrincioides* are subdivided into three groups corresponding to Coastal (pops. 41, 53-55, 57), Central Valley (pops. 50, 51, 59, 60), and Andes Mountains (pops. 42-49, 52), no significant differences are seen between groups. The same results occur within *H. spathulata* when subdivided into two groups, corresponding the northern (pops. 28-33) and southern (pops. 34, 37, 38) populations.

Conclusion

The results of morphological and molecular analyses reveal a process of adaptive radiation in the *H. apargioides* complex. The group is evolutionarily young, certainly less than one million years in the South American continent (Tremetberger *et al.*, 2005, 2006). The presence of key innovations in the different species of the complex (i.e., succulence of the leaves, floret color, and vesture of phyllaries) plus facility of long distance dispersal, have provided the opportunity to successfully colonize new habitats (Hughes & Eastwood, 2006). The presence of numerous characters with intermediate stages, high morphological variation within and between species, and the molecular evidence of populational groups with low levels of genetic cohesion, suggest that the process of adaptive radiation may still be in an early stage of development.

Acknowledgements

The authors wish to thank J. Cáceres-Chamizo, C. López Cáceres, P. Peñailillo, and L. Letelier for help with collecting population samples; Dr. Prof. J. Greimler for help with analysis of morphometric data; Departamento de Botánica, Universidad de Concepción, Chile, for logistical support; Corporación Nacional Forestal (CONAF) for permission to collect samples in Chilean National Parks; and Administración de Parques Nacionales, Argentina, for permission to make collections in Argentina. This project was supported by the Fonds zur Förderung der Wissenschaftlichen Forschung, Austria (FWF grant P18446 to TFS).

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Table 1 Collection data of populations of the *Hypochaeris apargioides* complex used for AFLP and morphometric analyses. Vouchers are on deposit at WU. A = AFLP analysis; M = Morphometric analysis; G = Greenhouse experiment; P.N. = Parque Nacional; R.N. = Reserva Nacional, M.N. = Monumento Natural; CB = Carlos Baeza; EU= Estrella Urtubey; PL = Patricio López; TS = Tod Stuessy.

	Analysis	Population	Collection number	Latitude	Longitude	Elevation (m)
Hypochaeris apargioides Hook. et Arn.	M	1: Chile, Trehuaco	PL *et al. 2642*	36°25’15” S	72°38’51” W	90
	M	2: Chile, Concepción	PL *et al. 2645*	36°47’57” S	73°07’00” W	10
	A	3: Chile, Shangri-La	PL *et al. 2550*	36°53'12" S	71°28'15" W	1430
	M	4: Chile, Camino a Las Trancas	PL *et al. 2546*	36°54'39" S	71°29'43" W	1215
	A	5: Chile, Termas de Chillán	PL *et al. 2542*	36°55'17" S	71°25'17" W	1650
	A, M	6: Chile, Yumbel	PL *et al. 2634*	37°09’00” S	72°32’00” W	112
	A	7: Chile, P.N. Laguna del Laja	PL *et al. 2585*	37°22'28" S	71°01'32" W	1430
	A, M	8: Chile, Chacay	PL *et al. 2586*	37°23’42” S	71°25’11” W	1120
	M	9: Chile, Ralco-Lepoy	PL *et al. 2663*	38°03’19” S	71°20’25” W	941
	A	10: Chile, R.N. Malalcahuello	PL *et al. 2552*	38°25'12" S	71°32'40" W	1430
	A	11: Chile, Volcán Lonquimay	PL *et al. 2558*	38°26'55" S	71°15'52" W	940
	A, M	12: Chile, Termas Rio Blanco	PL *et al. 2563*	38°34'34" S	71°37'38" W	1035
	A	13: Chile, Liucura	PL *et al. 2562*	38°37'29" S	71°06'51" W	1040
	A, M	14: Chile, Volcán Llaima	PL *et al. 2573*	38°41'14" S	71°50'39" W	1115
	A	15: Argentina, Villa Pehuenia	EU *et al. 312*	38°52’42” S	71°11’21” W	1686
	A	16: Chile, entre Aduana y Volcán Llaima	PL *et al. 2575*	39°33'49" S	71°30'36" W	1090
	A, M	17: Chile, Laguna Huinfica	PL *et al. 2576*	39°34'45" S	71°30'01" W	1170
	A	18: Argentina, Junin de Los Andes	EU *et al. 307*	39°54’30” S	71°18’28” W	1003
	A	19: Argentina, Lago Melinque	EU *et al. 305*	40°22’29” S	71°15’23” W	942
	M	20: Argentina, Cerro Buitrera	PL *et al. 2684*	41°13’09” S	71°09’21” W	917

Table 1 Continued

	Analysis	Population	Collection number	Latitude	Longitude	Elevation (m)
	G	21: Argentina, Cerro Buitrera	TS *et al*. 18021	41°16’51’’ S	71°08’57’’ W	1130
	G	22: Argentina, Cerro Buitrera	TS *et al*. 18023	41°17’09’’ S	71°08’32” W	1150
Hypochaeris gayana (DC) Cabrera	A, M	23: Chile, P.N. Nahuelbuta	PL *et al*. *2622*	37°47'59" S	73°00'53" W	1260
	A, M	24: Chile, P.N. Nahuelbuta	PL *et al*. *2591*	37°48'33" S	73°00'57" W	1215
	A	25: Chile, P.N. Nahuelbuta	PL *et al*. *2589*	37°49'01" S	73°01'25" W	1320
	A, M	26: Chile, P.N. Nahuelbuta	PL *et al*. *2590*	37°49'06" S	73°01'54" W	1390
	A, M	27: Chile, P.N. Nahuelbuta	PL *et al 2588*	37°49'08" S	72°59'51" W	1295
Hypochaeris spathulata (Remy) Reiche	A	28: Chile, Llico	PL *et al*. 2649	34°45’17” S	72°05’24” W	4
	A, M	29: Chile, Playa Duao	PL *et al*. 2647	34°51’49” S	72°08’53” W	8
	A	30: Chile, Constitución	PL *et al*. 2644	35°19’44” S	72°25’55’’ W	10
	A, M	31: Chile, Playa Loanco	PL *et al*. 2616	35°34’43” S	72°37’07” W	15
	A, M	32: Chile, Curanipe	PL *et al*. 2614	35°50’17” S	72°37’36” W	15
	A, M	33: Chile, Cobquecura	PL *et al*. 2540	36°05’38” S	72°48’47” W	8
	A, M	34: Chile, Punta Lavapié	PL *et al*. 2618	37°08’58” S	73°35’06” W	5
	G	35: Chile, Caleta Rumena	CB 257	37°10’31” S	73°36’49” W	2
	M	36: Chile, Lebu	PL *et al*. 2620	37°34’22” S	73°38’32” W	3
	A, M	37: Chile, Quidico	PL *et al*. 2651	38°14’33” S	73°29’30” W	20
	A, M	38: Chile, Lobería	PL *et al*. 2652	38°36’55” S	73°29’30” W	19
	M	39: Chile, Playa Curiñanco-Niebla	PL *et al*. 2669	39°46’29” S	73°23’31” W	50
	M	40: Chile, Cucao	PL *et al*. 2670	42°34’53” S	74°07’56” W	5

Table 1 Continued.

	Analysis	Population	Collection number	Latitude	Longitude	Elevation (m)
Hypochaeris thrincioides (Remy) Reiche	A, M	41: Chile, La Serena	PL *et al. 2641*	29°55’36” S	71°16’35” W	5
	A	42: Chile, Villa El Colorado	PL *et al. 2535*	33°20'33" S	70°17'16" W	2775
	A	43: Chile, La Parva-Farellones	PL *et al. 2538*	33°20'40" S	70°18'04" W	2550
	A	44: Chile, Camino al Colorado	PL *et al. 2533*	33°20'48" S	70°17'41" W	2662
	A	45: Chile, Villa El Colorado	PL *et al. 2534*	33°20'51" S	70°17'26" W	2785
	A	46: Chile, El Colorado	PL *et al. 2536*	33°21'00" S	70°17'48" W	2695
	A, M	47: Chile, Farellones	PL *et al. 2529*	33°21'58" S	70°18'20" W	2350
	A, M	48: Chile, M.N. El Morado	PL *et al. 2525*	33°49'03" S	70°03'55" W	2030
	A, M	49: Chile, Camino Central Pangal	PL *et al. 2637*	34°14’57” S	70°23’55” W	1150
	A	50: Chile, Termas de Cauquenes	PL *et al. 2636*	34°15’05” S	70°33’03” W	800
	A	51: Chile, Talca, desvío San Rafael	PL *et al. 2635*	35°15'32" S	71°28'52" W	180
	A, M	52: Chile, R.N. Altos de Lircay	PL *et al. 2656*	35°36’21” S	71°02’15” W	1712
	A, M	53: Chile, R.N. Los Ruiles	PL *et al. 2613*	35°50'04" S	72°30'24" W	193
.	A, M	54: Chile, Talcahuano-Penco	PL *et al. 2628*	36°44'02" S	73°03'23" W	4
	A, M	55: Chile, Lenga	PL *et al. 2629*	36°45'53" S	73°09'20" W	4
	G	56: Chile, Talcahuano	TS *et al*. 18092	36°46’01” S	73°09’07” W	5
	A	57: Chile, Cerro Pon-Pon	PL *et al. 2581*	36°48'44" S	73°10'22" W	20
	M	58: Chile, Arauco, Licauquen	PL *et al. 2650*	37°45’44” S	73°24’09” W	95
	A	59: Chile, Ercilla, puente Dumo	PL *et al. 2579*	38°08'52" S	72°18'25" W	340
	A, M	60: Chile, Cerro Ñielol	PL *et al. 2654*	38°43’23” S	72°35’13” W	255

Table 2 Estimates of divergence and genetic diversity within the *Hypochaeris apargioides* complex based on AFLP analyses, from a total of 225 individuals in 47 populations. SD = standard error; *= significant differences between species, below 0.05

Species	Population	Number of private bands	Rarity Index	Total number of bands	Percentage of polymorphic bands	Shannon Diversity index
Hypochaeris apargioides	3	6	6.19	309	13.77	95.82
	5	14	15.23	521	23.36	158.22
	6	14	14.02	341	14.57	96.57
	7	28	26.81	606	25.85	177.27
	8	33	21.11	501	22.23	150.04
	10	8	12.23	478	21.53	146.26
	11	14	15.10	510	22.99	157.65
	12	4	11.16	481	21.01	140.64
	13	20	17.35	573	25.48	172.69
	14	12	15.40	559	24.82	169.31
	15	0	3.83	219	9.97	67.93
	16	10	21.05	346	13.02	96.00
	17	3	9.05	415	18.48	125.23
	18	3	2.82	161	7.05	48.90
	19	0	2.91	192	7.99	54.47
	Mean (±SD)	**11.27 (±9.76)**	**12.95 (±7.13)**	**414.13 (±144.49)**	**18.14 (±6.53)**	**123.80 (±43.89)**
H. gayana	23	2	5.72	255	10.63	74.66
	24	11	10.85	378	16.46	111.20
	25	4	5.32	278	12.08	82.89
	26	7	7.53	307	13.40	89.39
	27	4	6.17	307	12.88	87.52
	Mean (±SD)	**5.60 (±3.51)**	**7.12 (±2.25)**	**305.00 (±46.28)**	**13.09 (±2.15)**	**89.13 (±13.58)**

Table 2 Continued.

Species	Population	Number of private bands	Rarity Index	Total number of bands	Percentage of polymorphic bands	Shannon Diversity index
H. spathulata	28	1	3.42	192	8.46	57.40
	29	7	5.70	193	8.51	60.11
	30	3	6.35	282	12.55	91.84
	31	6	6.69	306	13.26	88.89
	32	6	9.47	360	15.00	101.79
	33	5	9.49	367	15.61	105.56
	34	3	6.19	313	13.68	91.84
	37	3	4.41	221	9.69	65.02
	38	8	7.98	295	12.79	86.12
	Mean (±SD)	**4.67 (±2.29)**	**6.63 (±2.07)**	**281.00 (±65.91)**	**12.17 (±2.67)**	**83.17 (±17.91)**
H. thrincioides	41	12	7.49	197	8.60	57.87
	42	12	10.51	373	16.27	108.58
	43	3	8.68	346	15.04	101.03
	44	7	12.45	469	20.78	140.75
	45	2	11.16	456	20.31	136.36
	46	6	9.87	409	18.62	125.57
	47	3	6.30	262	11.52	78.40
	48	15	10.94	310	13.63	91.61
	49	15	9.52	246	10.72	76.68
	50	10	7.36	252	11.24	76.68
	51	6	6.96	251	11.19	76.45
	52	9	14.63	480	22.24	150.81
	53	5	9.70	326	14.38	99.77
	54	8	6.97	310	13.68	91.77

Table 2 Continued.

Species	Population	Number of private bands	Rarity Index	Total number of bands	Percentage of polymorphic bands	Shannon Diversity index
	55	2	3.58	214	9.59	65.05
	57	0	5.84	185	6.86	50.60
	59	7	8.62	362	16.22	109.55
	60	8	10.98	397	17.96	120.98
	Mean (±SD)	**7.22 (±4.40)**	**8.98 (±2.66)**	**324.72 (±93.32)**	**14.38 (±4.42)**	**97.70 (±29.12)**
ANOVA one way	**F**	**2.44**	**4.62**	**3.67**	**3.50**	**3.68**
	significance	**0.077**	**0.007 ***	**0.019 ***	**0.023 ***	**0.019 ***

Table 3 Estimates of divergence and genetic diversity within species of the *Hypochaeris apargioides* complex based on AFLP analyses.

Species	Number of private bands	Rarity Index	Total number of bands	Percentage of polymorphic bands	Shannon Diversity index
H. apargioides	311	12.41	1661	78.09	279.82
H. gayana	30	7.18	750	34.93	159.44
H. spathulata	55	6.65	999	46.97	186.73
H. thrincioides	236	9.08	1566	73.62	244.96

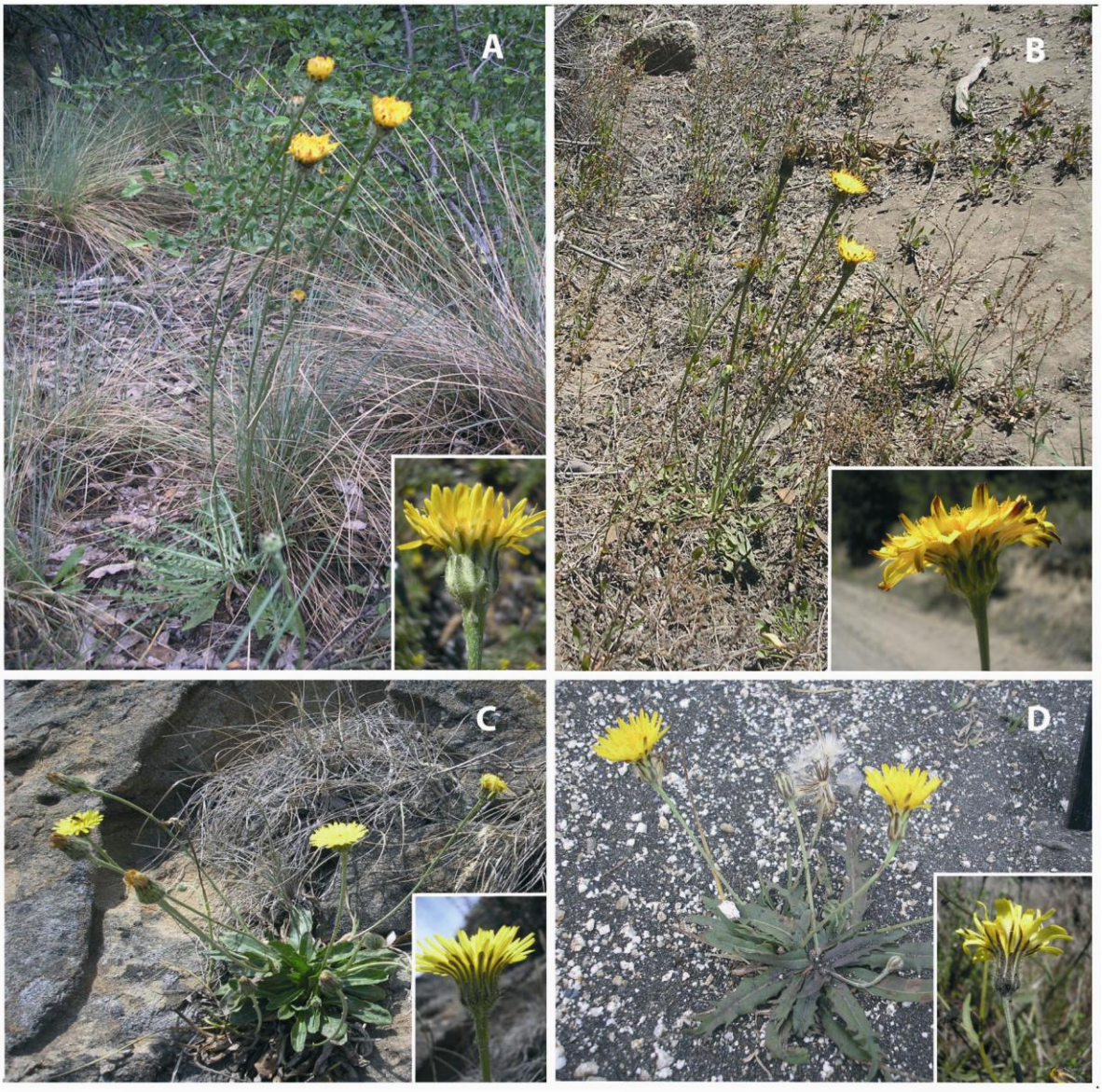

Figure 1. Species of the *Hypochaeris* a*pargioides* complex (insets showing flowering heads). A, *H. apargioides*; B, *H. gayana*; C, *H. spathulata*; D, *H. thrincioides*.

Figure 2. Typical habitats of species of the *Hypochaeris apargioides* complex. A, *H. apargioides*, *Nothofagus* woodland, near Parque Nacional Tolhuaca; B, *H. gayana*, *Araucaria* forest, Parque Nacional Nahuelbuta; C, *H. spathulata*, rocky coastal cliffs at Caleta Rumena; D, *H. thrincioides*, Mediterranean deciduous forest in Reserva Nacional Altos de Lircay, central Chile.

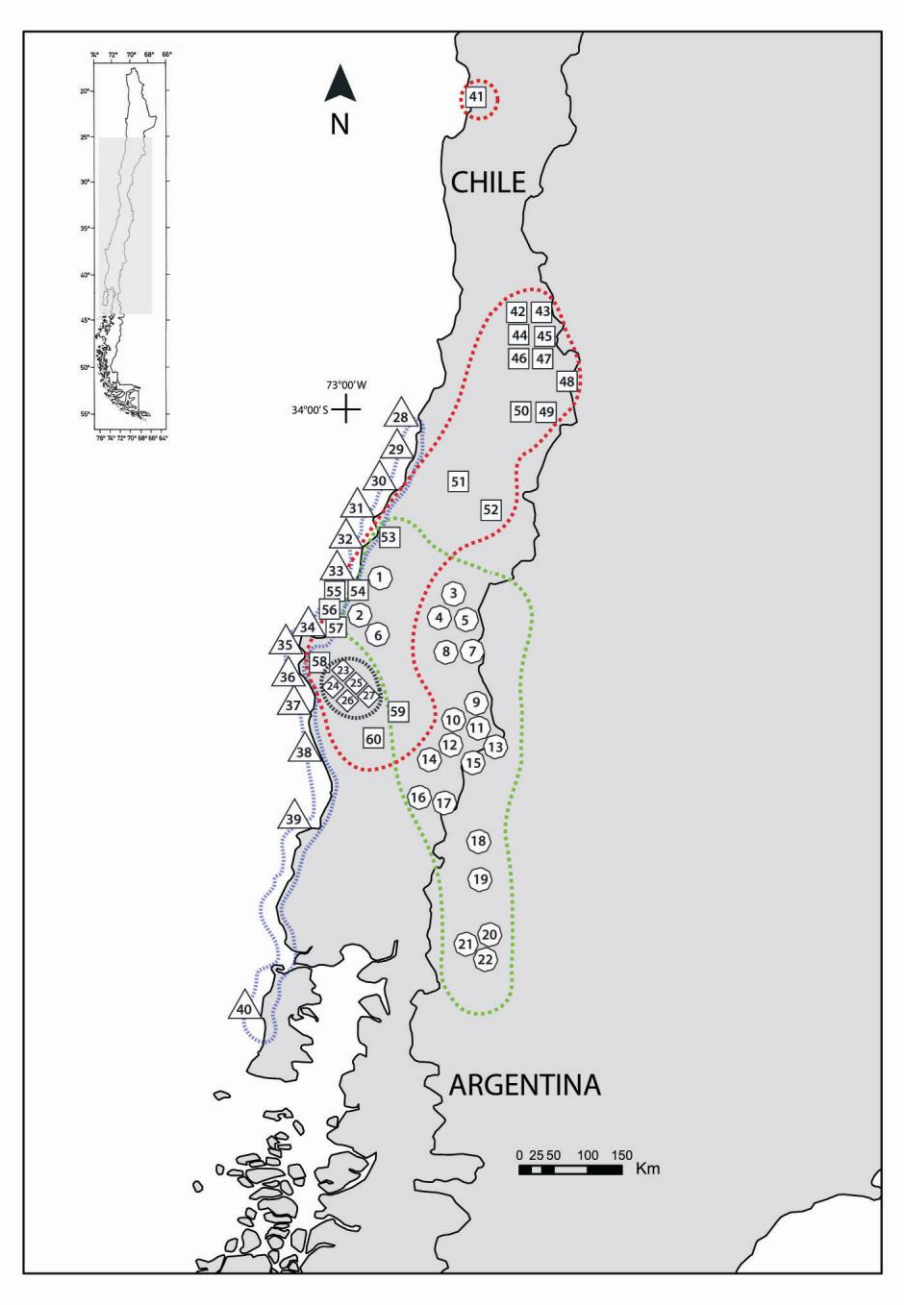

Figure 3. Distribution of the species of the *Hypochaeris apargioides* complex (dotted lines) and populations sampled (*H. apargioides*, circles; *H. gayana*, diamonds; *H. spathulata*, triangles; *H. thrincioides*, squares). See Table 1 for details of localities.

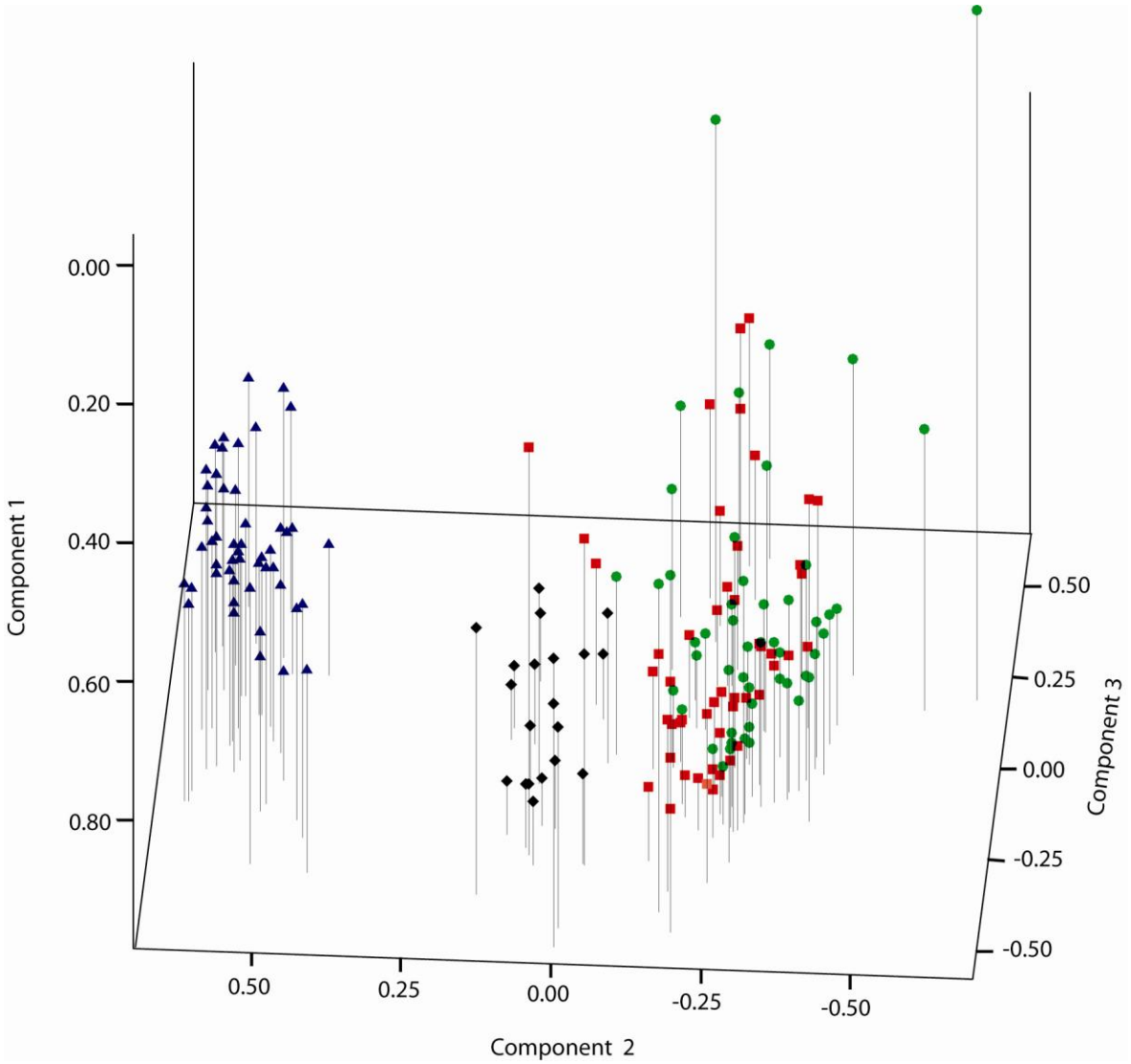

Figure 4. Principal components analysis of morphometric data showing three major groupings (symbols represent individuals): blue triangles = *Hypochaeris spathulata*; black diamond= *H. gayana*; red square = *H. thrincioides;* green circles = *H. apargioides*.

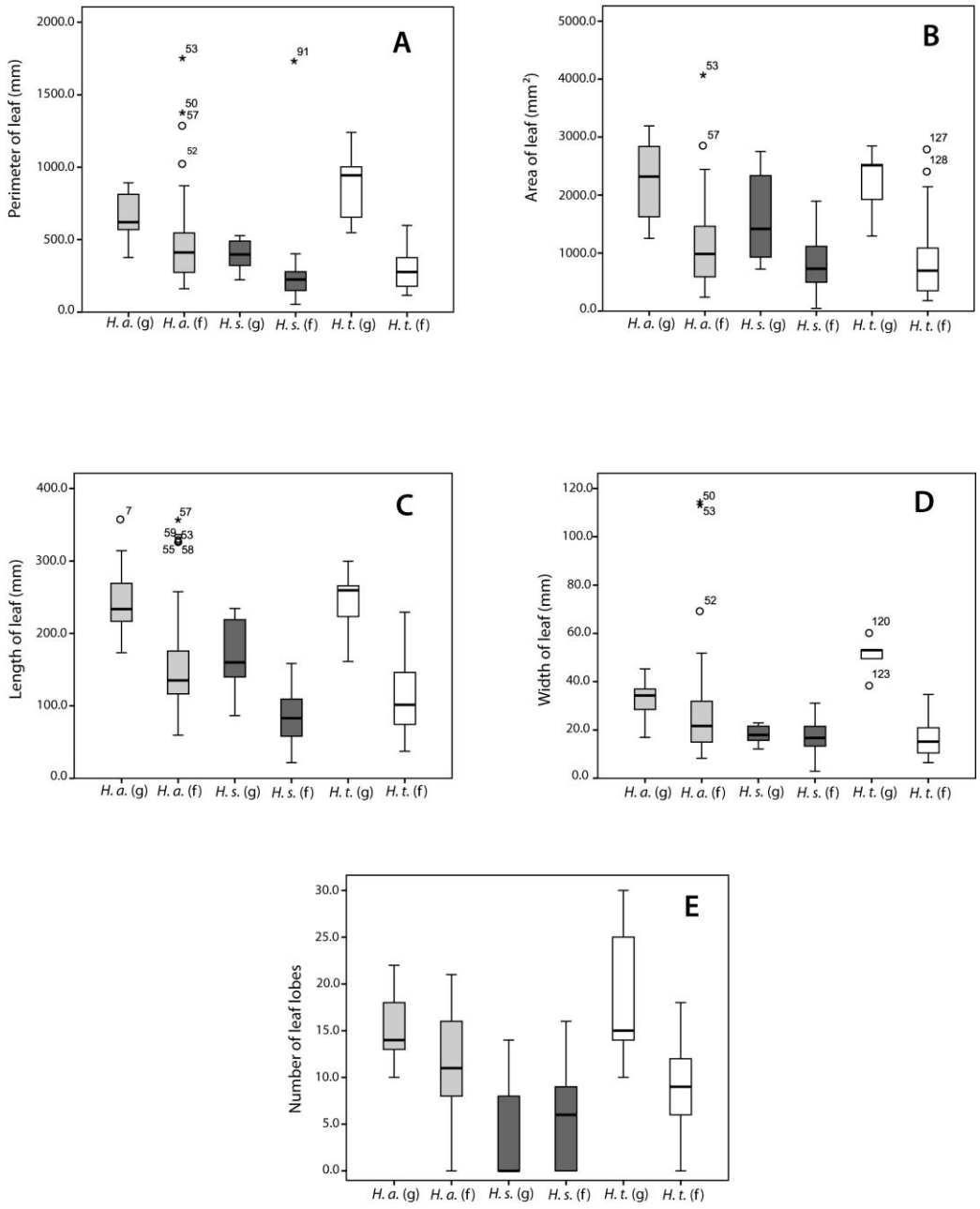

Figure 5. Boxplot analyses of leaf features (A, perimeter; B, area; C, length; D, width; E, number of lobes) between individuals of *H. apargioides* (*H. a.*), *H. spathulata* (*H. s.*), and *H. thrincioides* (*H. t.*) from the greenhouse (g) and field (f).

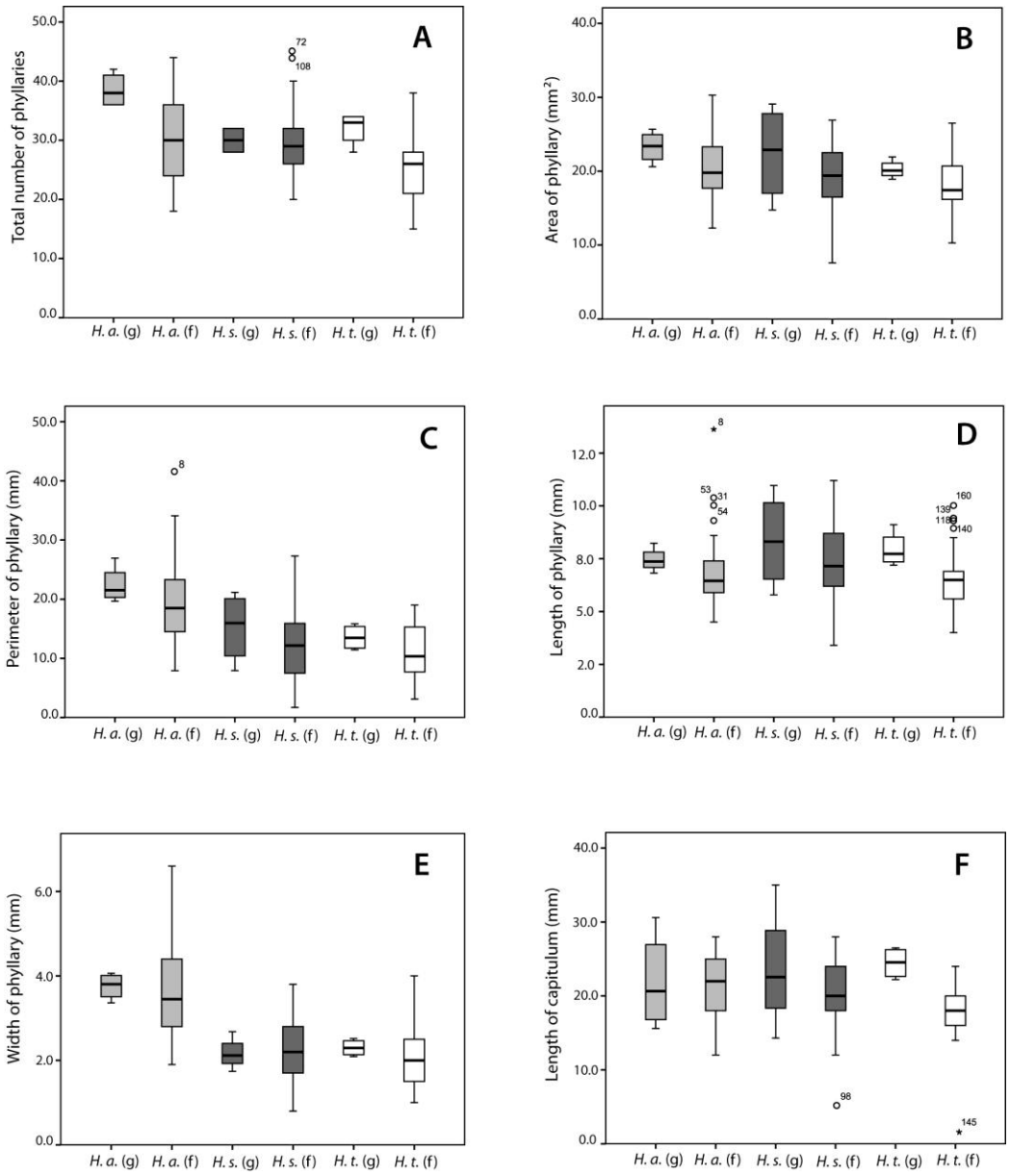

Figure 6. Boxplot analyses of phyllaries and capitulum features between individuals of *H. apargioides* (*H. a.*), *H. spathulata* (*H. s.*), and *H. thrincioides* (*H. t.*) from the greenhouse (g) and field (f). A, total number of phyllaries; B, area of phyllary; C, perimeter of phyllary; D, length of phyllary; E, width of phyllary; F, length of capitulum.

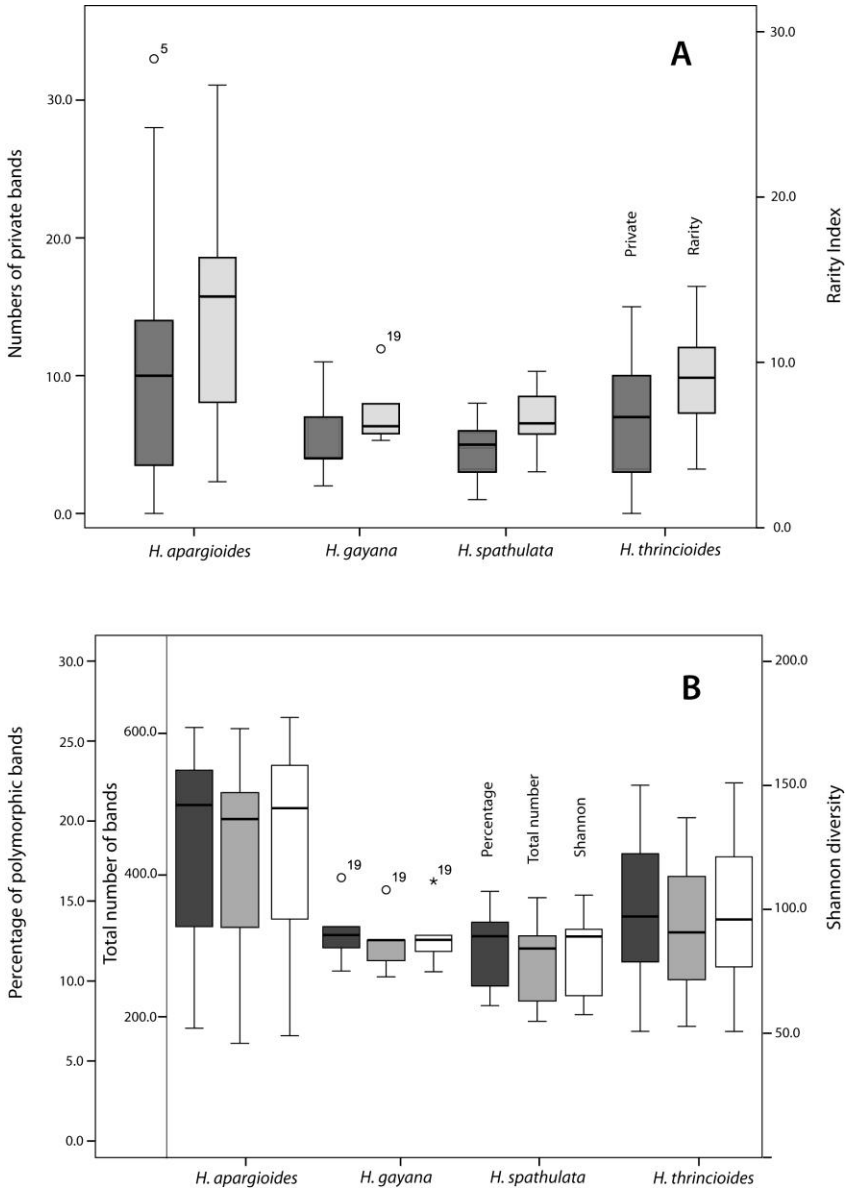

Figure 7. Boxplots of AFLP data showing the median, 25%, and 75% quartile in the *Hypochaeris apargioides* complex. A, number of private bands and Rarity Index; B, percentage of polymorphic bands, total number of bands, and Shannon Diversity index. Population numbers with circle or asterisk represent outliers.

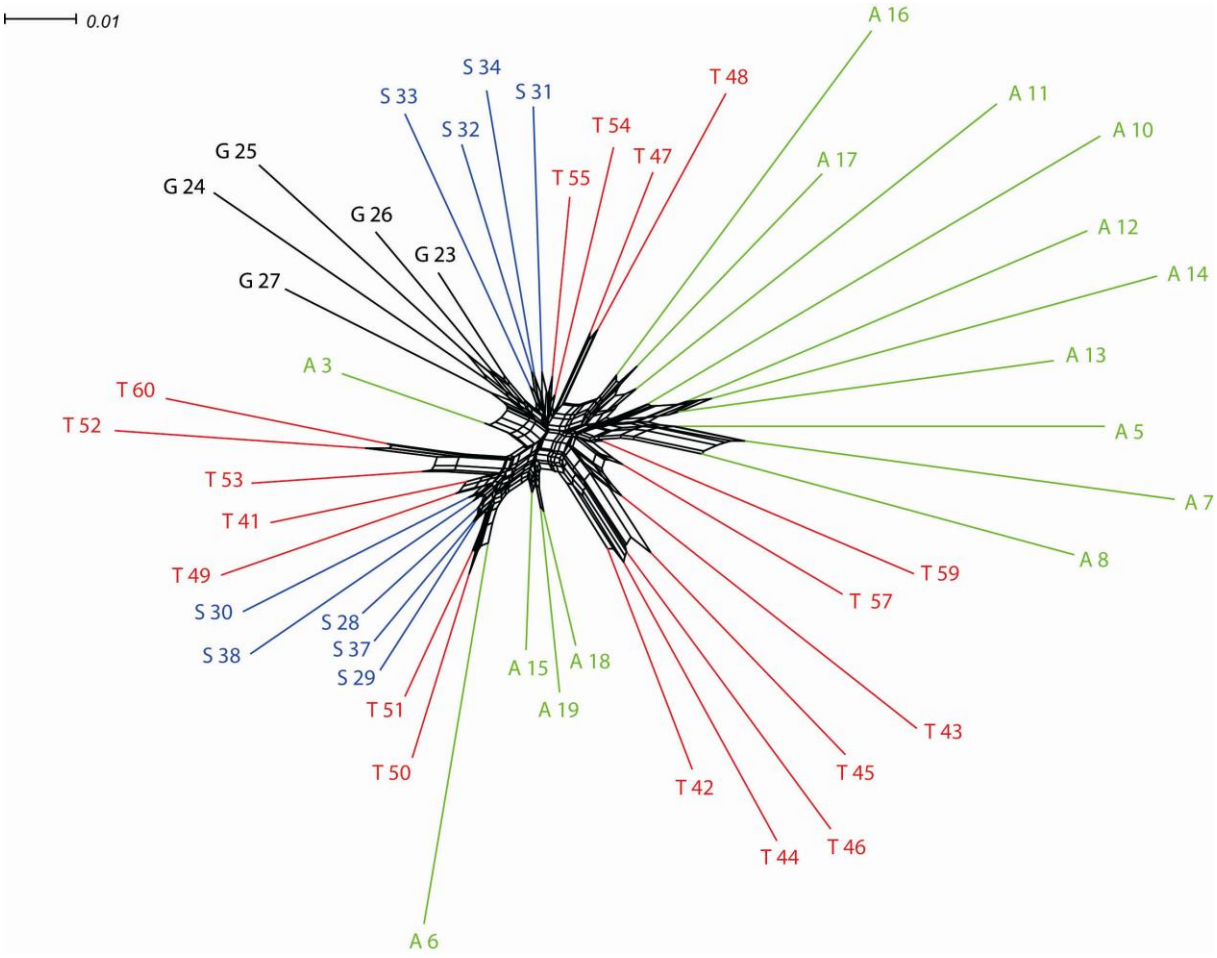

Figure 8. SplitsTree Neighbor net analysis of AFLP data showing genetic variation among populations of the *Hypochaeris apargioides* complex. The scale indicates percentage of base pair changes. A = *H. apargioides* (green); G = *H. gayana* (black); S = *H. spathulata* (blue); T = *H. thrincioides* (red). See Fig. 3 and Table 1 for distributions of numbered populations.

General Conclusion

The Andes Mountains of southern of South America is an area in wich have developed innumerable evolutionary processes in different organisms, and which have allowed colonization of a wide variety of environments. Patterns of genetic diversity in colonizing plant species, geographic speciation, and adaptive radiation are only a small sampling of the spectrum of evolutionary mechanisms that have occurred.

Study of the pattern of genetic diversity in colonizing *Nassauvia lagascae* var. *lagascae* (Asteraceae) in gap areas after the eruption of Volcán Lonquimay (Araucanía Region, Chile), shows the occurrence of a founder effect. Limited numbers of founding propagules, derived from nearby source populations, has led to reduction in levels of genetic diversity that have not yet been compensated by subsequent population growth and migration. The recovery of genetic diversity must occur through slow population growth, kin-structure within populations, and low rates of secondary dispersal.

Progenitor-derivative speciation in the genus *Pozoa* (Apiaceae) was also examined. Based on chloroplast markers and AFLP analysis, using *Asteriscium* and *Gymnophyton* as outgroups, *Pozoa* is shown to be monophyletic. The geography, ecology, and populational genetic data from AFLPs, together with a high genetic and morphological similarity between the species, plus lower genetic variation in *P. volcanica,* confirm that *P. volcanica* is a species derived from its progenitor *P. coriacea*. Local glaciers in the Andes Mountain and volcanic activity may have provided new ecological opportunities that stimulated speciation within the genus.

Adaptive radiation in the *Hypochaeris apargioides* complex has also been investigated. Morphometric studies show that two species, *H. spathulata* and *H. gayana,* are clearly separated phenotypically from the others, but that *H. apargioides* and *H. thrincioides* are more similar to each other. The principal environmental conditions influencing morphology and distribution of species in this complex appear to be salinity and elevation in *H. spathulata* and *H. gayana,* respectively, and ambient temperature in *H. thrincioides*. The presence of numerous characters with intermediate stages, high morphological variation within and between species, and the molecular evidence of populational groups with low levels of genetic cohesion, suggest that the process of adaptive radiation may still be in an early stage of development.

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EDUCATION

2007 – at present:	Doctoral thesis: Genetic diversity, speciation and evolutionary relationships in *Pozoa* (Apiaceae), *Nassauvia*, and the *Hypochaeris apargioides* complex (Asteraceae) in southern South America.
1988	Magister in Biology. Faculty of Natural Sciences and Oceanography. University of Concepción, Concepción, Chile.
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ACADEMIC EXPERIENCE

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OTHER ACTIVITY

1995-1998. Technical director of the project "Botanical Garden", Sociedad Agrícola del Sur, Coronel, Chile. Design, establishment and maintenance of different plants species. Collection and exchange of seeds. Development of nursery with forests species.

FIELD WORK

2008. January-February. Chile-Argentina. "Evolution of genus *Hypochaeris* (Asteraceae) in Southamerica", University of Vienna, Austria.

2007. January-February. Chile. "Evolution of genus *Hypochaeris* (Asteraceae) in Southamerica",University of Vienna, Austria.

2005. October. Chile, "The vegetation in Termas de Chillán, National Reserve Radal Siete Tazas and Nahuelbuta National Park". California State Language and Culture Program-University of Concepción.

2004. November. Chile, "The vegetation of Termas de Chillán, National Reserve Ralco and Nahuelbuta National Park". California State Language and Culture Program-University of Concepción.
"The vegetation in Chillan mountains". St. Cloud University-University of Concepción.

2003. December. Chile, "The vegetation of Termas de Chillán and Nahuelbuta National Park". St. Cloud University-Universty of Concepción and California State Language and Culture Program-University of Concepción.
2002. November. Chile, "Vegetation in Nevados of Chillán and Nahuelbuta National Park". University of Concepción (Chile) and St. Cloud University.
2002. September. Chile, "Florid Desert at the North of Chile". University of Concepción.
2002. January. Chile, "Ornamental Plants of Bío-Bío region", Anglatin.
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2001. October. Chile, "Chiloé Island". University of Concepción.
2001. January. Chile, "Ornamental Plants of the Bío-Bío region", Anglatin.
2000. November. Chile, "Wildlife protected areas of National Park Nahuelbuta", University of Concepción.
2000. April. Chile, "Traditional and not traditional crops in central Chile", University of Concepción.
2000. February. Chile, Confection of a vegetational map and analysis of the flora of Juan Fernández Island, Chile. University of Concepción (Chile) and University of Vienna, Austria.
1999. January-February: Chile, Study and preliminary valorization of cartografic-phytosociology metodology in Juan Fernandez Island, Chile. University of Concepción (Chile) and University of Vienna, Austria.
1996. January: Chile, Reproductive biology in endemic species of Juan Fernandez Island, Chile. University of Concepción (Chile), University of Connecticut (USA) and Multidisciplinary Institute of Plant Biology (Argentina).
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PUBLICATIONS

Papers:

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López, P, K. Tremetsberger, M.A. Ortiz, T.F. Stuessy. Adaptive radiation in the *Hypochaeris apargioides* complex (Asteraceae, Cichorioideae) of southern South America.

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López, P. y O. Matthei. 1991. Malezas de la familia Cyperaceae A.L.Juss. en Chile. VIII National meetings of Botany. Santiago, Chile.

López, P. y S. Palma-Heldt. 1988. Contribución al conocimiento de la tafoflora terciaria chilena sobre la base de improntas. V Chilean Geological Congress. Santiago, Chile.

López, P. y M. Rondanelli. 1986. Contribución al conocimiento de la flora terciaria en la zona carbonífera de Arauco-Concepción, Chile. XXIX Annual meetings of Biological Society of Chile. Puyehue, Chile.

Teaching manuals

2004. Botany in veterinary medicine. University of Concepción, Concepción, Chile.
1993. Vegetal Biology I (243102), Botany Department, University of Concepción, Chile.
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PARTICULARLY RELEVANT BACKGROUND

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2002. Assistant of the proyect "Study of the Mosses of South-Central Chile". Funded by National Geographic Science.

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2000-2001. Assitant of the project FONDECYT 100364 "Analysis of the vascular flora at zone temperate-mediterranean of Chile". University of Concepción, Chile.

1999. Assistant of the proyect "Update of native vegetation in the region of Coquimbo" University of Concepción, Chile.

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Fellowships

1992-1993: Investigation in Botany. University of Concepción. Concepción, Chile.
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PARTICIPATION IN ANNUAL MEETING AND SEMINAR

2008. Systematics 2008. Göttingen, Germany.
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1994. IX. Annual meeting of the Botanical Society. Valdivia, Chile.
1992. International meeting of Territorial Planning. Concepción, EULA Center, Chile.
International meeting of Environmental Legislation. Concepción EULA Center, Chile.
International meeting of Coastal and Ocean Management. Concepción, EULA Center, Chile.
XXXV Annual meeting of the Biological Society of Chile. Puyehue, Chile
1991. VIII Annual meeting of Botany. Santiago, Chile
XLII Congress of the Agricultural Society. Chillán, Chile.
1990. II Congress of Students of Biological Sciences and Natural Resources. Concepción, Chile.
1988. V Chilean Geological Congress. Santiago, Chile.
1987. VII Argentine Symposium of Paleobotany and Palynology. Buenos Aires, Argentina.
Seminar: "A Kaleidoscopic view of Natural Resources". Biological Society of Chile, Concepción, Chile.
1986. II Meetings Environmental Sciences. Talca, Chile.
XXIX Annual meeting of the Biological Society of Chile, Concepción, Chile.
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Seminar : "Genetic and his Impact in the actual and future Society". Biological Society of Chile, Concepción, Chile.