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Evolutionary processes underlying plant diversification in the tropical Andean highlands

Evoluční procesy podmiňující diverzifikaci rostlin v tropických Andách

Ph.D. Thesis

Supervised by Petr Sklenář, Ph.D.

Prague, 2018

Hereby I declare that I wrote this thesis independently, using the mentioned references. I have not submitted any part of this thesis to obtain any other academic degree.

Diana Libeth Aparicio Vásquez, Prague, July 2018

Author contribution statement

I declare that I have substantially contributed to all papers included in the thesis. My contributions to particular papers are as follows:

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Contents

Abstract

The highlands in the northern Andes, which are known as the páramo, are recognized worldwide for their unique and species-rich flora. Many páramo plant groups underwent radiations, which have been shown to be very recent and outstandingly fast. These radiations have usually been linked to (1) the uplift of the northern Andes, which provided new ecological opportunities in the highlands that originated in this process, (2) Quaternary climate change that produced range shifts of the páramo, resulting in periods of páramo contraction and isolation, during cold periods, and periods of páramo expansion and connection, during warm periods (3) the Andean physiographical and ecological heterogeneity, which provides extent oppornuties for isolation and for ecological divergence. In spite of increasing research efforts to understand the evolution of the páramo flora, the actual processes underlying species diversification remain unclear. The main aim of this thesis is to contribute to the understanding of these processes. We use three different approaches in two different study systems: (1) A population genetics approach, which remains rare among páramo plant studies, focuses on three páramo *Lupinus* species (*Lupinus alopecuroides, L. nubigenus, L. microphyllus*). These species belong to one of the best studied páramo plant radiations, the Andean *Lupinus* radiation, which is also one of the fastest radiations reported for plants to date. (2) A phylogenetic approach on a group of páramo *Senecio* species (former *Lasiocephalus*), implementing large-scale sampling and two different molecular markers. (3) A taxonomic approach applied also to a *Senecio* species. We find that most of the populations in the studied *Lupinus* species became genetically differentiated when warming forced the páramo to migrate to the isolated, colder mountaintops during the Holocene. Besides isolation, populations' differentiation was also driven by founder events during the colonization of the mountaintops. On the other hand, we find that the main genetic structure within the studied *Senecio* species corresponds to differences in ecological niches (elevation zones), suggesting that ecological divergence underlies its diversification. We also find that homoploid hybridization was involved in the origin of several *Senecio* species. We describe a new species (*Senecio sangayensis*), which accurately exemplifies the importance of ecological divergence in driving diversification of the páramo flora. We suggest that the interaction between geographic isolation, founder events, ecological divergence, and hybridization underlies plant diversification in the páramo. The relative role of each of these processes varies depending on species-specific traits, such as dispersal ability, and on the stage of speciation. The role of isolation may be particularly important at the initial stages of speciation because, further during the speciation process, this role is obscured by the influence of selection and gene flow. Founder events may be also important at the initial stages of speciation, particularly in plant groups with limited long-distance dispersal. The interaction between homoploid hybridization, geographic isolation, and ecological divergence was also particularly important in the diversification of the páramo flora.

Abstrakt

Vysoké horské polohy severních And, známé jako páramo, jsou celosvětově proslulé svojí jedinečnou, druhově bohatou květenou. Řada skupin rostlin párama prodělala velmi nedávnou a výjimečně rychlou radiaci. Tyto radiace bývají dávány do souvislosti s: (1) orogenezí severních And, jejímž důsledkem byly nově vytvářené proměnlivé ekologické podmínky, (2) čtvrtohorními změnami klimatu, které zapříčinily výškové posuny párama, tj. kontrakci a izolaci jednotlivých oblastí v chladných obdobích a naopak jejich expanzi a vzájemné propojování v teplých obdobích, (3) fyziografií a ekologickou heterogenitou And, které nabízejí široké možnosti pro geografickou izolaci a ekologickou diferenciaci. Přes stále intenzivnější výzkum, který se zabývá otázkami evoluce páramové květeny, zůstávají vlastní procesy podmiňující diverzifikaci druhů jen málo známé. Hlavním cílem této disertační práce je přispět k poznání těchto procesů s použitím třech rozdílných přístupů u dvou studovaných systémů. (1) Populačně-genetický přístup, který byl u páramových skupin rostlin dosud použit jen vzácně, se zaměřuje na tři druhy rodu *Lupinus* (*Lupinus alopecuroides*, *L. nubigenus*, *L. microphyllus*). Tyto druhy náleží do jedné z nejlépe zdokumentovaných radiací u páramových skupin rostlin, tj. radiace andských vlčích bobů, a zároveň představuje vůbec nejrychlejší známou radiaci u rostlin. (2) Fylogenetický přístup se prostřednictvím rozsáhlého vzorkování a za použití dvou rozdílných molekulárních markerů zaměřuje na skupinu páramových druhů rodu *Senecio* (dříve řazených do rodu *Lasiocephalus*). (3) Taxonomický přístup zaměřen taky na jeden drůh rodu *Senecio*. Zjistila jsem, že většina populací studovaných druhů rodu *Lupinus* se začala geneticky diferencovat v průběhu Holocénu, kdy se v důsledku oteplování klimatu začalo páramo kontrahovat na izolované, chladnější horské vrcholy. Společně s geografickou izolací populací byla jejich diferenciace podmíněna efektem zakladatele během osídlování vrcholů. Naproti tomu hlavní genetická struktura studovaných druhů rodu *Senecio* odpovídá diferenciaci ekologických nik (podle výškových stupňů), což naznačuje, že divergence druhů byla podmíněna jejich rozdílnou ekologií. Původ několika druhů byl navíc podmíněn homoploidní hybridizací. Popisuji nový druh, *Senecio sangayensis*, který příkladně dokumentuje význam ekologické divergence pro diversifikaci páramových rostlin. Na základě výsledků svého výzkumu navrhuji, že diversifikace rostlin párama je výsledkem vzájemného spolupůsobení geografické izolace, efektu zakladatele, ekologické divergence a homoploidní hybridizace. Relativní význam jednotlivých procesů se liší v závislosti na druhově specifických znacích, jakým je například schopnost šíření, a na fázi probíhající speciace. Izolace může být významná především na začátku speciačního procesu, neboť její role v pozdějších fázích může být upozaděna působením selekce a genovým tokem. Podobně významnou roli v počátcích speciace může hrát efekt zakladatele, a to především u rostlin s omezenou schopností dálkového šíření. Pro diversifikaci páramových rostlin je zejména důležitá interakce mezi homoploidní hybridizací, geografickou izolací a ekologickou divergencí.

INTRODUCTION

The Andes are one of the richest biodiversity hotspots in the World (Myers et al., 2000; Olson & Dinerstein, 2002). The highlands in the tropical Andes, which are known as the páramo, have also been considered a biodiversity hotspot given their outstanding plant diversity (Madriñán et al., 2013), which is under threat due to mining activities and global warming (Morueta-Holme et al., 2015; Pérez-Escobar et al., 2018). Specifically, the páramo is found at the upper part of the highest mountains (approx. 3000−4900 m a.s.l) in the northern Andes (from Venezuela to Ecuador), in northern Peru, and in the Central American Cordillera in Panama and Costa Rica (van der Hammen & Cleef, 1986; Sklenář et al., 2011) (Figure 1). Hence, the páramo forms an archipelago of sky-islands geographically and ecologically separated by deep valleys, where distinct lowland ecosystems are found (Hughes & Eastwood, 2006). The environmental conditions of the páramo are very specific − climate is in general cold and humid, but temperatures fluctuate highly throughout the day from below freezing to as much as 30°C, solar input is continuously high, as well as ultraviolet radiation (Luteyn, 1999). Due to its climate and high water-retention capacity, the páramo is a reliable and constant source of water, which is used by several millions of people in the Andes for drinking, agricultural, and industrial purposes (Hofstede, 1995; Buytaert, 2006).

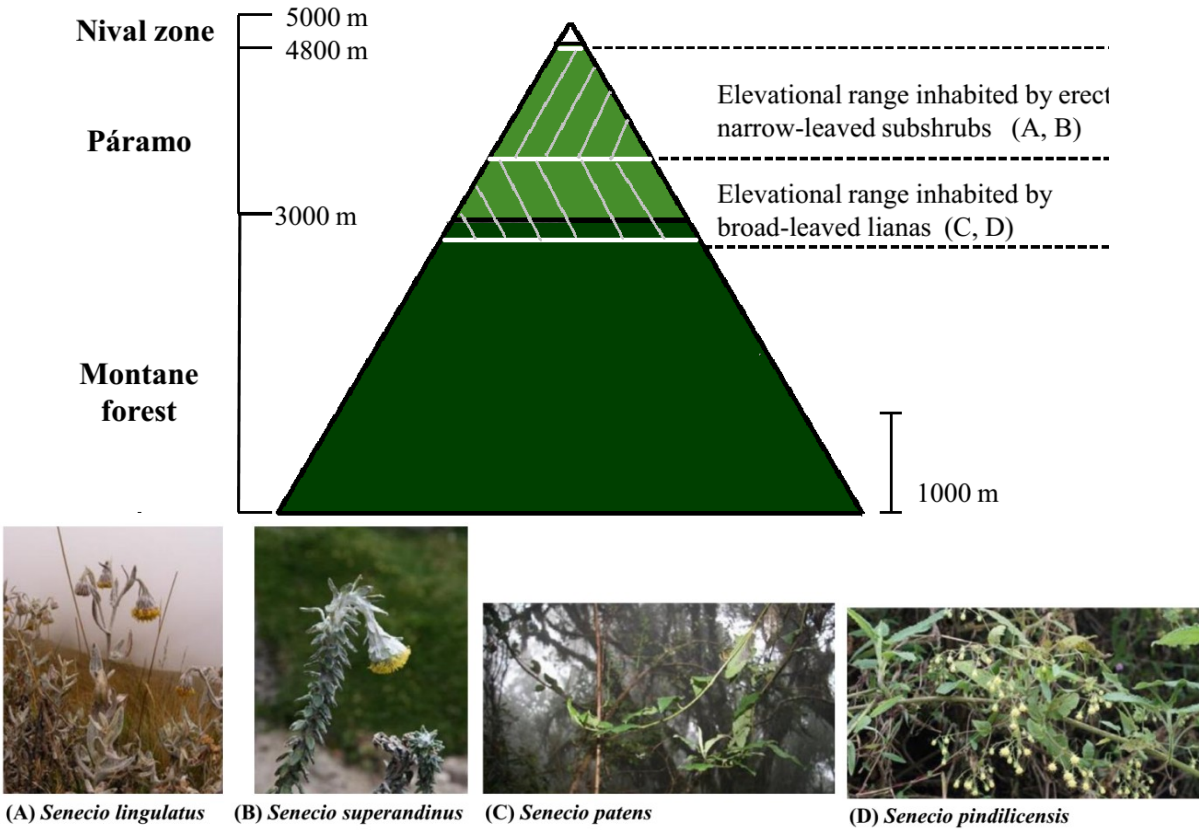
Figure 1. Geographic extension of the páramo ecosystem with the names of the major Andean mountain ranges (taken from Sklenář et al., 2011).

The páramo flora is the most species-rich tropical-alpine flora in the World (Smith & Cleef, 1988; Sklenář et al., 2011). Besides species richness, the páramo is also particulary rich in endemic species, the majority of which are geographically very restricted (Luteyn, 1999). Much of this diversity can be attributed to the numerous species radiations that have happened in several páramo plant groups (Linder et al., 2008). Besides species and endemic richness, the páramo radiations also have their outstandingly high diversification rate in common

(Madriñán et al., 2013; Luebert & Weigend, 2014; Hughes & Atchison, 2015). They are also of relatively recent origin within the last five million of years, with most of their species diversity having arisen in the Pleistocene (Madriñán et al., 2013; Luebert & Weigend, 2014; Nevado et al., 2018). Most of the páramo plant radiations also display a remarkable diversity of growth forms, including stem and acaulescent rosettes of various sizes, microphyllous upright and prostrate shrubs, cushion plants, and erect and trailing herbs (Ramsay & Oxley, 1997)

The subtribe Espeletiinae constitutes perhaps the most classical example of the páramo plant radiations. In particular, the giant stem rosettes of the genus *Espeletia* Mutis ex Bonpl., that have an up to 15 m tall stem that is densely covered with marcescent leaves, are an icon of the páramo. The Espeletiinae comprises about 144 morphologically highly variable species distributed mainly in the páramo in Venezuela and Colombia. The great majority of these species (ca. 90%) are local endemics (Diazgranados & Barber, 2017). Other good examples of the fast and recent diversification characteristic of the páramo flora are seen, for instance, in *Puya* Molina (Jabaily & Sytsma, 2013), *Valeriana* L. (Bell & Donoghue, 2005), *Bartsia* L. (Uribe-Convers & Tank, 2015), and in *Lasiocephalus* Willd. ex Schltdl. (Cuatrecasas, 1978), which has turned out to belong to the genus *Senecio* L. (Pelser et al., 2007; Pelser et al., 2010). The former *Lasiocephalus* comprises about 25 species, which diversified within the last 2 Myr (Pelser et al., 2010). Most of these species are found in the northern Andes, where they inhabit the páramo and the montane forest just below the páramo (2800–3800 m a.s.l). Some species also extend into the highlands of the central Andes in Peru and Bolivia. Most of the former *Lasiocephalus* species are morphologically distinct and readily identifiable, however, some are very variable in leaf size and shape. Two main growth forms, which are restricted to different elevation zones, can be recognized – broad-leaved lianas, which inhabit the montane forest and the lower part of the páramo, the so-called subpáramo (3000–3600 m a.s.l.), and ascending or erect, narrow-leaved subshrubs, which are found across the páramo between the upper limit of the subpáramo and the snow-line (3600–4900 m a.s.l.) (Figure 2).

Figure 2. Elevational distribution of two main growth forms (broad-leaved lianas and erect, narrow-leaved subshrubs) identified within the former *Lasiocephalus.*



Another well-known example of the páramo species radiations is found in the Andean *Lupinus* L. The so-called Andean *Lupinus* radiation is perhaps the best studied so far. In particular, the páramo lupins have been the focus of several recent studies that have made significant contributions to understanding the extraordinarily fast and recent diversification observed in this groups and in the páramo in general (Hughes & Eastwood, 2006; Drummond et al., 2012; Hughes & Atchison, 2015; Nevado et al., 2016; Contreras-Ortiz et al., 2018; Nevado et al., 2018). Hughes and Eastwood (2006) were the first authors to assess the tempo, the origin, and the causes of the Andean *Lupinus* radiation. They generated a well-resolved phylogeny based on nuclear ITS and the CYCLOIDEA gene sequence, and identified a monophyletic clade composed of high-elevation Andean lupins, including the páramo lupins and other species occurring in the highlands from Peru to Argentina. The age of this clade was estimated to be 1.18–1.76 Myr and the diversification rate 2.49–3.72 species per Myr. This diversification rate was the highest documented for plants and comparable to rates reported for the cichlid fish radiations from the African rift lakes, which are the highest estimated across the tree of life. To explain the extraordinarily fast and recent diversification of the Andean lupins, Hughes and Eastwood (2006) presented several ideas that have had an important influence on subsequent studies. The authors, for instance, suggested that the Andean *Lupinus* radiation is comparable in stimulus with species radiations in oceanic islands or island-like formations – after the final Andean uplift, the Andean high-elevation habitats emerged as "islands in the sky" that were subsequently colonized by plant groups migrating from the north temperate zone, such as *Lupinus*, from the south temperate zone, and also from

the lower tropical ecosystems (Sklenář et al., 2011). Ecological opportunities and lack of competition in the recently formed sky-islands facilitated diversification in the newly arrived groups. This hypothesis is nowadays widely recognized and has been suggested to explain fast and recent diversification in other páramo plant groups (Madriñán et al., 2013; Diazgranados & Barber, 2017).

In their seminal work, Hughes and Eastwood (2006) also suggested that climate change in the Quaternary promoted diversification in the Andean lupins by producing repeated range shifts that led to geographic isolation. Quaternary climate change has for a long time been considered a key factor in the formation of the páramo flora (e.g. Simpson, 1974; Van der Hammen & Cleef, 1986; Hooghiemstra & Van der Hammen, 2004). However, the evolutionary mechanisms by which past climate change influenced plant species diversification in the páramo remain to be clearly identified (Madriñán et al., 2013; Kolář et al., 2016; Contreras-Ortiz et al., 2018). Jabaily and Sytsma (2013), who examined the biogeographical history of the Andean *Puya*, also suggested that Quaternary climate change stimulated speciation by producing opportunities for geographical isolation. Madriñán and collaborators (2013), and Nevado and collaborators (2018), brought further support to this hypothesis by showing that numerous páramo plant groups diversified profusely during the Pleistocene. By analysing patterns of genetic diversity and divergence in several Andean lupins, Nevado and collaborators (2018) also showed that Quaternary climate change promoted speciation by allowing secondary contact of species during glacial periods, when the high-elevation vegetation extended over continuous lower elevations.

Another factor that was suggested by Hughes and Eastwood (2006) and also by other authors (e.g. Jabaily & Sytsma, 2013; Givnish, 2015) to explain the fast and recent diversification of the páramo flora is the physiographic and environmental gradient of the Andes, which are among the steepest in the World (Kreft & Jetz, 2007; Hughes & Atchison, 2015). This extreme physiographical and environmental heterogeneity stimulated speciation by producing opportunities for isolation and ecological divergence. The importance of ecological divergence in driving speciation in the páramo is supported by the noticeable disparification of growth forms observed in several plant groups, which exhibit striking adaptations to environmentally distinct elevation zones (Hughes & Atchison, 2015; Contreras-Ortiz et al., 2018). Nevado and collaborators (2016) demonstrated the importance of adaptive processes in the diversification of the Andean lupins by finding evidence of accelerated natural selection acting on regulatory and coding regions across the genome. Pioneer studies suggested that in particular niche shifts between elevation zones promoted diversification in the páramo (Cuatrecasas, 1978; Cuatrecasas, 1986). Recent studies have provided genetic evidence that supports this hypothesis. For instance, based on phylogenetic inference Dušková and collaborators (2010) suggested that niche shifts occurred in several páramo *Senecio* species that were accompanied by growth form changes. The importance of niche shifts in driving diversification was also highlighted by Kolář and collaborators (2016), who assessed the evolutionary history of *Loricaria*. Recently, Contreras-Ortiz and collaborators (2018) found evidence that suggest that the rosette growth form originated in the Andean lupins multiple times probably from shrubs and treelets occurring at lower elevations.

In spite of increasing research efforts to understand the evolution of the páramo flora, the actual processes underlying species diversification in the páramo remain unclear (Madriñán et al., 2013; Kolář et al., 2016; Contreras-Ortiz et al., 2018). This is mainly a

consequence of limited taxon sampling and lack of resolution in the majority of the phylogenetic studies published so far (Kolář et al., 2016; Contreras-Ortiz et al., 2018). The main aim of this thesis is to contribute to the understanding of the processes underlying plant diversification in the páramo. To achieve this aim, we used three different approaches in two different plant systems: 1. a population genetic approach, which has only rarely been used to assess the evolutionary drivers of species diversification in the páramo, focuses on three high-elevation *Lupinus* species (Chapters 1 and 2), 2. a phylogenetic approach applied to a group of *Senecio* species (the former genus *Lasiocephallus*) (Chapter 3), and 3. a taxonomic approach applied also to a *Senecio* species (Chapter 4). These different research approaches with their respective goals are described below.

Population genetic approach

Population genetics seeks to understand how and why allele-frequencies change over time within and between populations (Clark, 2001). Genetic drift, gene flow, natural selection, and mutation are the microevolutionary mechanisms that cause change in allele-frequencies over time. When one or more of these mechanisms act in a population, the Hardy-Weinberg assumptions are violated and evolution occurs. The Hardy-Weinberg Theorem thus provides a null model and population genetics focuses on the causes and consequences of violating these assumptions (Andrews, 2012). Population genetic studies are, therefore, fundamental to understand the detailed processes that drive differentiation among populations and species (Charlesworth, 2015).

In Chapter 1, we examined patterns of genetic variation across the populations of *Lupinus alopecuroides* and the causes underlying these patterns. We sampled 220 individuals from the ten known populations of that species. Each population is located on a different mountain at the upper part of the páramo, the so-called superpáramo (4100−4900 m a.s.l.) (Cuatrecasas, 1969). To our knowledge, this was the first population genetic study undertaken in the superpáramo. Due to the effect of enhanced genetic drift and lack of gene flow, geographically isolated populations may display low levels of within-population genetic diversity and high genetic differentiation between them (Frankham et al., 2002; Allendorf & Luikart, 2009). Moreover, in tropical sky-islands founder effects may occur, as founders arrive over long distances from similar habitats, rather than from adjacent sites at lower elevations, which are ecologically different (Carson & Templeton, 1984). In view of this, we expected the populations of *L. alopecuroides* to be impoverished in genetic diversity. Extremely low levels of genetic variation were found in populations of *Puya raimondii*, which occur around 4000 m a.s.l from Peru to northern Bolivia, probably as a consequences of repeated bottlenecks and autogamy (Sgorbati et al., 2004). High homozygote excess was also found in populations of *Phaedranassa tunguraguae* growing at 1500–2500 m a.s.l in Ecuador, which may have resulted from restricted gene flow between populations and enhanced genetic drift due to small effective population size (Oleas et al., 2012).

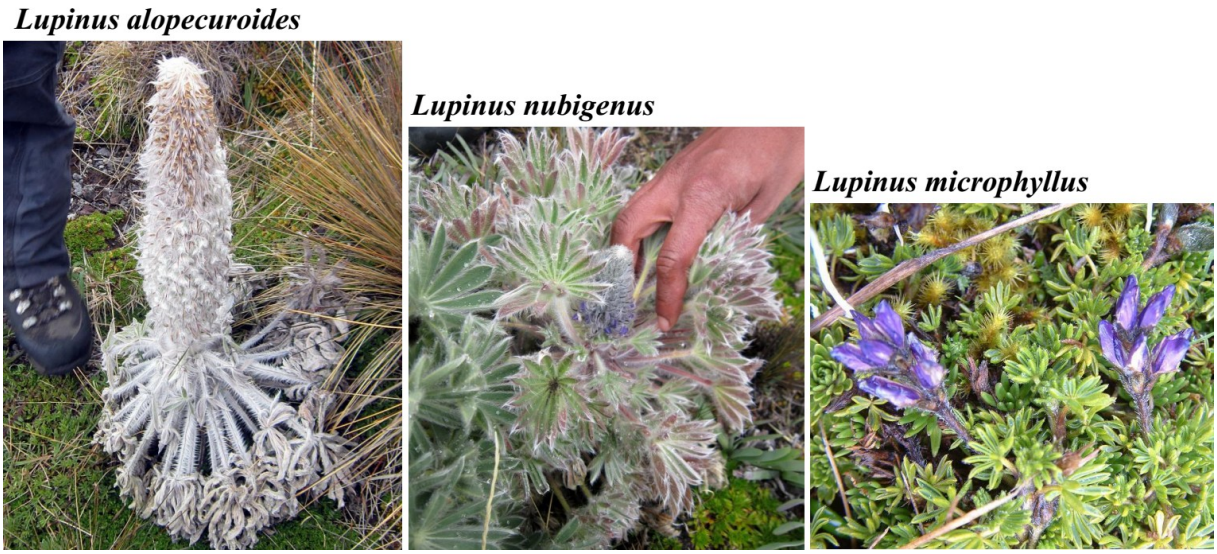
In Chapter 2, we reconstructed the divergence history of the populations in *Lupinus alopecuroides,* and also in *Lupinus nubigenus*, and *Lupinus microphyllus.* Our main goal was to examine the role of population-history in driving genetic differentiation. We were particularly interested in the role played by Quaternary climate change, which has been invoked repeatedly to explain the outstanding diversity of the páramo flora (e.g., Simpson, 1974; Hooghiemstra & Van der Hammen, 2004; Hughes & Eastwood, 2006; Jabaily &

Sytsma, 2013), but even so, the mechanisms by which past climate change influenced species diversification remained unclear (Madriñán et al., 2013; Contreras-Ortiz et al., 2018). We used Approximate Bayesian Computations (ABC) (Beaumont et al., 2002) as implemented in DIYABC 2.0 (Cornuet et al., 2008, 2014). Specifically, we tested whether populations divergence was associated with the restriction of the páramo to the mountaintops during warmer periods, and whether the colonization of these mountaintops was accompanied by founder events.

In Chapters 1 and 2 we used microsatellites DNA molecular markers, which is one of the most widely used marker in population genetics (Zhang & Hewitt, 2003). Microsatellites are suitable for population genetic studies addressing evolutionary young species, such as the páramo lupins, because they are highly informative and highly variable. Unlike multilocus markers, such as RAPDs (randomly amplified polymorphic DNA) and AFLP (amplified fragment length polymorphic DNA), single-locus microsatellites are codominant. This allows the identification of each of the two alleles at a locus and also makes it possible to distinguish heterozygotes from homozygotes (Sunnucks, 2000). Moreover, thanks to their high mutation rate, microsatellites are far more variable than other molecular markers, such as allozymes and chloroplast DNA. Microsatellites are also considered to be more variable than Single Nucleotide Polymorphisms (SNPs) (Dardé et al., 2007).

Lupinus alopecuroides, which was studied in Chapter 1 and 2, is one of the most conspicuous species of the páramo flora. It forms a giant semelparous acaulescent rosette (Figure 3), which is a distinctive life form of the páramo and, in general, of the tropical, high-elevation floras (Rundel et al., 1994). The abundant rosette's leaves are sericeous and marcescent, protecting the meristem from freezing temperatures. The inflorescence is up to 1 m tall, cylindrical, hollow, and densely covered by lanate hairs. *Lupinus nubigenus*, which was addressed in Chapter 2, resembles *L. alopecuroides* in its habit and life-history traits, but *L. nubigenus* is smaller (Figure 3). *Lupinus nubigenus* is also restricted to the superpáramo and it is also known from very few populations. This species was considered endemic to Ecuador (IUCN, 2004), where only three populations are known, but it has now been documented in northern Peru (Contreras-Ortiz et al., 2018). *Lupinus alopecuroides* and *L. nubigenus* are considered endangered (IUCN, 2004). On the other hand, *L. microphyllus*, which is also addressed in Chapter 2, is a common species generally found at 3000–4900 m a.s.l from Colombia to Bolivia. Its geographic distribution is also archipelagic, with each of its populations restricted to a different mountaintop. The populations of *L. microphyllus* co-occurred with the populations of *L. alopecuroides* or *L. nubigenus*, or both. *Lupinus microphyllus* is an iteroparous, prostrate, dwarf shrub (Figure 3). All the three studied species have a uniform chromosome number of 2n=48 (Conterato & Schifino-Wittmann, 2006).

Figure 3. Typical habit of *Lupinus alopecuroides*, *L. nubigenus* (acaulescent rosette), and *L. microphyllus* (prostrate dwarf shrub).



Lupinus alopecuroides, *L. nubigenus,* and *L. microphyllus*, and in general the páramo lupins, provide an ideal study system for addressing the micro-evolutionary processes underlying species diversification. Genetic signatures of several evolutionary forces, such as founder effects, are more likely to persist in recently diversified lineages than in lineages of evolutionary older origin (Spurgin et al., 2014). Because genetic drift, gene flow, mutation, and selection do not act in isolation, disentangling the signatures of different evolutionary processes is extremely challenging (Marie Curie SPECIATION Network, 2012). In this regard the páramo lupins provide and advantage because gene flow between the populations may be weak or almost absent. This is because the populations of the páramo lupins are geographically and ecologically isolated. At the population level selection may be also considered to be weak, because they usually occur in ecologically similar habitats.

Phylogenetic approach

Phylogenetics seeks to reconstruct evolutionary relationships between species. Comparisons of species in a phylogenetic context provide significant insights to understanding both patterns and processes of evolution (Soltis & Soltis, 2000). In particular, character state reconstructions on phylogenetic trees can be used to test hypotheses about how and why characters evolve (Harvey, 2001). In this regard, character reconstructions can provide important insights to understand, for instance, growth form evolution. Contributions from phylogenetic studies to understand the processes underlying species diversification in the páramo have often been constrained by lack of phylogenetic resolution. This is mainly a consequence of limited sampling and the use of little variable DNA regions, which present important limitations when addressing recent and fast diversifying species (Kolář et al., 2016; Contreras-Ortiz et al., 2018).

In Chapter 3, we sought to reconstruct the phylogenetic relationships between *Senecio* species of the former genus *Lasiocephalus* Willd. ex Schltdl. (Cuatrecasas, 1978). We also examined overall genetic structure and used character state reconstruction to investigate growth form evolution. Our main aim was to test whether niche shifts between the Andean

montane forest and the páramo occurred and were accompanied by growth form changes. This hypothesis was put forward by Dušková and collaborators (2010), who studied several of the former *Lasiocephalus* species to examine the evolutionary forces underpinning genome size variation, including the role of phylogeny. To achieve this goal, they assessed genome size variation in 20 species using flow cytometry and karyology, and amplified ITS (Internal transcribed spacer) sequences in 13 species. Phylogenetic analysis of these ITS sequences identified two well-supported clades – one clade comprising the forest *Lasiocephalus* species and the other clade composed exclusively of páramo *Lasiocephalus* species. Dušková et al. (2010) found that these clades also differed in genome size, with smaller genomes recorded in the páramo clade. The authors concluded that intrageneric genome size variation reflects primarily phylogenetic effects, while intraspecific genome size variation is mainly shaped by ecological factors.

For the work presented in Chapter 3, we used large-scale sampling, which involved 108 populations and 28 *Senecio* species of the former genera *Lasiocephalus* and *Culcitium* Bonpl. (Pelser et al., 2007; Pelser et al., 2010). We used two molecular markers – AFLP (amplified fragment length polymorphism) and ITS (Internal transcribed spacer). AFLP and ITS markers are commonly used for phylogenetic inference. In particular, AFLP is considered to be suitable for studies of closely related species given its relatively high variability (Koopman, 2005). AFLP and ITS markers, as well as other restriction fragment markers such as RAPD (random amplified polymorphic DNA) and microsatellites, present some limitations for phylogenetic reconstruction because homology of the fragments is not known. However, this problem may not represent a serious limitation in the case of closely related species (Koopman, 2005), in which the proportion of nonhomologous fragments might be low (O'Hanlon & Peakall, 2000). High-throughput sequencing technologies have developed rapidly during the last years. Recent studies show that in particular multi-gene DNA sequencing and genome skimming have been extremely useful for phylogenetic reconstruction of plant groups undergoing recent and rapid diversification (Uribe-Convers & Tank, 2015; Vargas et al., 2017; Contreras-Ortiz et al., 2018)

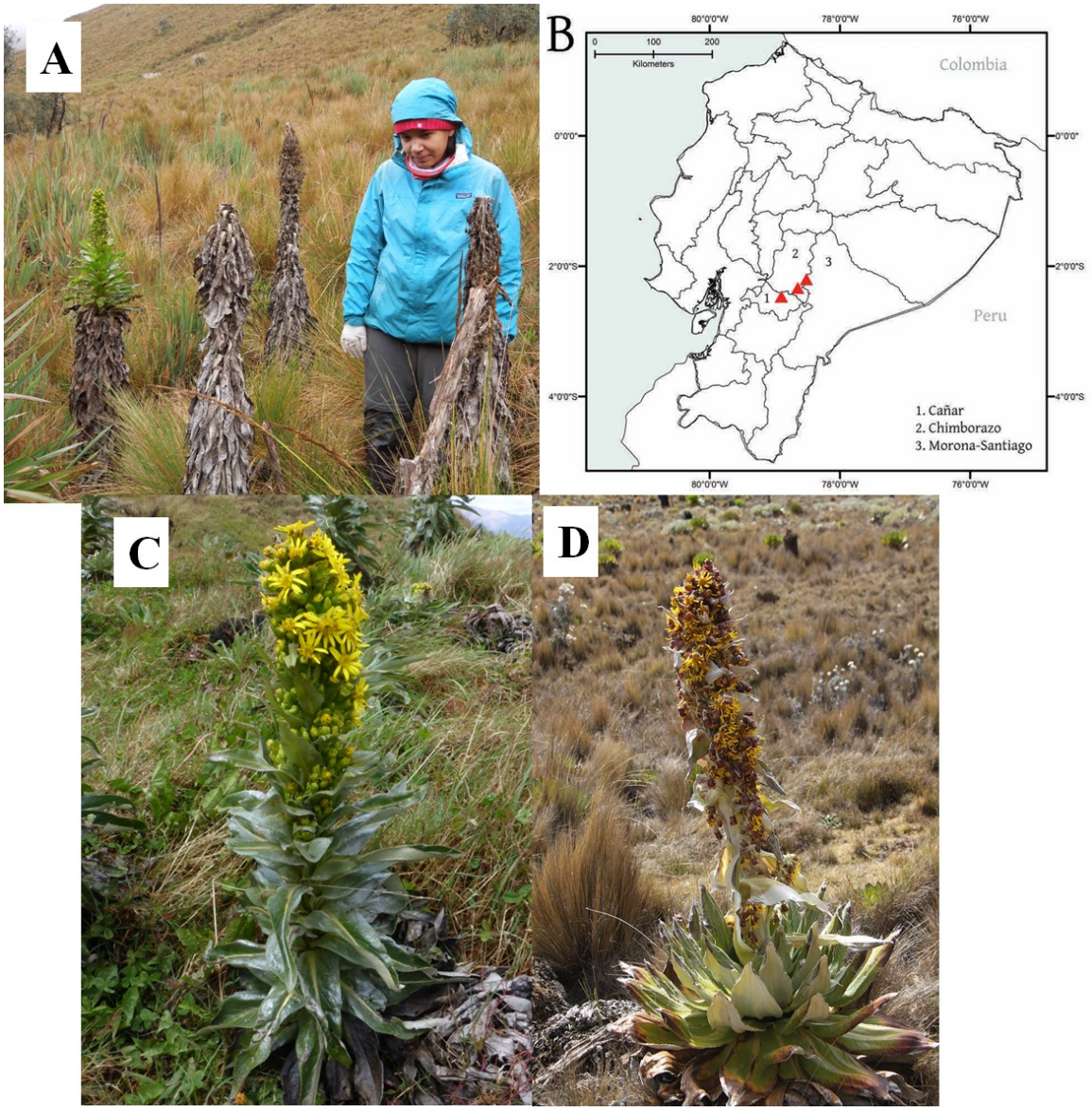
Taxonomic approach

Taxonomy seeks to classify organisms using different criteria, usually morphological, behavioural, and phylogenetical (Holstein & Luebert, 2017). In the páramo, most of the plant groups remain taxonomically unresolved. This may be a consequence of the proliferation of species names (Contreras-Ortiz et al., 2018), the fact that many páramo taxa are morphologically indistinguishable (Kolář et al., 2016), the limited number of well-resolved phylogenies (Contreras-Ortiz et al., 2018), and the fact that most of the páramo plant species actually remain unknown (Madriñán et al., 2013) because vast regions of the páramo remain completely unexplored (Peyre et al., 2015).

In Chapter 4, we described a new species named *Senecio sangayensis* (Figure 4). We found this species when we were undertaking the field-work required by the studies presented in this thesis. We found two populations in a hardly accessible place located on the east-oriented slopes of the eastern Ecuadorian Cordillera, in an area protected by the Sangay National Park. The species is also known from a third population occurring in the same region (Figure 4, B). *Senecio sangayensis* markedly differs morphologically and ecologically from all other Andean *Senecio* species growing in the páramo and the montane forest. The singular

morphology of *S. sangayensis* resembles that of *Dendrosenecio keniensis* (Figure 4, D), which inhabits the highlands of Mount Kenya – both species have a striking terminal racemiform synflorescence with large triangular-ovate bracts. The architecture of the capitulum is also similar, with a few subulate supplementary bracts almost as long as the involucral bracts and long yellow ray florets. The fact that a noticeable species such as *S. sangayensis,* which reaches almost 2 m tall (in bloom), remained so long undescribed proves that there are still large areas of páramo unexplored and that a large proportion of its species richness remains probably undiscovered.

Figure 4. *Senecio sangayensis.* (A) Mature and senescent individuals of *Senecio sangayensis*, (B) Distribution map, (C) Typical habit of *Senecio sangayensis*, (C) Tpical habit of *Dendrosenecio keniensis* (photo taken from Wikipedia).



Main results and discussion

Our results presented in Chapter 1 of this thesis reveal a so far unknown pattern – all populations of *Lupinus alopecuroides* are depauperate in genetic diversity while, at the same time, they are highly genetically differentiated. Extremely low levels of within-population genetic diversity have been reported in other species, but such low levels are generally found only in part of the species' geographical range (e.g., Habel et al., 2009; Aguilar et al., 2004; Furlan et al., 2012) and not in all extant populations, as was the case in *L. alopecuroides.* There are cases in which low diversity is observed across all populations, for instance, the case of the Hawaiian monk seal (*Monachus schauinslandi*) (Schultz, 2009) or the case of *Puya raimondii*, a semelparous giant rosette ocurring in the highlands of Peru and Bolivia (Sgorbati et al., 2004). In those cases, however, overall genetic variation at the species level was low. In *L. alopecuroides*, different populations are fixed for different alleles, therefore, overall genetic variation is high, although within-population genetic diversity is extremely low. Genome-scale nextRADseq also revealed genetic uniformity and marked genetic differentiation between populations in *L. alopecuroides* and other páramo lupins (Contreras-Ortiz et al., 2018).

We suggested three factors that may underlie the patterns of genetic variation observed in *L. alopecuroides*: geographic isolation between the populations, autogamy, and founder events. Lack of gene flow between geographically isolated populations and enhanced genetic drift within them result in the fixation of random alleles, and thus in loss of within-population genetic diversity and high genetic differentiation among the populations (Frankham et al., 2002; Allendorf & Luikart, 2009). Low genetic diversity in the populations and high differentiation among them have also been observed in autogamous species (Hamrick & Godt 1996). The reproductive biology of the Andean lupins has not been studied yet. However, high-rates of self-fertilization in the páramo lupins may be expected because outcross pollination is limited by the low availability of pollinators in the harsh alpine environment (Arroyo et al., 1985; Berry & Calvo, 1989). Genetic differentiation and loss of within-population genetic diversity may also result from founder events (Frankham et al., 2002; Hedrick, 1996). Founder events in *L. alopecuroides* are likely, given its limited long-distance dispersal. Like most *Lupinus* species, *L. alopecuroides* relies on ballistichory to disperse its seeds (Nevado et al., 2018). These seeds are relatively big and have a smooth surface without any adaptations to anemochory or zoochory. Moreover, other potential dispersal vectors, such as rodents (Milla & Iriondo, 2011; Maron & Kauffman, 2006), are virtually absent in the superpáramo. Founder events in *L. alopecuroides* are also suggested by the lack of isolation by distance found in the species. The establishment of a population by a small number of individuals leads to a pattern of "isolation by colonization", wherein genetic differences between populations reflect colonization history, rather than contemporary patterns of gene flow (Spurgin et al., 2014).

Our analyses presented in Chapter 2 largely confirm these hypotheses. The ABC analyses showed that the populations in *L. alopecuroides* and *L. microphyllus* became isolated during the last 50,000 years. They also demonstrated that several populations were founded by a small number of individuals. We did not use ABC analyses to test for founder events in all the populations because, when all populations are included in the analysis, the program failed to generate simulated datasets that were congruent with the observed summary

statistics. This was probably a consequence of the fact that the historical model created when all populations are included is too complex to generate reliable results. However, very low values of the Garza-Williamson modified index (Garza & Williamson, 2001) estimated in all populations indicate that all populations underwent size reductions in the past. Populations of high-elevation plants are usually founded by a small number of individuals due to the abrupt topography of mountains (Stöcklin et al., 2009). In the studied species, founder events are particularly likely given their limited long-distance dispersal. Like *L. alopecuroides*, *L. microphyllus* and *L. nubigenus* also rely on ballistichory to disperse their smooth seeds. The ABC analyses also support the occurrence of founder events by showing that populations' genetic structure is largely determined by colonization history. Finally, high-rates of self-fertilization are supported by the heterozygote deficit observed in the three species.

Our analyses presented in Chapter 2 help clarify how Quaternary climate change influenced genetic differentiation. The ABC analyses show that most of the populations in *L. alopecuroides* and *L. microphyllus* became genetically differentiated at the beginning and by the Middle-Late Holocene, when the páramo was confined to isolated mountaintops due to warming trends (van der Hammen & Cleef, 1986). This shows that, although gene flow probably occurred between populations during cold periods, when the páramo extended at lower elevations (Hooghiemstra & Van der Hammen, 2004; Nevado et al., 2018), the colonization of the isolated mountaintops during warm periods resulted in genetic differentiation among the populations. This is in line with previous studies, which have suggested that past climate changes promoted diversification in the páramo by producing isolation (Hughes & Eastwood, 2006; Jabaily & Sytsma, 2013; Madriñán et al., 2013). Our results also agree with those of Nevado et al. (2018), who found that some Andean *Lupinus* species, including *L. alopecuroides,* diverged as recently as by the Late Pleistocene. They suggested that geographic isolation during warm periods initiated divergence, which was further enhanced by selection. Finally, our ABC analyses also suggest that some populations of *L. alopecuroides* and *L. microphyllus* could be glacial relict populations that survived at the upper part of relatively lower mountains. Glacial cover at these mountains was limited (Clapperton, 1983), which could facilitate the survival of populations there. We suggested that survival was also facilitated by lack of volcanic activity, however, since very little is known about the occurrence of mountain glacial refuges in the northern Andes, further research is needed to test our hypotheses.

Our results presented in Chapters 1 and 2 show that isolation and founder events result in the divergence of populations of *L. alopecuroides*, *L. nubigenus,* and *L. microphyllus*, suggesting that genetic drift played an important role in driving diversification among páramo *Lupinus* species. Genetic drift was probably enhanced by small effective population size related to the supposed autogamous reproductive mechanism of the studied species. Although, it is acknowledged that genetic drift and founder events can contribute to speciation (Lande, 1981; Lynch, 2007), their roles in producing genetic differences among populations and species have remained unclear. This is, in part, a consequence of the fact that genetic drift is usually considered a null hypothesis (Marie Curie SPECIATION Network, 2012; Spurgin et al., 2014). The impact of drift on speciation primarily depends on the extent of gene flow. Therefore, the role of genetic drift may vary during the speciation process. In the case of the high-elevation Andean *Lupinus* species, we suggest that genetic drift related to geographic isolation and founder events played an important role in the initial stages of differentiation

and speciation, while its further role is obscured by the influence of gene flow and selection. Genetic drift probably interacted with selection to contribute to species differentiation (Nevado et al., 2018) – genetic drift provides the initial divergence on which selection subsequently acted. To clarify the role of genetic drift and founder events in driving speciation in the páramo, future studies should focus on quantifying gene flow throughout the speciation process, which remains challenging (Marie Curie SPECIATION Network, 2012). To disentangle the influence of genetic drift in the presence of selection is also difficult. In this regard, high-elevation Andean lupins provide an ideal study system because genetic signatures of drift and founder events are likely to persist in populations that diverged recently. Moreover, since different *Lupinus* species are usually restricted to different elevation zones, populations of single species are usually under similar selective pressures.

Unlike Chapters 1 and 2, which focus in the non-adaptive processes underlying diversification (isolation, founder events), Chapter 3 contributes to the understanding of adaptive processes. Although, the phylogenetic relationships between the studied *Senecio* species (former *Lasiocephalus*) were only partially resolved, both ITS and AFLP data show that the main genetic structure within this group corresponds to differences in ecological niches (elevation zones), suggesting that ecological divergence underlies its diversification. Our results also indicate that, as expected, multiple independent shifts between different elevation zones occurred, which were accompanied by growth form changes. Growth form changes associated with shifts between habitats at different elevation zones have been reported also in other páramo plant groups, such as *Chusquea* (Fisher et al., 2009), *Puya* (Jabaily & Sytsma, 2013), *Loricaria* (Kolář et al., 2016), and *Lupinus* (Contreras-Ortiz et al., 2018). Recently, Contreras-Ortiz et al., (2018) found that rosettes lupins, which are restricted to the highest parts of the páramo, evolved multiple times in different parts of the Andes from shrubs and treelets that grow at lower elevations. We suggest that similar cases of convergent evolution in growth forms will be also uncovered in *Lasiocephalus*, as the phylogenetic relationships between these recently diverged species will be fully resolved.

Further, our analyses in Chapter 3 suggest that homoploid hybridization was involved in the origin of several páramo *Senecio* species. This is suggested by admixed AFLP profiles, incongruences between AFLP and ITS data, and also by morphological observations. Hybridization was also suggested to underlie diversification in the Andean *Lupinus* (Nevado et al., 2018) and *Diplostephium* radiations (Vargas et al., 2017). The importance of hybridization in the speciation process is widely acknowledged. Also, it is clear that, during this process, hybridization interplays with other processes. Particularly, in the case of homoploid hybridization, other processes are crucial to assure reproductive isolation from the parental populations (Marie Curie SPECIATION Network, 2012). In this regard, the extreme physiographical and ecological heterogeneity in the Andes may play a key role by providing opportunities for geographical and ecological separation of the newly admixed populations from the parental populations (Gompert et al., 2006).

In Chapter 4, we described a new *Senecio* species, which we called *Senecio sangayensis*, found only in the southern part of the Sangay National Park in Ecuador (Figure 4, B). We consider that this species accurately exemplifies the importance of ecological divergence in driving diversification in the páramo flora. *Senecio sangayensis* is morphologically and ecologically different from all other *Senecio* species inhabiting the páramo and the Andean montane forest but, at the same time, it possess several biological

traits that are seen in other tropical high-elevation plant groups, which are ofted considered to be adaptations to the specific environmental conditions of the tropical, high-elevation ecosystems. For instance, *S. sangayensis* is probably the only semelparous páramo *Senecio*. However, semelparity is observed in other páramo plant genera, such as *Lupinus* and *Puya*, and also in high-elevation African groups, such as, *Lobelia* L. Semelparity is thought to be favoured in high-elevation environments because future survival and reproduction are less likely due to the harsh environmental conditions (Young & Augspurger, 1991). *Senecio sangayensis* also owns an up to 1 m tall, erect stem densely cover by marcescent leaves (Figure 4, A). Stem rosettes covered with marcescent leaves are seen, for instance, in members of *Espeletia*, or in the African high-elevation genera *Dendrosenecio* B. Nord. and *Lobelia*. The pith tissue in the center of the stem stores water that is used when intense insolation or frozen soil result in drought stress. The marcescent leaves protect the stem from freezing temperatures (Goldstein et al., 1984; Smith & Young, 1987). Another striking character observed in *S. sangayensis* is the presence of a mucilaginous substance between the young leaves, which are arranged in a rosette before the stem develops. This mucilage is observed in some other páramo species, such as *Valeriana plantaginea* and *Oritrophium peruvianum*, and in some high-elevation African plants, such as *Lobelia keniensis*, *Lobelia aberdarica*, and *Dendrosenecio keniensis* (Young & Orden-Robe, 1986; Beck et al., 1982). This substance probably acts as a thermal buffer that protects the plant from freezing temperatures (Beck, 1994). The fact that *S. sangayensis* possess a set of striking adaptation, which are unique among the Andean *Senecio* but are seen in other tropical, high-elevation plant groups, suggest that they may be an example of adaptive convergent evolution to the specific environmental conditions of the tropical, high-elevation ecosystems. Given the very restricted geographic distribution of *S. sangayensis*, it's likely that geographic isolation also was involved in the origin of this species.

Altogether, our results suggest that the interaction between geographic isolation, founder events, ecological divergence, and hybridization underlies species diversification in the páramo. The relative importance of each of these processes in driving speciation varies depending on the species and on the stage of speciation. Geographic isolation and founder events were probably more relevant in páramo plant groups with limited long-distance dispersal, such as the Andean *Lupinus*, than in wind-dispersed plant groups, such as the Andean *Senecio.* In both cases, however, geographic isolation may have played a key role in the initial stages of speciation by providing the initial divergence on which selection and gene flow subsequently acted. Hybridization, in combination with geographic isolation and ecological divergence, also contributes to species diversification, even in plant groups with poor long-distance dispersal. In these plant groups, inter-specific gene flow was possible during cold periods, when the páramo expanded over continuous areas at lower elevations (Nevado et al., 2018). Although, the importance of ecological divergence in driving species diversification in the páramo is indisputable, further research is still needed to fully understand its role and, in particular, the role played by repeated shifts between different elevation zones. In this regard, to disentangle the role of phenotypic plasticity in the origin of the páramo species becomes a challenging and urgent need that should be approached in future studies.

Conclusions

The outstanding fast diversification characteristic for several páramo plant groups results from the interaction between a number of processes. The role of each of these processes varies depending on the species and on the stage of speciation. Several authors have highlighted the role of geographic isolation prompted by past climate change (e.g. Hughes & Eastwood, 2006; Jabaily & Sytsma, 2012; Madriñán et al., 2013). Our results support this hypothesis by showing that the colonization of the isolated mountaintops during warm periods in the Holocene resulted in genetic differentiation of populations. However, our results also suggest that, besides isolation, founder events during the colonization of the mountaintops also played an important role in driving differentiation of populations. The role of founder events in the diversification of páramo plants has been ignored so far. We suggest that founder events in the interplay with isolation played a key role in the diversification of páramo plant groups characterized by limited long-distance dispersal, as is the case of the Andean *Lupinus* species. This role is particularly important at the initial stages of speciation because, further during the speciation process, this role is obscured by the influence of selection and gene flow (Marie Curie SPECIATION Network, 2012; Spurgin et al., 2014). Our results also support the importance of ecological divergence in driving species diversification in the páramo. In this regard, repeated shifts between different elevation zones within the páramo and between the páramo and the Andean mountain forest, were particularly important. The importance of ecological divergence and niche shifts was highlighted already by pioneer studies mainly on the basis of morphological observations (Cuatrecasas, 1978; Cuatrecasas, 1986). Genetic evidences, however, remain incomplete, given the technical difficulties behind the reconstruction of well-resolved phylogenies for young, rapidly diversifying clades (Contreras-Ortiz et al., 2018). We also provide evidence to suggest that homoploid hybridization contributes to speciation in the páramo. We suggest that the interaction between homoploid hybridization, ecological divergence, and geographic isolation was particularly important since ecological or geographic isolation is needed to assure reproductive isolation between new admixed populations and parental populations.

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Chapter 1

Low genetic variation and high differentiation across sky island populations of Lupinus alopecuroides (Fabaceae) in the northern Andes

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Abstract

The tropical alpine flora in the northern Andes has caught the attention of evolutionary biologists and conservationists because of the extent of its diversity and its vulnerability. Although population genetics studies are essential to understand how diversity arises and how it can be maintained, plant populations occurring above 4100 m a.s.l. in the so-called superpáramo have rarely been stud-ied at the molecular level. Here, we use 11 microsatellite DNA markers to examine genetic structure in populations of *Lupinus alopecuroides*, a long-lived semelparous giant rosette known from only 10 geographically isolated populations. Each population is located on a different mountaintop, of which three are in Colombia and seven in Ecuador. We analysed 220 individuals from all the ten known populations. We find low genetic variation in all but one of the populations. Four populations are completely monomorphic, and another five show only one polymorphic locus each. On the other hand, we find extremely high genetic differentiation between populations. We discuss the mechanisms that might cause this pattern, and we suggest that it is related to founder effects, lack of gene flow, and autogamy. The genetic relationships among the populations, and the lack of correlation between the genetic and geographic distances also point to the importance of founder effects and colonization history in driving differentiation among the populations.

Keywords: population genetics, genetic diversity, founder effects, páramo, Andean flora, Lupinus alopecuroides.

Introduction

The highlands in the northern Andes host one of the World's most species-rich tropical alpine floras (Smith & Cleef, 1988; Sklenář et al., 2011), characterized by rapid and recent evolution (van der Hammen et al., 1973; Hughes & Eastwood, 2006; Madriñán et al., 2013). This is the so-called páramo ecosystem that lies between 3000 and 4900 m a.s.l., and the highest tiers of it, above 4100 m, is called the superpáramo (Cuatrecasas, 1968; van der Hammen & Cleef, 1986). Given its restricted geographic distribution, páramo plant diversity is severely threatened by habitat destruction due to global warming and land-use changes (Balslev & Luteyn, 1992; Morueta-Holme et al., 2015; Vásquez et al., 2015). Although the páramo flora has received significant attention from evolutionary biologists and conservationists, population genetic studies are scarce (Oleas et al., 2012). To our knowledge, superpáramo plant populations have never been studied at the molecular level.

Patterns of genetic variation across populations are determined by various interrelated factors, and population history plays an important role (Ellstrand & Elam, 1993; Hewitt, 2000). In the northern Andes, Pleistocene climatic oscillations produced elevational range shifts that enormously impacted páramo species. During glaciations, species expanded across broader landscapes at lower elevations, establishing new populations and gene flow between populations that had been isolated. During interglacials, species were again restricted to geographically isolated ''islands in the sky'' (van der Hammen et al., 1973; Hooghiemstra et al., 2006; Graham, 2009; Sklenář et al., 2011). Another historical factor that impacted the pa´ramo populations is volcanism (Oleas et al., 2012). Volcanic activity is a driver of population extinction and recolonization (Carson et al., 1990; Beheregaray et al., 2003), and thus influences evolutionary trajectories of the populations.

We examined the genetic structure of populations of *Lupinus alopecuroides* Desr.—a long-lived semelparous giant rosette restricted to the superpáramo in Colombia and Ecuador. Semelparous giant rosettes are a distinctive feature of tropical alpine floras, and a fascinating example of convergent adaptive evolution to harsh high-elevation environments (Rundel et al., 1994; Sgorbati et al., 2004). Marcescent dead leaves of the rosette protect the meristem from nocturnal temperatures, which often are below zero (Monasterio, 1986; Rundel et al., 1994), and lanate hairs on the up-to 1 m high inflorescence provide insulation for reproductive organs (Miller, 1986) (Figure 1). Semelparous plants maximize reproduction by investing all resources in a single reproductive episode because, due to harsh environmental conditions, future survival and reproduction are less likely (Young & Augspurger, 1991).

Figure 1. Typical habit and local distribution of *Lupinus alopecuroides* in the superpáramo of Sincholagua, Ecuador (Photo by Diana L. A. Vásquez).

The genetic structure of populations is also determined by species-specific biological traits such as dispersal ability and breeding system. One of the most common seed dispersal modes observed in lupines is ballistichory—a mechanical process in which the walls of the mature pod twist in opposite directions, firing the seeds 1–3 m away from the parent plant. Long-distance dispersal of lupine seeds is usually afforded by waterways, human activities, and animals, mainly rodents (Milla & Iriondo, 2011; Fagan et al., 2005; Maron & Kauffman, 2006; Australian Department of Health and Ageing, 2013). The reproductive biology of *L. alopecuroides* has remained unstudied. Other perennial species of Lupinus have asexual reproduction by vegetative regeneration, and there is no evidence of apomictic reproduction in the genus (Richards, 1986). Most annual lupines are self-compatible while perennials are self-incompatible (Kittelson & Maron, 2000). However, in the papilionoid legumes, to which Lupinus belongs, self-sterility is rare (East, 1940; Kittelson & Maron, 2000). For facultatively autogamous species, selfing may occur either as a result of insufficient pollinator visitation, or as a result of pollinator visits due to the close proximity of viable pollen to the receptive stigma (Pazy, 1984; Karoly, 1992), or due to transfer of pollen between flowers on the same individual (de Jong et al., 1993).

Another important factor influencing the genetic structure of natural populations is landscape (Slatkin, 1987; Manel & Holderegger, 2013). Populations of *Lupinus alopecuroides* are known from only ten geographically isolated mountaintops (Figure 2). In geographically isolated populations, enhanced genetic drift and lack of gene flow result in genetic differentiation and loss of genetic diversity (Lesica & Allendorf, 1995; Frankham et al., 2002; Allendorf & Luikart, 2009). In these tropical ‘‘islands in the sky’’, founder effects may also occur, as founders arrive over long distances from similar habitats, rather than from adjacent sites at lower elevations, which are ecologically different (Carson & Templeton, 1984). Enhanced genetic drift, restricted gene flow, and founder events put the sky island populations of *L. alopecuroides* at risk of inbreeding depression, and potentially make them

vulnerable to an extinction vortex (Frankham et al., 2002). In populations of *Puya raimondii*, which is another sky island long-lived semelparous giant rosette occurring around 4000 m a.s.l from Peru to northern Bolivia, low genetic variation was reported across 217 AFLP marker loci in 160 individuals from eight populations. Four populations were completely monomorphic, and each of the others displayed only one to three polymorphic loci. It was suggested that the causes of this pattern are high rates of selfing and repeated bottlenecks (Sgorbati et al., 2004). *Phaedranassa tunguraguae*, which is a long-lived, partially selfing plant, showed homozygote excess at 12 microsatellite loci. Its populations were restricted to a single valley in the Ecuadorian Andes, at 1500–2500 m a.s.l., and the most likely explanation suggested for this pattern was genetic drift due to small population size and restricted gene flow (Oleas et al,. 2012).

It is well established that founding of a new population by a small number of individuals causes abrupt changes in allele frequencies that can lead to loss of genetic variation within populations and increased genetic differentiation among populations (Mayr, 1954, Allendorf & Luikart, 2009). However, founder effects are hard to detect, because it is difficult to disentangle their signatures from those of other evolutionary forces (Hoeck et al., 2010; Spurgin et al., 2014). In island populations of Berthelot's pipit (*Anthus berthelotii*), Spurgin et al. (2014) detected genetic and phenotypic changes that resulted from founder events, and suggested that, under lack of gene flow and selection, founder effects can persist for evolutionary time scales. In populations of silvereyes (*Zosterops lateralis*), Clegg et al. (2002) demonstrated that sequential founder events produced divergence among populations. Finally, Prugnolle et al. (2005) have suggested that patterns of neutral genetic differentiation in human populations are the result of sequential founder events that occurred as small populations of modern humans migrated out of Africa.

Population genetics studies can help us understand how contemporary diversity arises and how it can be maintained (Frankham et al., 2002; Allendorf & Luikart, 2009). Here, we use microsatellite markers to examine patterns of genetic variation in the sky island populations of *Lupinus alopecuroides*, and we discuss the factors underlying genetic variation and population structure. In particular, we address the following questions. (1) How high is genetic diversity within populations? (2) Are populations genetically differentiated, and how is genetic variation distributed within and among the populations? (3) Is there gene flow between the populations? (4) How are the different populations related, and is there a geographical correlation?

Figure 2. Geographic distribution of 10 locations (mountaintops), where populations of Lupinus alopecuroideswere sampled. Map by Flemming Nørgaard.

Materials and methods

The species

Lupinus alopecuroides Desr. is a long-lived semelparous giant rosette known from only ten geographically isolated populations. Each population is located on a different mountaintop, of which three are in Colombia and seven in Ecuador (Figure 2). Within the populations, individuals are found forming dense clusters (Figure 1). This might be related to the species' ballistic seed dispersal. We assume that long-distance dispersal of *L. alopecuroides* seeds, which weigh approx. 40 mg and measure 5 x 3 mm, is limited. Elsewhere rodents and human activity are important dispersers of Lupinus (Milla and Iriondo, 2011; Fagan et al., 2005, Maron & Kauffman, 2006; Australian Department of Health and Ageing, 2013), but these agents are nearly absent in the superpáramo (Sklenář & Ramsay, 2001; Rangel, 2006). Anemochory or epizoochory are also unlikely in *L. alopecuroides*, since the seeds have a smooth surface without appendages or other adaptations that could facilitate external transport by animals. Some long-distance dispersal may be provided by waterways, white-tailed deer (*Odocoileus virginianus*), and domestic cattle, which have been seen to feed on *L. alopecuroides* pods (Vásquez, personal observation). The reproductive biology of the species has remained unstudied. Bumblebees are the most likely pollinator of *L. alopecuroides*, but

their activity may be reduced by the harshness of the environment where this species grows (Dillon et al., 2006). During our fieldwork for this (10 weeks) and previous studies, we never observed insects visiting the flower, even on the sunniest days. We also observed that flowers remain closed for a long time after anthesis.

Population sampling

Exhaustive field and herbarium work in 2013–2014 permitted us to sample all ten known populations of *L. alopecuroides*, each of them located on a different mountain (Figure 2). On the Cayambe and Sincholagua mountains, populations consist of three subpopulations clearly separated by 1–10 km (Table 1). Population size, elevation, and slope were recorded for each population and subpopulation (Table 1). Population size was estimated by counting all individuals or, when population size exceeded 50, by extrapolating it from the average number of individuals counted in subplots. Population area was estimated from the polygon obtained from GPS-delimited boundaries using Google Earth Pro. Leaf tissue was randomly collected from 8 to 67 clearly distinct individuals, and stored in silica gel.

Molecular methods

DNA was extracted from leaf tissue of 220 individuals. Nine loci (Luna1, Luna3, Luna4, Luna6, Luna8, Luna12, Luna15, Luna17, Luna20) developed for *Lupinus nanus* (Molecular Ecology Resources Primer Development Consortium et al., 2012), and two loci (AG55-20-22, AG55-26-16) developed for *Lupinus microcarpus* (Drummond & Hamilton, 2005) were amplified in four separate multiplexes, using QIAGEN Multiplex PCR Maxter Mix (Qiagen, Valencia, CA, USA). PCR products were electrophoresed on an automated capillary sequencer (3130xl Genetic Analyzer, Applied Biosystems, Foster City, CA, USA) with Genescan-600 (LIZ) size standard (Applied Biosystems). Sizes of alleles (in base pairs) were determined using GeneMarker (Soft Genetics).

Statistical analysis

To estimate within-population genetic diversity, allelic richness as well as observed heterozygosity (Ho), and unbiased expected heterozygosity (He) according to Nei (1987) were calculated for each population. Genetic differentiation among all populations was estimated using Weir and Cockerham's θ (1984), an estimator of F_{ST}, and R_{ST}, an F_{ST} analogue based on the stepwise mutation model (Slatkin, 1995). The program FSTAT 2.9.3 (Goudet, 2001) was used for these analyses. Tests for significant deviation from Hardy–Weinberg equilibrium were conducted using exact tests implemented in Arlequin 3.5 (Excoffier & Lischer, 2010). Genetic relationships among the populations were estimated using POPTREEW (Takezaki et al., 2014) to construct a neighbour-joining phenogram based on dmu2 distances (Goldstein et al., 1995). Bootstrap values across loci were based on 10000 permutations by locus. Spatial genetic structure was further explored by testing for isolation by distance using a Mantel test between a matrix of dmu2 genetic distances (Goldstein et al., 1995) and a matrix of geographic distances as implemented in R package adegenet 2.0.0 (Jombart & Ahmed, 2011).

Results

A total of 46 unambiguously scorable and reproducible alleles ranging from 75 to 346 bp were detected at 11 microsatellite loci across 220 individuals, representing 10 populations distributed throughout the species' geographic range. Numbers of alleles per locus ranged from 2 to 6. Monomorphic loci were observed in all the populations except COCUY, where all eleven loci were polymorphic. Five populations showed only one polymorphic locus each. The other four populations (CHIMBORAZO, IMBABURA, CAYAMBE, SINCHOLAGUA), were completely monomorphic (Table 1). Consequently, there was no genetic variation between the subpopulations neither in CAYAMBE nor in SINCHOLAGUA. Moreover, CAYAMBE and SINCHOLAGUA were fixed for the same alleles (Online Resource 1). Therefore, all individuals within these populations were identical multilocus genotypes. Significant deviation from Hardy–Weinberg equilibrium was found across all non-monomorphic loci except Luna 3 (p value = 0.23) and Luna 17 (p value = 0.02) in the population COCUY. Genetic differentiation among all populations was high: $R_{ST} = 0.65$ and $\theta = 0.84$ (95 % CI 0.753–0.900).

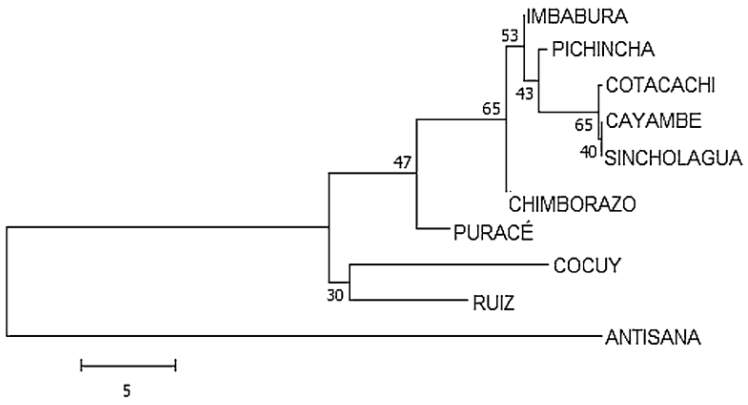
Table 1. *Lupinus alopecuroides* populations and subpopulations with their respective population size, sample size, elevation, slope, area, mean allelic richness per loci, observed heterozygosity (Ho), expected heterozygosity (He), and number of polymorphic loci among the 11 analysed loci.

Population	Subpopulation	Pop. Size	Sample size	Elevation (m a.s.l.)	Slope (L)	Area (m^2)	Mean allelic richness	Ho	He	No. polymor loci/pop
COCUY		*50	14	4150–4300	0–10	400000	2.72	0.144	0.51	1
RUIZ		*400	22	4450–4600	20–30	35000	1.08	0.01	0.04	1
PURACE		*600	23	4100–4200	20	22000	1.08	0	0.04	1
COTACACHI		*60	19	4340–4350	30	700	1.11	0	0.036	1
IMBABURA		10	10	4350–4370	35	150	1	0	0	0
CAYAMBE	CAY1	*80	8	4468	0–20	22500	1	0	0	0
	CAY2	*300	50	4425–4450	35	35000				
	CAY3	9	9	4100–4200	40	300				
PICHINCHA		*250	18	4550–4600	20–30	38000	1.09	0	0.04	1
ANTISANA		*50	12	4400–4450	35	600	1.16	0	0.06	1 0
SINCHOLAGUA	SINCH1	*100	19	4430–4438	30	5000	1	0	0	
	SINCH2	*40	9	4430–4440	35	250				
	SINCH3	*100	11	4490–4495	40	4500				
CHIMBORAZO		8	8	4500–4600	10–20	300000	1	0	0	0

*Populations are sorted by latitude

The neighbour-joining phenogram (Figure 3) constructed from dmu2 distances (Goldstein et al., 1995) grouped all Ecuadorian populations except ANTISANA in a well-supported group (65 % bootstrap value). COTACACHI, CAYAMBE and SINCHOLAGUA were also a group with high bootstrap value (65 %). Test of isolation by distance yielded a non-significant outcome (p value = 0.416).

Figure 3. Neighbour-joining dendrogram relating 10 *Lupinus alopecuroides* populations based on dmu2 distances (Goldstein et al.,1995). Bootstrap values across loci were based on 10000 permutations by locus.



Discussion

Genetic diversity and genetic differentiation

Our analyses revealed extremely low genetic diversity in populations of *L. alopecuroides* throughout its geographic range. From the total of ten known populations, four were completely monomorphic (all individuals within the population were identical multilocus genotypes) and another five showed only one polymorphic locus each (Online Resource 1). On the other hand, the high θ and R_{ST} values show that the ten populations of *L. alopecuroides* are highly genetically differentiated. R_{ST} incorporates molecular distances between alleles, and if R_{ST} is substantially higher than θ, then this might indicate that mutations have contributed to differentiation, indicating a substantial phylogeographical signal (Slatkin, 1995). In the present case, however, $\theta > R_{ST}$ suggesting that drift is the predominant force underlying differentiation. Our results also show that populations of *L. alopecuroides* are not in Hardy–Weinberg equilibrium. We suggest that this disequilibrium is due to selfing and enhanced genetic drift rather than null alleles, as it seems unlikely that null alleles should occur at all loci and populations, whereas selfing would be expected to affect all loci. Finally, we suggest that low genetic diversity within populations and high genetic differentiation between populations are related to founder effects, lack of gene flow, and/or autogamy.

Founder effects—in tropical sky islands, populations are likely founded by individuals arriving from long distances, rather than from the warm tropical surroundings (Carson & Templeton, 1984). Long-distance dispersal of *L. alopecuroides* seeds might be limited since rodents and human activity, important dispersers of *Lupinus* elsewhere (Milla & Iriondo, 2011; Fagan et al., 2005, Maron & Kauffman, 2006; Australian Department of Health and Ageing, 2013), are nearly absent in the superpáramo (Sklenář & Ramsay, 2001; Rangel, 2006). Therefore, *L. alopecuroides* populations are likely founded by a small number of individuals. This produces severe bottlenecks that cause loss of genetic variation and increases genetic differentiation (Mayr, 1954). Furthermore, Pleistocene climatic oscillation and intensive volcanic activity could also promote founder and bottleneck events by causing population expansions and contractions, and by affecting the populations' extinction–

recolonization rate (Carson & Templeton, 1984; Carson et al., 1990; Beheregaray et al., 2003). Repeated founder events may have triggered genetic divergence among populations (Clegg et al., 2002), and loss of genetic variation through sequential bottlenecks, as has previously been invoked to explain low variation in other species (e.g. Hedrick, 1996). Interestingly, COCUY is the only population that is not located on a volcanic mountain massif (Kroonenberg et al., 1990) and it showed the highest genetic diversity. However, further research is needed to test the influence of volcanic activity and Pleistocene climatic oscillations on within-population genetic variation.

Lack of gene flow—in isolated populations, genetic drift leads to fixation of random alleles, and hence to loss of genetic variation and high genetic differentiation due to the absence of gene flow (Lesica & Allendorf, 1995; Frankham et al., 2002; Allendorf & Luikart, 2009; Bech et al., 2009). The high θ value in our data indicates that populations of *L. alopecuroides* are fixed for different alleles, suggesting an absence of gene flow.

Autogamy—low within-population diversity and high differentiation among populations are often observed in selfing species (Hamrick & Godt, 1996). In *L. alopecuroides*, high rates of selfing may be expected due to low availability of pollinators (Vásquez personal observation). Autogamous self-fertilization, which is common among islands plants (Baker, 1967; Carlquist, 1974), provides reproductive assurance when outcross pollination is limited by low availability of mates and/or pollinators (Karoly,1992). In the Venezuelan highlands, Berry and Calvo (1989) found near absence of pollinators and higher levels of selfing in four species of *Espeletia* (Asteraceae) growing above 4000 m a.s.l. Like lupines, *Espeletia* species are mainly pollinated by bumblebees. Significant decrease of diversity and activity levels of pollinators with elevation was also found in the Chilean Andes (Arroyo et al., 1985). Although, high inbreeding was indicated in the COCUY population and self-sterility is rare among lupines (East, 1940; Kittelson & Maron, 2000), further research is needed to demonstrate autogamy in *L. alopecuroides* and its role in determining the populations genetic structure.

Genetic relationships between populations

The neighbour-joining phenogram (Figure 3) identified a well-supported group consisting of all Ecuadorian populations except ANTISANA. Within that group, CAYAMBE and SINCHOLAGUA, which were shown to be genetically indistinguishable (Online Resource 1), are the only populations located on the eastern Ecuadorian Cordillera. The population ANTISANA was shown to differ genetically from the rest of the populations. Interestingly, this population was also phenotypically different from the rest. In comparison with other populations, individuals have smaller leaves and inflorescence, and the overall size of the plants was also smaller (Vásquez, personal observation).

Finally, correlation between genetic and geographic distances of pair of populations was not significant, as was shown by the test for isolation by distance. This indicates that the genetic structure of *L. alopecuroides* populations is not the simple product of geographic spatial structure, pointing towards the importance of colonization history and founder effects in driving differentiation among populations. Although it has been heavily debated, the role of founder effects in evolution remains poorly understood (Clegg et al., 2002; Spurgin et al., 2014). It is unclear if founder effects can persist through evolutionary time when at the same

time ongoing selection, mutation, gene flow, and drift affect the genetic composition of populations (Hoeck et al., 2010; Spurgin et al., 2014). In this regard, it has been suggested that, depending on the severity and the continuality of the founder effects (Clegg et al., 2002), and on the extent of gene flow and selection, founder effects may be detectable in present populations, and may play an important role in the initial stages of speciation (Spurgin et al., 2014).

Possible effects of microsatellite cross-species amplification

Success of cross-species transfer of microsatellite markers depends on the evolutionary distance between the source and the target species (Rossetto, 2001; Selkoe & Toonen, 2006; Barbara et al., 2007). *Lupinus alopecuroides*, *L. microcarpus*, and *L. nanus* belong to a large western New world ‘‘super-radiation’’ (5.0–13.2 Mya) that comprises the western lupines from North America, Mexico and the Andes (Drummond et al., 2012). Low sequence divergence in adaptive radiations allows transfer of polymorphic microsatellite markers between species of the same sub-family and beyond (Barbara et al., 2007; Bezault et al., 2012). Otherwise, in plants, cross-species transfer of polymorphic markers is likely to be successful mainly within genera (Rossetto, 2001; Barbara et al., 2007).

Cross-species transfer of microsatellites markers is often accompanied by a decrease in allelic diversity (Selkoe & Toonen, 2006). However, if cross-species amplification underlies the low variation observed in the present study, then this should be a species-wide effect, whereas low variation within populations but higher variation in the species as a whole would suggest that low variation reflects demographic history. The latter was the case in our study; the total number of alleles for the species ranged from 2 to 6 across loci (Online Resource 1). One population (COCUY) in fact exhibited most of the observed alleles, whereas the other populations were mostly fixed for different alleles. We, therefore, conclude that the observed patterns of microsatellite DNA variation reflect genuine demographic processes and are not a result of reduced variation due to cross-species transfer.

Conclusions

Our study reveals extremely low genetic diversity within populations and high genetic differentiation between populations of *L. alopecuroides* across its range. We suggest that this pattern is related to founder effects, lack of gene flow, and possibly autogamy. However, further research is needed to provide evidence for autogamy in *L. alopecuroides*. The genetic relationships between the populations and the lack of correlation between genetic and geographic distances point to the importance of colonization history and founder effects in determining the populations’ genetic structure. Although based on a limited number of markers, our study gives insights into the evolution of the unexplored but fascinating sky island Andean flora. Moreover, *L. alopecuroides* provides an exceptional opportunity for understanding the role of founder effects in evolution. Future studies should focus on reconstructing the colonization history of *L. alopecuroides*, including the impact of volcanism and Pleistocene climatic oscillations on their population dynamics. Moreover, the low genetic variation and supposedly low adaptive potential in most populations suggest that the species could be particularly susceptible to anthropogenic disturbance. Formulation of a conservation strategy to protect *L. alopecuroides* is, therefore, strongly recommended.

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Online Resource 1. Allele frequencies (percentage) at eleven microsatellite loci in 10 populations of *Lupinus alopecuroides*. Allele size in base pairs. Populations are sorted by latitude and only the first four letters of their names are shown.

Locus	Allele size	COCU	RUIZ	PURA	COTA	IMBA	CAYA	PICHI	ANTI	SINCH	CHIM
Luna1	128								100		
	140	30.8									
	142	69.2	100	100	100	100	100	100		100	100
Luna3	140		28.1	68.4	100	100	100	100		100	100
	144	84.6	71.9	31.6					100		
	148	15.4									
Luna4	105	23.1									
	108		100	100	100	100	100	100	100	100	100
	111	23.1									
	114	53.8									
Luna6	162	7.7									
	165	38.4									
	168	15.4	100	100	100	100	100	100	33.3	100	100
	171	23.1							25		
	174								41.7		
	177	15.4									
Luna8	178		100								
	180	69.2							100		
	186	15.4		100	100	100	100	100		100	100
	204	7.7									
	206	7.7									
Luna12	290	20									
	306	10			26.3			35.3			
	314	20	100	100	73.7	100	100	64.7		100	100
	326	5							100		
	346	45									
Luna15	216	15.4									
	222	84.6	100								
	228			100	100	100	100	100	100	100	100
Luna17	231	53.8	100	100	100	100	100	100		100	
	234	46.2							100		100
Luna20	270	7.7							100		
	282	46.1	100	100		100		100			100
	288	15.4			100		100			100	
	290	15.4									
	306	7.7									
	312	7.7									
AG-55-20-22	75	15.4									
	78	69.2		100	100	100	100	100	100	100	100
	81		100								
	87	15.4									
AG55-26-16	168	69.2									
	171		100	100							
	177	7.7									
	180				100	100	100	100	100	100	100
	183	23,1									

Chapter 2

Genetic consequences of Quaternary climate change on high-elevation Andean *Lupinus* populations

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Abstract

Elevation shifts prompted by Quaternary climate change are considered one of the main factors underlying the outstanding diversity and fast evolution of the páramo, which is the name given to the high-elevation vegetation in the northern Andes. However, the genetic consequences of these elevation shifts on páramo plant populations remain poorly explored and little is known about how they influenced intraspecific genetic differentiation. Here, we use a multi-species approach, micrositellites DNA markers, and Approximate Bayesian Computation (ABC) to test whether upslope shifts of the páramo resulted in high genetic differentiation between populations of páramo *Lupinus* species, and whether the colonization of higher elevations was accompanied by founder events. Our results indicate that most of the populations became differentiated during the Holocene, when climate warmed and the páramo was restricted to isolated mountaintops at similar elevations as today. Our results also suggest that founder events occurred during the colonization of these mountaintops. Founder events led to a pattern of isolation by colonization, wherein genetic structure is primarily determined by colonization history. The observed patterns of genetic variation are also influenced by the autogamous reproductive mechanism of the studied species. We also find evidence, which indicates that some populations are glacial relict populations. We propose that in-situ survival was facilitated by limited glacier cover at relatively lower mountains and lack of volcanic activity.

Keywords: climate change, genetic differentiation, founder event, *Lupinus,* páramo.

Introduction

Depending on the landscape and on species-specific biological traits, past climate change affected intraspecific differentiation and species diversification by producing range shifts, population size changes, extinction, and recolonization (Hamrick & Godt, 1996; Avise, 2000; Hewitt, 2000). The genetic consequences of these changes have been widely studied, however, alpine regions remain less explored (Galbreath et al., 2009), in particular tropical-alpine regions (Hensen et al., 2012; Oleas et al., 2012; Vásquez et al., 2016). Studies in the Northern Hemisphere have shown that, unlike low-land species, many high-elevation species overcame climate change by shifting in elevation and not in latitude. Therefore, they underwent isolation during interglacials, when they were restricted to the higher part of the mountains, and experienced increased gene flow during glacial periods, when they had a broader distribution at lower elevations (Avise, 2000; Hewitt, 2000). Given the abrupt mountain topography, founder events during postglacial recolonization are likely in high-elevation species (Stöcklin, 2009). Also, it has been suggested that, in view of the narrow temperature tolerance of high-elevation species, they were especially affected by extinction during climate changes (Galbreath et al., 2009).

Range shifts of the high-elevation vegetation in the northern Andes, which is known as the páramo, is well documented in paleoenvironmental studies. During warm periods, the páramo occupied the same elevation as today (3000–4900 m a.s.l), forming an archipelago of isolated sky-islands. During cold periods, the páramo expanded to become continuous over much larger areas at lower elevations (e.g., Van der Hammen, 1974; Hooghiemstra, 1984; van't Veer & Hooghiemstra, 2000). Van der Hammen and Cleef (1986) found evidence of elevational shifts during the late Quaternary. They showed that by the Late Pleniglacial (21,000–14,000 yr BP) climate was cold and dry, glaciers and forest were reduced, but the páramo, on the contrary, expanded above 2000 m forming a continuous belt. This belt became fragmented at the beginning of the Holocene (ca. 10,000 yr BP), and between ca. 7500–3000 yr BP, when climate became warmer and the páramo was restricted to isolated mountaintops at similar elevations as today. More recent paleoenvironmental studies, however, suggest that species responded individually to climate change and that the páramo elevational shifts were more complex than originally thought (Colinvaux et al., 1997; Hooghiemstra & Van der Hammen, 2004).

Elevation shifts prompted by climate change during the Quaternary are considered by many authors as one of the main factors underlying the outstanding diversity and extremely fast evolution of the páramo flora (e.g., Simpson, 1974; Hooghiemstra & Van der Hammen, 2004; Hughes & Eastwood, 2006; Sklenář et al., 2011; Madriñán et al., 2013). Increased gene flow during cold periods alternating with isolation during warm periods is considered to have stimulated speciation (Van der Hammen & Cleef, 1986; Luteyn, 1999; Hooghiemstra & Van der Hammen, 2004). In particular, it has been proposed that opportunities for isolation during these range shifts accelerated species diversification (Hughes & Eastwood, 2006; Jabaily & Sytsma, 2012; Madriñán et al., 2013). These hypotheses, however, were prompted by paleoenvironmental findings, patterns of species distribution, and phylogenetic studies that showed that several speciation events took place during the Pleistocene. This means that the genetic consequences of the elevational shifts on páramo plant populations remain poorly

explored and little is known about how these shifts influenced intraspecific genetic differentiation.

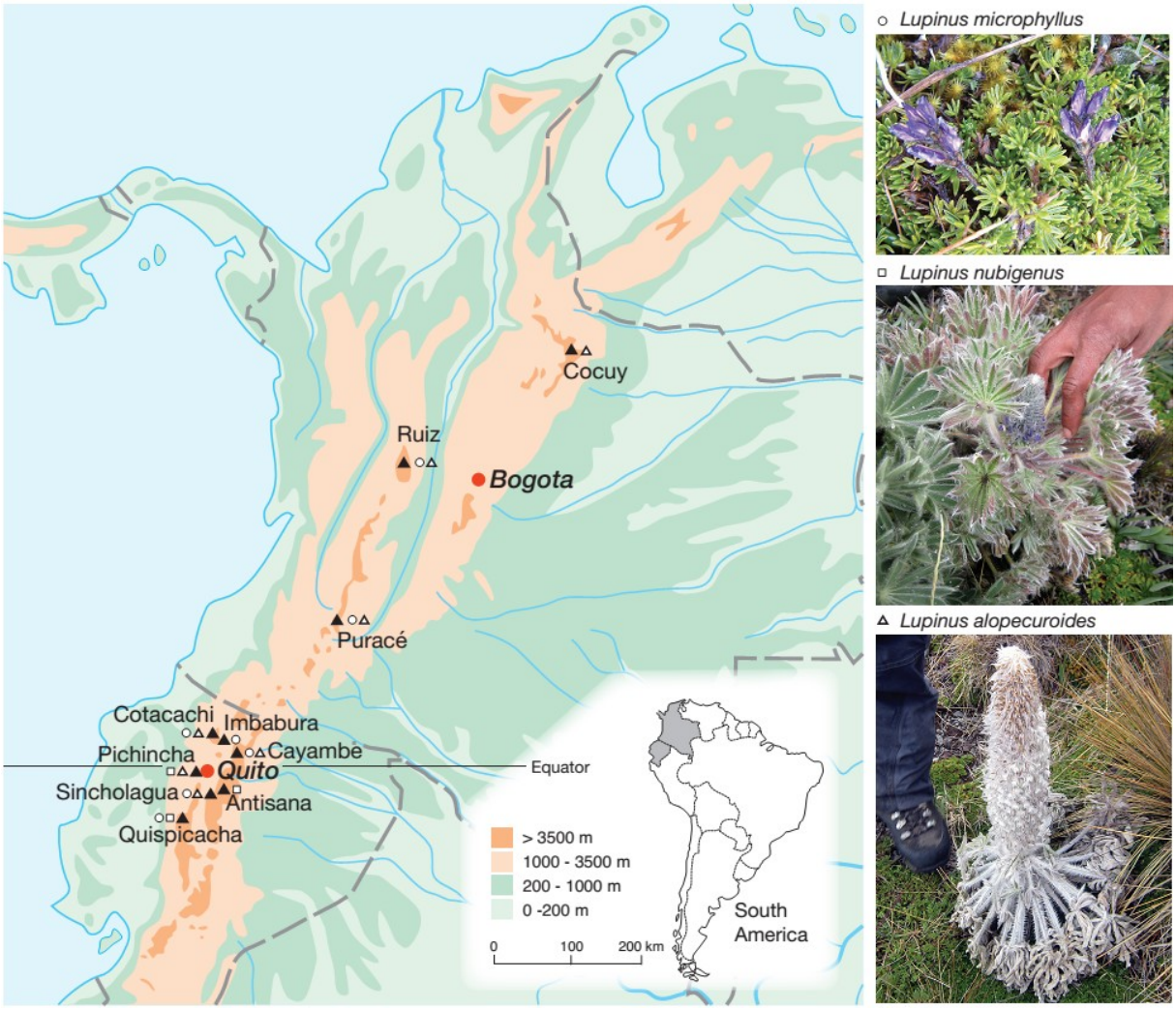
Population genetic studies provide valuable insights to understand the factors underlying genetic differentiation (Spurgin et al., 2014), including the role of history (Hansen & Taylor, 2008). However, available population genetic studies of páramo plant species are very scarce and show conflicting patterns, in which the role of Quaternary climate change remains mostly speculative. For instance, Hensen and collaborators (2012) found low genetic diversity and substantial differentiation between Ecuadorian populations in the wind-pollinated, high-elevation tree *Polylepis incana*. They suggested that this pattern is related to upward shifts of the vegetation during warmer periods in the Holocene, which led to founder events and geographic isolation. Peng and collaborators (2015), on the other hand, found high within-population genetic diversity and low genetic differentiation between populations in *Polylepis tarapacana* at 4100–5000 m a.s.l. in Bolivia and Chile and suggested that this pattern is the result of increased gene flow during colder periods in the Pleistocene. Kolář and collaborators (2016) also found lack of differentiation between currently geographically isolated populations in páramo species of *Loricaria*. The authors suggested that in *Loricaria* gene flow is not hampered by the deep Andean valleys that separate the populations, and that migration probably took place during both, cold and warm periods.

The páramo *Lupinus* species, are one of the best studied groups within the páramo flora. They belong, along with other Andean species, to a clade of about 85 species, which exhibit a wide range of growth forms and ecologies. The age of this clade was estimated to be 1.18–1.76 Myr implying a diversification rate of 2.49–3.72 species per Myr, which is one of the highest reported for plants (Hughes & Eastwood, 2006). Recent findings demonstrated that some páramo lupins originated as recently as the Late Pleistocene (Nevado et al., 2018). One very recently originated species is *Lupinus alopecuroides*. In a previous study, we examined patterns of genetic variation in this species and found that all its ten extant populations, each of which is restricted to a different mountaintop (4100–4600 m a.s.l.), are highly genetically differentiated (Vásquez et al., 2016). Contreras-Ortiz and collaborators (2018) also found marked genetic differentiation in this species and in other páramo lupins. On the other hand, Nevado and collaborators (2018) found evidence of gene flow between currently isolated populations in several páramo lupins. Given that pollen and seed long-distance dispersal is probably very limited in páramo lupins, the authors suggested that gene flow occurred during the last glacial period, when the páramo expanded at lower elevations (Nevado et al., 2018).

Here, we examine how elevation shifts, prompted by Quaternary climate change, influenced the genetic structure of populations and intraspecific differentiation in the páramo flora. To address these questions, we selected the páramo lupins. Since paleoenvironmental studies (Van der Hammen & Cleef, 1986) and the available genetic evidence (Nevado et al., 2018) suggest that the populations of páramo lupins were genetically connected by the Late Pleniglacial, we hypothesized that the populations of páramo lupins became genetically differentiated after the last glacial period, when climate warmed and the páramo shifted to isolated, higher elevations. Given the poor long-distance dispersal of the páramo lupins, we also predicted that genetic differentiation was triggered by founder events that occurred during postglacial colonization of the mountaintops. To test these hypotheses, we used microsatellites DNA markers and Approximate Bayesian Computation (ABC) to reconstruct the populations' history in three páramo *Lupinus* species – *Lupinus alopecuroides, L.*

nubigenus, and L. microphyllus. The first two species are known from only a few populations in Colombia and Ecuador, both species are endangered (IUCN, 2004), and are semelparous rosettes (Figure 1). *Lupinus microphyllus* has also an archipelagic geographic distribution but is otherwise more widespread and is an iteroparous, dwarf, sprawling shrub (Figure 1).

Figure 1. Map showing the geographic distribution of the mountaintops, where we studied populations of *Lupinus microphyllus* (7), *Lupinus nubigenus* (3), and *Lupinus alopecuroides* (7). The species co-occur at some mountain tops. Map by Flemming Nørgaard.



Methods

Sampling and molecular methods

Populations were sampled between the years 2011 and 2014. Seven of the ten extant populations of *Lupinus alopecuroides* (Vásquez et al., 2016) and all known Ecuadorian populations of *L. nubigenus* were included. Each of these populations is restricted to a different mountaintop at 4100–4600 m a.s.l (Figure 1). *Lupinus nubigenus* was considered endemic to Ecuador (IUCN, 2004), but it is now also known in northern Peru (Contreras-Ortiz et al., 2018). *Lupinus microphyllus* occupies a wider elevational (3000–4800 m a.s.l) and latitudinal range from Colombia to Bolivia, with disjunct populations on different mountains.

Seven populations of *L. microphyllus* included in this study co-occurred with *L. alopecuroides* or *L. nubigenus*, or both (Figure 1). Leaf tissue was randomly collected from 7–67 clearly distinct individuals. Some populations were small (<12 individuals), which explains why the size of some populations' samples were also small (Table 1).

DNA was extracted from leaf tissue of 35 individuals of *Lupinus nubigenus,* and 93 individuals of *L. microphyllus*. In *L. nubigenus*, a total of 10 loci were amplified*,* of which eight (Luna1, Luna3, Luna4, Luna6, Luna8, Luna12, Luna15, Luna17) were developed for *L. nanus* (Molecular Ecology Resources Primer Development Consortium et al., 2012), and two (AG55-20-22, AG55-26-16) were developed for *L. microcarpus* (Drummond & Hamilton, 2005). All loci except AG55-26-16 were also amplified in *L. microphyllus,* plus Luna13 and Luna18 (Molecular Ecology Resources Primer Development Consortium et al., 2012). Loci were amplified in four separate multiplexes, using QIAGEN Multiplex PCR Master Mix (Qiagen, Valencia, CA, USA). PCR products were electrophoresed on an automated capillary sequencer (3130xl Genetic Analyzer, Applied Biosystems, Foster City, CA, USA) with Genescan-600 (LIZ) size standard (Applied Biosystems). Sizes of alleles (in base pairs) were determined using GeneMarker (Soft Genetics). For *L. alopecuroides*, microsatellite data from the amplification of 11 loci in 202 individuals was obtained from our previous study (Vásquez et al., 2016).

Statistical Analysis

Within-population genetic diversity was estimated using standard diversity measures (Table 1). Genetic differentiation was estimated calculating R_{ST} (Slatkin, 1995), and θ (Weir & Cockerham, 1984) in FSTAT 2.9.3 (Goudet, 2001). Deviations from Hardy–Weinberg equilibrium were tested using exact tests as implemented in Arlequin 3.5 (Excoffier & Lischer, 2010). Genetic relationships among the populations were explored using POPTREEW (Takezaki et al., 2014) to construct a neighbour-joining phenogram based on dmu2 distances (Goldstein et al., 1995) and 10,000 bootstrap permutations. Isolation by distance was tested using a Mantel test between a matrix of delta-mu squared (dmu2) genetic distances (Goldstein et al., 1995), and a matrix of geographic distances as implemented in the R package adegenet 2.0.0 (Jombart & Ahmed, 2011).

Population history was examined using the Approximate Bayesian Computation (ABC) (Beaumont et al., 2002) framework as implemented in DIYABC 2.0 (Cornuet et al., 2008, 2014), which has proven to be useful for inference of complex population histories (e.g. Guillemaud et al., 2010; Spurgin et al., 2014). Populations were identified using the name of the mountains, where they are located, written with capital letters (Table 1). In order to examine the pattern and the timing of population divergence, five contrasting divergence scenarios were defined for *L. alopecuroides* (Figure 3), *L. microphyllus* (Figure 4), and *L. nubigenus* – the first two scenarios describe a North to South and South to North colonization of the mountaintops, respectively, the third scenario was constructed based on the genetic relationships reflected by the Neighbour-joining phenograms (Figure 2), the fourth scenario describes a random colonization, and the fifth a simultaneous colonization.

Divergence scenarios are defined by a historical model, which describes how the sampled populations are connected to their common ancestor, and a mutational model, which describes how allelic states of the studied genes change along their genealogical trees. DIYABC provides four categories of events to characterize the historical models (population

divergence, discrete change of effective population size, admixture, and sampling). We took into account only population divergence to simplify the models. We also used wide and flat priors for effective population size (*Ne*) [10; 1000], and divergence time (*t*) [10;10,000]. The mutational model was characterized using the default settings provided by the program. Based on these models, 10^6 data sets were simulated. A set of summary statistics (Table S1, Supporting information) was calculated for the observed and simulated data sets. We pre-evaluated each scenario and set of priors using a Principal Component Analysis (PCA) to check whether the simulated and the observed summary statistics were congruent. Further, DIYABC identified the most likely divergence scenario (the closest to the observed data), using a polychotomic weighted logistic regression. Based on this scenario, divergence times, along with other model parameters, were estimated using the logit approach. To verify that the chosen scenario is correct, new datasets were simulated using the posterior distribution of the parameters, and a PCA to check that these simulated datasets were congruent with the observed data. Here, we used the summary statistics that were not chosen for estimating posterior distribution of parameters.

Since DIYABC estimates divergence time between populations in terms of number of generations, information on generation times is required to calculate the time of divergence in years. Exact generation times are unknown for the páramo lupins. *Lupinus alopecuroides* is a long-lived, semelparous plant. Therefore, its generation time may be relatively long. This is in line with our demographic data (unpublished) collected in the population CAYAMBE. In 2011, we marked 20 individuals there and measured different characteristics such as overall height and width of the leaf rosette and the inflorescence, overall number of leaves, length and width of the largest and of the smallest leaf, and proportion of withered leaves against green leaves in the rosette. The studied individuals belong to different cohorts from seedlings (individuals with 2–10 leaves up to 15 cm long and 7 cm wide) to senescent individuals (individuals that die after reproduction, see photo *L. alopecuroides* in Figure 1). After 4 years, we measured the same individuals again. The collected data suggests that the growth rate of *L. alopecuroides* may be very slow, as has been shown for other páramo plants (e.g. Cavelier et al., 1992; Ramsay & Oxley, 1996). Moreover, the individuals that flowered after these four years were mature individuals with leaf rosettes at least 30 cm tall and 35 cm wide, in which the largest leaves were at least 25 cm long and 15 cm wide. Based on that information, we assumed a generation time of 5–10 years for *L. alopecuroides*. The generation time in *L. microphyllus* may be shorter (D. Vásquez pers. obs.), which is in line with the fact that this species is iteroparous. For *L. microphyllus* we assumed a generation time of 3–7 years.

The ABC approach was also used to test if founder events occurred during the colonization of the mountaintops. To do so, we compared only two scenarios – the scenario chosen as the most likely in the analysis described above and a modified version of it, which involves population size reductions (Figure 6). The historical and mutational models of the scenarios were otherwise defined as in the first analysis. A polychotomic weighted logistic regression was also used to compare them. Further, we used the Garza-Williamson modified index (*M*) (Garza & Williamson, 2001) to detect evidence of ancestral population decline. *M* was calculated for each population in Arlequin 3.5 (Excoffier & Lischer, 2010) as the ratio of the number of alleles to range in allele size. We also used the program BOTTLENECK (Piry et al., 1999) to detect recent bottleneck events by testing for a significant difference between the heterozygosity expected (*He*) given the number of alleles and a specified mutation model,

and *He* at mutation-drift equilibrium. This test was performed assuming the two phase model with 70% stepwise mutation model and 30% infinite allele model, and the Wilcoxon 1-tail test and the sign test.

Results

In *Lupinus nubigenus,* a total of 15 alleles were found across all 10 loci. Only 4 out of 10 loci were polymorphic, with a range of 2–3 alleles per locus. Within-population genetic diversity was extremely low. Within the QUISPICACHA population, all loci were monomorphic. The other two populations showed only one polymorphic locus each (Table 1). An overall heterozygote deficit was found. Among all 35 individuals sampled, only one heterozygote was found. All non-monomorphic loci showed departure from HWE. In *Lupinus microphyllus*, a total of 63 alleles were successfully amplified across all 11 loci. All loci were polymorphic, however, the number of polymorphic loci varied within each population. The RUIZ population had only one polymorphic locus, while most of the populations had more than seven polymorphic loci. Number of alleles per locus ranged from 2–14. Except for the RUIZ population, estimates of within-population genetic diversity were similar across all populations. Heterozygote deficit was found in all populations. Five of the seven populations studied had fixation indices higher than 0.72 (Table 1). All loci showed a significant departure from HWE in at least one population. *Lupinus alopecuroides* also showed extremely low within-population genetic diversity, high heterozygote deficit, and departure from HWE across all non-monomorphic loci. Two from its seven studied populations were completely monomorphic and the other six populations showed only one polymorphic locus each (Table 1).

Genetic differentiation and population structure

Populations of *L. nubigenus* were highly differentiated with a θ of 0.757 (95% CI 0.328–1), and R_{ST} of 0.63. In *L. microphyllus*, genetic differentiation was high among all populations, as suggested by both estimates, θ=0.545 (95% CI 0.445–0.653), and R_{ST} =0.6. The populations of *L. alopecuroides* were also highly differentiated (θ=0.83 (95% CI 0.74–0.900) and R_{ST}=0.65). The neighbour-joining phenogram based on dmu2 distances showed that, in *L. nubigenus*, the QUISPICACHA population is relatively well differentiated from the ANTISANA and PICHINCHA populations (Figure 2A), however, the analysis failed to identify any group with good bootstrap support, probably due to the overall low genetic variation found in the species. In *L. microphyllus*, two well-supported groups were identified (65% bootstrap value). One formed by the populations CAYAMBE, COTACACHI, and IMBABURA, and the other comprising the Colombian populations and the population QUISPICACHA (Figure 2B). In *L. alopecuroides*, the Colombian population PURACÉ and all the Ecuadorian populations were grouped together (76% bootstrap value). Within that group, only the Ecuadorian populations were also grouped together (76% bootstrap value) and within the Ecuadorian populations CAYAMBE, COTACACHI, and SINCHOLAGUA formed another well-supported group (63% bootstrap value) (Figure 2C). Test of isolation by distance was not significant in *L. nubigenus* (*p* value = 0.37) and *L. alopecuroides* (*p* value = 0.38), but it was significant in *L. microphyllus* (*p* value = 0.001).

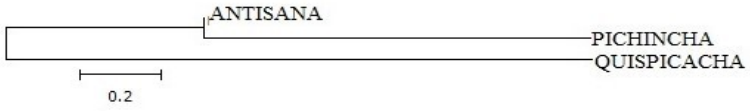
Table 1. Populations of *Lupinus nubigenus*, *L. microphyllus*, and *L. alopecuroides* with their respective mean allelic richness, observed heterozygosity (Ho), expected heterozygosity (He), number of polymorphic loci, inbreeding coefficient (Fis), and Garza-Williamson modified index (*M*). Populations are identified with the name of the mountains, where they are located.

Population name	Mean allelic richness	Ho	He	No. polymorphic loci/pop	Sample size	Fis	*M*
Lupinus nubigenus							
PICHINCHA	1	0	0.0523	1	9	1	0.816
ANTISANA	1.2	0.0048	0.0633	1	21	0.926	0.85
QUISPICACHA	1.1	0	0	0	5	NA	0.783
Lupinus microphyllus							
RUIZ	1.08	0	0.04	1	9	1	0.2
PURACÉ	1.70	0.09	0.21	5	9	0.56	0.28
COTACACHI	2.36	0.215	0.39	10	26	0.45	0.52
IMBABURA	1.64	0.048	0.19	7	10	0.75	0.3
CAYAMBE	1.84	0.006	0.24	8	15	0.97	0.35
SINCHOLAGUA	2.14	0.032	0.32	8	17	0.9	0.35
QUISPICACHA	1.95	0.078	0.27	7	7	0.72	0.32
Lupinus alopecuroides							
COCUY	2.72*	0.144*	0.51*	11*	14*	0.72	0.52
RUIZ	1.08*	0.01*	0.04*	1*	22*	0.75	0.22
PURACÉ	1.08*	0*	0.04*	1*	23*	1	0.22
COTACACHI	1.11*	0*	0.036*	1*	19*	1	0.20
CAYAMBE	1*	0*	0*	0*	67*	NA	0.19
PICHINCHA	1.09	0	0.04	1	18	1	0.19
SINCHOLAGUA	1	0	0	0	39	NA	0.19

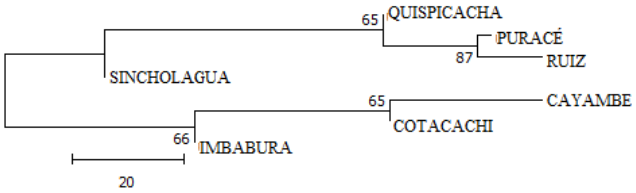
* from Vásquez et al. (2016).

Figure 2. Neighbour-joining phenograms based on dmu2 distances (Goldstein et al., 1995) (A) *Lupinus nubigenus* (B) *L. microphyllus* (C) *L. alopecuroides*. Bootstrap values across loci were based on 10,000 permutations by locus.

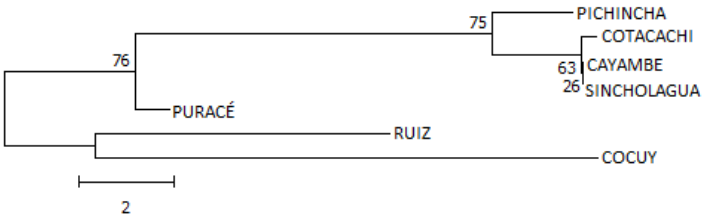
(A) *Lupinus nubigenus*



(B) *Lupinus microphyllus*



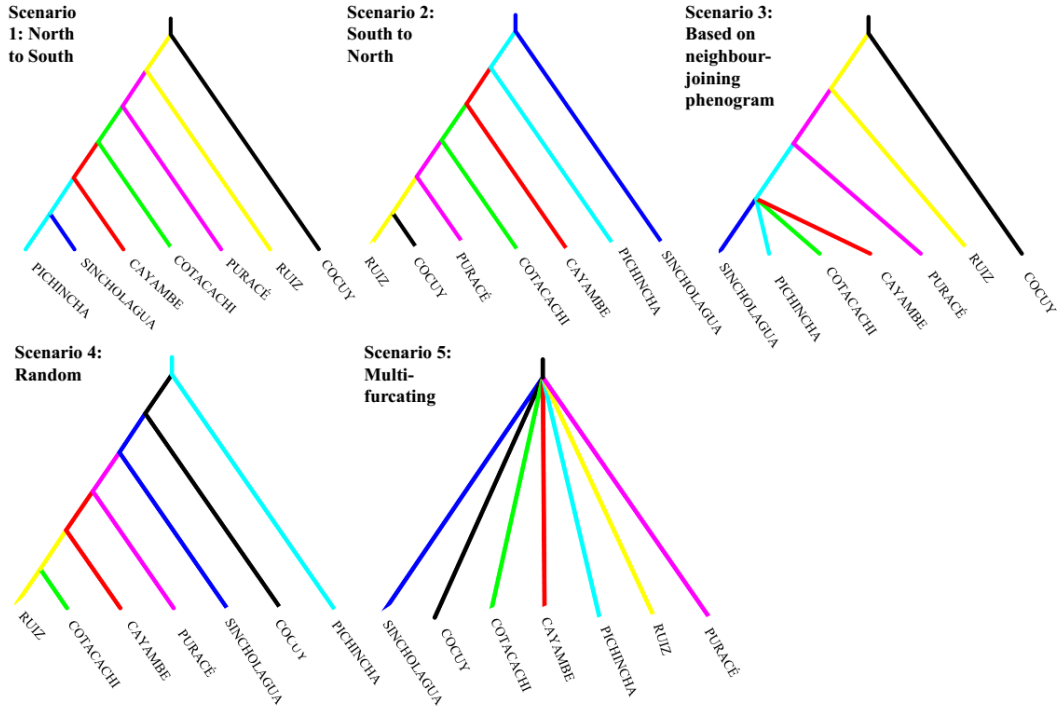
(C) *Lupinus alopecuroides*



Population history

In *L. alopecuroides*, the divergence scenario 3 is the most likely (Figure 3). It was constructed based on the populations' relationships suggested by its neighbour-joining phenogram (Figure 2C). According to this scenario, the COTACACHI, CAYAMBE, and SINCHOLAGUA populations are the evolutionarily youngest populations, and they diverged simultaneously from the PICHINCHA population. Estimates of divergence time (Figure 3) suggest that this split took place ca. 500 generations ago. The PICHINCHA population itself, diverged from PURACÉ ca. 900 generations ago, PURACÉ diverged from RUIZ ca. 1500 generations ago, and RUIZ diverged from COCUY ca. 3500 generations ago. In *L. microphyllus*, the scenario that was based on its neighbour-joining phenogram was also chosen as the most likely (Figure 4). According to this scenario, SINCHOLAGUA is the ancestral population from which IMBABURA and QUISPICACHA diverged. Estimates of divergence time (Figure 4) suggest that this split took place ca. 4680 generations ago. Further, COTACACHI diverged from IMBABURA and PURACÉ diverged from QUISPICACHA ca. 1380 generation ago, and CAYAMBE diverged from COTACACHI, and RUIZ from PURACÉ ca. 298 generations ago. In *L. nubigenus*, the comparison of scenarios was not performed because the simulated data was not congruent with the observed data. This was probably a consequence of the overall low genetic variation displayed by the species and the limited number of individuals sampled in two of its three studied populations (Table 1).

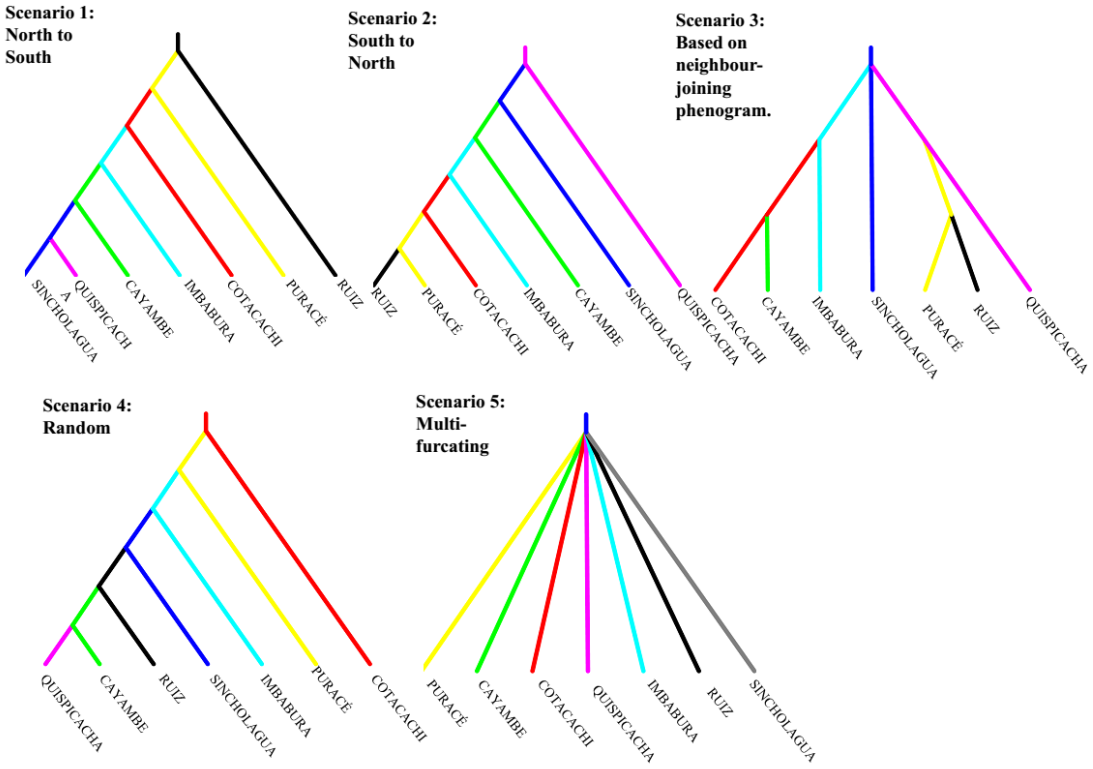
Figure 3. Comparison of divergence scenarios for seven populations of *Lupinus alopecuroides* with Approximate Bayesian Computations. Upper row, representation of the scenarios; middle row, model choice and performance; bottom row, mean and quantiles of posterior distribution samples for divergence times estimated among populations. Times of divergence, given in generations and year (in brackets), are estimated using the most likely scenario (highlighted in bold).



Scenario	Posterior Probability	Summary statistics out of the range of the observed data		
		P<0.05	P<0.01	P<0.001
1	0,06	7	8	0
2	0	9	16	25
3	**0,7**	**7**	**4**	**0**
4	0,2	7	14	15
5	0,2	12	12	12

	Times of divergence in generations and years (in brackets)		
	Mean	*Q*0.05	*Q*0.95
CAYAMBE, SINCHOLAGUA, COTACACHI–PICHINCHA	533 (2665–5330 yr)	182 (910–1820 yr)	1100 (5500–11,000 yr)
PICHINCHA–PURACÉ	1110 (5550–11,100 yr)	320 (1600–3200 yr)	2560 (12,800–25,600 yr)
PURACÉ–RUIZ	1790 (8950–17,900 yr)	486 (2430–4860 yr)	4350 (21,750–43,500 yr)
RUIZ–COCUY	4090 (20,450–40,900 yr)	1080 (5400–10,800 yr)	8760 (43,800–87,600 yr)

Figure 4. Comparison of divergence scenarios for seven populations of *Lupinus microphyllus* with Approximate Bayesian Computations. Upper row, representation of the scenarios; middle row, model choice and performance; bottom row, mean and quantiles of posterior distribution samples for divergence times estimated among populations. Times of divergence, given in generations and year (in brackets), are estimated using the most likely scenario (highlighted in bold).



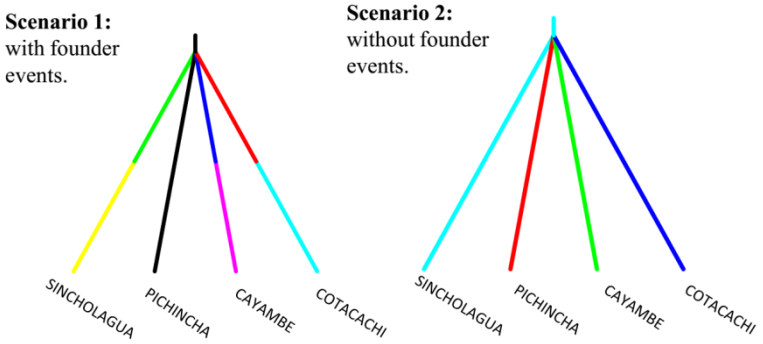
Scenario	Posterior Probability	Summary statistics out of the range of the observed data		
		P<0.05	P<0.01	P<0.001
1	0,1	5	0	0
2	0	0	0	0
3	**0,9**	**0**	**0**	**0**
4	0,009	0	0	0
5	0	0	0	0

	Times of divergence in generations and years (in brackets)		
	Mean	*Q*0.05	*Q*0.95
CAYAMBE–COTACACHI, RUIZ–PURACÉ	298 (894–2086 yr)	66 (198–1386 yr)	949 (2847–6643yr)
COTACACHI–IMBABURA, PURACÉ–QUISPICACHA	1380 (4140–9660 yr)	446 (1338–3122 yr)	3740 (11,220–26,180 yr)
IMBABURA–SINCHOLAGUA QUISPICACHA–SINCHOLAGUA	4680 (14,040–32,760 yr)	1550 (4650–10,850 yr)	9250 (27,750–64,750 yr)

The second analysis with ABC shows that, for *L. alopecuroides* and *L. microphyllus* the scenario that involves population size reductions is more likely than the scenario that does not involve such reductions (Figure 5). Further, *M* was very low across all populations of *L. alopecuroides* and *L. microphyllus* (Table 1). In *L. alopecuroides*, the population COCUY had the highest value (0.52), while the index in the remaining populations ranged from 0.19–0.22. Likewise, in *L. microphyllus*, the population COTACACHI had the highest value (0.52), while the index in the remaining populations ranged from 0.19–0.35. In *L. nubigenus,* the values estimated were all above 0.78. Tests performed with BOTTLENECK failed to find significant heterozygous excess (p>0.05) in any of the studied species.

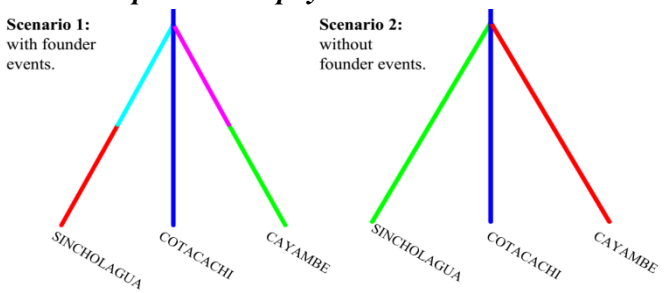
Figure 5. Test for founder events with Approximate Bayesian Computations. (A) Comparison of two divergence scenarios for four populations of *Lupinus alopecuroides*. Above, representation of the scenarios; bottom, model choice and performance. The most likely scenario is highlighted in bold. (B) Comparison of two divergence scenarios for three populations of *Lupinus microphyllus*. Above, representation of the scenarios; bottom, model choice and performance. The most likely scenario is highlighted in bold.

(A) Test for founder events in *Lupinus alopecuroides*



Scenario	Posterior Probability	Summary statistics out of the range of the observed data		
		P<0.05	P<0.01	P<0.001
1	**0,75**	**0**	**0**	**0**
2	0,25	0	0	0

(B) Test for founder events in *Lupinus microphyllus*



Scenario	Posterior Probability	Summary statistics out of the range of the observed data		
		P<0.05	P<0.01	P<0.001
1	**0,95**	**10**	**5**	**0**
2	0,05	10	2	4

Discussion

The influence of elevation shifts on genetic differentiation

The results of the ABC analyses largely support our hypothesis, according to which, populations in páramo lupins became genetically differentiated after the last glacial period, when warm climate restricted the páramo to the isolated and colder mountaintops. Although, all the populations of *Lupinus alopecuroides* and *L. microphyllus* did not diverge at the same time, most of them diverged during the Holocene, roughly at the beginning and by the Middle-Late Holocene (Figure 3, Figure 4). Although, estimates of divergence involved large uncertainty and should be interpreted with caution, they agree with paleoenvironmental evidence that shows that the early and Middle-Late Holocene were characterized by warming trends and upwards shifts of the páramo (Van der Hammen & Cleef, 1986). The estimates of divergence time also show that some populations diverged just over the last 3000 years or less (Figure 3, Figure 4). This is in line with the high diversification rate estimated for the radiation of the páramo *Lupinus* species (Hughes & Eastwood, 2006) and for other páramo plant groups (Madriñán et al., 2013), and brings support to the theory put forward by these authors, according to which, the elevational shifs of the páramo contributed to this fast diversification by isolating populations.

Estimates with ABC also indicate that some populations diverged before the last glacial period ended. Specifically, in *L. alopecuroides* (Figure 3), the PURACÉ and RUIZ populations diverged 8950–17,900 years ago and, in *L. microphyllus* (Figure 4), the SINCHOLAGUA, QUISPICACHA, and IMBABURA populations diverged 14,040–32,760 years ago. During these time periods, the climate was cold and paleoenvironmental studies showed that the páramo extended over large continuous areas at lower elevations (Van der Hammen & Cleef, 1986). We suggest that, given the abrupt Andean topography, populations could be isolated and diverged even during these periods of páramo expansion. For instance, isolation could be allowed by in-situ survival in mountain glacial refuges, such as nunataks or peripherial refuges (Schönswetter et al., 2005). Survival at high elevations could be facilitated by limited glacier cover at the summits of relatively lower mountains. In general, ice caps did not descend below 4000 m during cold periods (Clapperton, 1983). Therefore, lower mountains could remain at least partially unglaciated during these periods. This is the case of the mountains Puracé, Sincholagua, Imbabura, and Quispicacha, which all have summit elevations of 4300–4800 m. These mountaintops also harbour the populations that diverged before the last glacial period ended. Besides their relatively lower height, Sincholagua, Imbabura, and Quispicacha also have their volcanic inactivity in common, which, in theory, could also facilitate the in-situ survival of the populations.

The ABC analyses also show that the COCUY and RUIZ populations of *L. alopecuroides* diverged 20,450–40,900 years ago (Figure 3). This agrees with Contreras-Ortiz and collaborators (2018), who reconstructed the phylogenetic relationships among the páramo rosette lupins, including *L. alopecuroides*. They found that the populations located on the Eastern Colombian Cordillera (COCUY) and the populations located on the Central Colombian Cordillera (RUIZ) belong to two different clades. Since the Eastern and Central populations differed also morphologically and ecologically, Contreras-Ortiz and collaborators (2018) proposed that the COCUY population indeed belong to a distinct, yet undescribed species (Contreras-Ortiz et al., in prep). The authors also suggested that the genetic distinction

between the Eastern and Central Colombian Cordilleras, which has been observed in other species (e.g. Cadena et al., 2007; Gutierrez-Pinto et al., 2012), is a consequence of geographic isolation between the Cordilleras, which are separated by the huge valley of the Magdalena river, which is one of the Colombian major rivers. This valley probably acts as an efficient genetic barrier that hampered gene flow even during colder periods, when the páramo extended at lower elevations. However, some studies have suggested that, during these periods, gene flow was facilitated between the Cordilleras in some areas (Hooghiemstra & Van der Hammen, 2004).

The influence of elevation shifts on historical demography

We predicted that founder events occurred during postglacial colonization of the mountaintops. The ABC analyses confirm that the SINCHOLAGUA, COTACACHI, and CAYAMBE populations in *L. alopecuroides*, and the CAYAMBE and COTACACHI populations in *L. microphyllus* were founded by a small number of individuals (Figure 5). We did not use ABC to test for founder events in the rest of the populations, but the low values of the Garza-Williamson modified index estimated across all the populations suggest that all the populations underwent size reductions in the past (Table 1). Populations of high-elevation plants are usually founded by a small number of individuals due to the abrupt topography of mountains (Stöcklin et al., 2009). Moreover, in the páramo lupins, founder events are likely because their long-distance dispersal is limited. The seeds of these species are relatively big and have a smooth surface without appendages or other adaptations to anemochory or epizoochory. Moreover, potential dispersal vectors, such as rodents and human activity (Maron & Kauffman, 2006; Milla & Irondo, 2011), are scarce in the páramo (Rangel, 2006).

The occurrence of founder events during the colonization of the mountaintops is also supported by the lack of isolation by distance found in *L. nubigenus* and in *L. alopecuroides.* Isolation by distance is usually expected, given that gene flow generally occurs between neighbouring populations. However, the establishment of a population by a small number of individuals results in genetic divergence from the parent population. This may lead to a pattern of "isolation by colonization", wherein differences between populations primarily reflect colonization history, rather than current patterns of gene flow (Spurgin et al., 2014). This is in line with the comparison of divergence scenarios in *L. microphyllus* and *L. alopecuroides*, which show that the genetic structure in these species is largely determined by colonization history. Founder events may also result in loss of genetic variation (Frankham et al., 2002) and, therefore, they may also underlie the extremely low within-population genetic diversity observed in *L. nubigenus* and *L. alopecuroides* (Table 1). However, why did founder events not result in loss of within-population genetic diversity in *L. microphyllus*? (Table 1). We suggest that founder events could result in loss of genetic diversity in this species, however, unlike *L. nubigenus* and *L. alopecuroides*, within-population genetic diversity in *L. microphyllus* was recovered thanks to gene flow between its populations. Gene flow in *L. microphyllus* is suggested by the positive correlation between genetic and geographic distances and is in line with its geographic distribution. Unlike *L. nubigenus* and *L. alopecuroides*, which occur only at a few sky-islands sparsely located along the Andean cordilleras (at 4100–4900 m a.s.l.), populations of *L. microphyllus* are widely distributed along the Andean highlands at 3000–4900 m a.s.l.

The influence of autogamy on genetic structure

Very little is known about the reproductive biology of the páramo lupins, however, the high heterozygote excess observed in the species studied here are incompatible with high rates of out-crossing (Table 1), indicating that these species must be capable of self-fertilization. We suggest that selfing or autogamy is the cause of high heterozygote excess and the deviation from HWE observed across all loci in all populations (Table 1). This disequilibrium could also be a consequence of null alleles, however, null alleles rarely occur at all loci whereas autogamy is expected to affect all loci. Also, low within-population genetic diversity and high differentiation between populations are usually observed in autogamous species (Hamrick & Godt, 1996). Therefore, the patterns of genetic variation observed in the studied species must also be the outcome of autogamy. It is likely that other páramo lupins and other páramo plant species are also capable of self-fertilization. This mechanism provides reproductive assurance when out-crossing is limited by low availability of mates and/or pollinators (Karoly, 1992). Autogamy is therefore common among island plants (Carlquist, 1974; Baker, 1967) and may be favoured in high-elevation plants because availability of pollinators is often low in alpine environments (Arroyo et al., 1985; Berry & Calvo, 1989). Particularly in the páramo lupins, high-rates of self-fertilization may also be expected because these species usually close their flowers to protect the reproductive organs from the harsh environmental conditions. This strategy, is observed in other high-elevation Andean plants such as *Puya raimondii* (Sgorbati et al., 2004).

Elevation shifts of the populations

Although, the ABC analyses indicated that colonization of the mountaintops took place roughly by the same time periods (in the late Pleniglacial, at the beginning of the Holocene, and in the Middle-Late Holocene), *L. alopecuroides* and *L. microphyllus* did not always colonize the same mountains by the same time (Figure 3, Figure 4). For instance, *L. alopecuroides* colonized the mountaintop of Puracé by the Late Pleniglacial, while *L. microphyllus* did it by the Middle-Late Holocene. On the other hand, *L. alopecuroides* colonized the mountaintop of Sincholagua by the Middle-Late Holocene and in *L. microphyllus* the population SINCHOLAGUA is the ancestral population. We suggest that these differences may reflect the complex dynamics that populations experienced in the past. Depending on species-specific traits and local environmental conditions, populations could overcome climate change by shifting in elevation or by surviving in-situ, or they could also go extinct. Therefore, the páramo could shift uniformly as a belt both downslope and upslope during Quaternary climate change, but at the population level this dynamic was probably much more complex (Colinvaux et al., 1997; Hooghiemstra & Van der Hammen, 2003). The differences in the colonization history of *L. alopecuroides* and *L. microphyllus* could also be the result of different biogeographical histories. For instance, a northern origin of *L. alopecuroides* followed by southward migration against a southern origin of *L. microphyllus* and a northward migration.

Implications for conservation

Protection of intraspecific genetic variation is vital to guarantee the evolutionary potential of the species (Crandall et al., 2000; Moritz, 2002). This is especially true in the case of *L. nubigenus,* and *L. alopecuroides.* On one hand their populations are depauperate in genetic

diversity and highly genetically differentiated and, on the other hand, these genetic differences are the result of historical processes. Therefore, in these species, conservation must focus below the species level to protect as many populations as possible to ensure that sufficient genetic variation is maintained and that the genetic legacy of the species is protected. In particular, populations located on lower mountains may be more threatened by the current accelerated warming trend. Paleoenvironmental studies (Van der Hammen & Cleef, 1986) and our results here suggest that *L. nubigenus* and *L. alopecuroides* overcame warmer climate in the Holocene by shifting to higher, colder elevations. Therefore, we can expect that if climate becomes warmer populations such as CAYAMBE or RUIZ, which currently extend between approx. 4100 m a.s.l. and the snow-line, will shift to higher elevations as glaciers retreat. This is not the case of the QUISPICACHA population in *L. nubigenus* or the PURACÉ population in *L. alopecuroides*, which currently lie at the unglaciated summits of these mountains (around 4600 m a.s.l.). These populations have nowhere to move, if climate warms further, and will probably go extinct. At the same time, they may in fact also be particularly important for conservation because they may represent glacial relict populations.

Conclusions

Overall, our results suggest that populations in páramo lupins became genetically differentiated as late as by the Holocene, when warming trends forced the páramo to shift to isolated, colder mountaintops. Besides isolation, genetic differentiation was also triggered by founder events that took place during the colonization of the mountaintops. Given the occurrence of founder events, the genetic structure of populations is largely determined by colonization history. Genetic structure was also influenced by the ability of self-fertilization in the studied species. We also found evidence, which indicates that some populations may be glacial relict populations. We suggest that in-situ survival of these populations was facilitated by limited glacier cover at relatively lower mountains and lack of volcanic activity. Since very little is known about the existence of mountain glacial refuges in the northern Andes, further research is needed to establish whether the occurrence of such refuges is linked to lower, volcanically inactive mountains.

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Supporting information

Table S1. Set of summary statistics used for parameter estimation in the Approximate Bayesian Computation analyses (the word "sample" is abbreviated as "s".).

Set of summary statistics use for parameter estimation in *Lupinus alopecuroides*																		
One s. summary statistics	S. 1	S. 2	S.3	S. 4	S.5	S.6	S.7											
Mean number of alleles							X											
Mean genic diversity	X	X	X	X	X	X												
Mean size variance																		
Mean Garza-Williamson's M	X	X	X	X	X	X												
Two s. summary statistics	S.1 &2	S.1 &3	S.1 &4	S.1 &5	S.1 &6	S.1 &7	S.2 &3	S.2 &4	S. 2 & 5	S.2 &6	S.2 &7	S. 3 & 4	S. 4 & 5	S4 & 6	S. 4 & 7	S5 & 6	S. 5 & 7	S.6 &7
Mean number of alleles						X					X	X			X		X	X
Mean genic diversity					X	X		X	X	X	X	X	X	X	X	X	X	
Mean size variance																		
Fst																		
Classification Index																		
Shared allleles distance	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
dmu2 distance																		
Set of summary statistics use for parameter estimation in *Lupinus microphyllus*																		
One s. summary statistics	S. 1	S. 2	S.3	S. 4	S.5	S. 6	S. 7											
Mean number of alleles	X	X	X	X	X	X	X											
Mean genic diversity	X	X	X	X	X	X	X											
Mean size variance	X	X	X	X	X	X	X											

Mean Garza-Williamson's M																		
Two s. summary statistics	S.1 &2	S.1 &3	S.1 &4	S.1 &5	S.1 &6	S.1 &7	S.2 &3	S.2 &4	S. 2 & 5	S.2 &6	S.2 &7	S. 3 & 4	S. 4 & 5	S4 & 6	S. 4 & 7	S5 & 6	S. 5 & 7	S.6 &7
Mean number of alleles																		
Mean genic diversity																		
Mean size variance	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Fst																		
Classification Index																		
Shared allleles distance																		
dmu2 distance	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Set of summary statistics use for parameter estimation in *Lupinus nubigenus*																		
One s. summary statistics	S. 1	S. 2	S.3	S. 4	S.5	S. 6	S. 7											
Mean number of alleles	X	X	X	X	X	X	X											
Mean genic diversity																		
Mean size variance	X	X	X	X	X	X	X											
Mean Garza-Williamson's M																		
Two s. summary statistics	S.1 &2	S.1 &3	S.1 &4	S.1 &5	S.1 &6	S.1 &7	S.2 &3	S.2 &4	S. 2 & 5	S.2 &6	S.2 &7	S. 3 & 4	S. 4 & 5	S4 & 6	S. 4 & 7	S5 & 6	S. 5 & 7	S. 6& 7
Mean number of alleles																		
Mean genic diversity																		
Mean size variance	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Fst																		
Classification Index	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Shared allleles																		

distance																		
dmu2 distance	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Set of summary statistics use for parameter stimation in the test for founder events in *Lupinus alopecuroides*

One s. summary statistics	S. 1	S. 2	S.3	S. 4	S.5	S. 6	S. 7
Mean number of alleles							
Mean genic diversity	X	X	X	X	X	X	X
Mean size variance							
Mean Garza-Williamson's M	X	X	X	X	X	X	X

Two s. summary statistics	S.1 &2	S.1 &3	S.1 &4	S.2 &3	S.2 &4	S.3 &4
Mean number of alleles						
Mean genic diversity						
Mean size variance	X	X	X	X	X	X
Fst						
Classification Index						
Shared allleles distance						
dmu2 distance	X	X	X	X	X	X

Set of summary statistics use for parameter stimation in the test for founder events in *Lupinus microphyllus*

One s. summary statistics	S. 1	S. 2	S.3	S. 4	S.5	S. 6
Mean number of alleles	X	X	X	X	X	X
Mean genic diversity	X	X	X	X	X	X
Mean size variance						

Mean Garza-Williamson's M	X	X	X	X	X	X
Two s. summary statistics	S.1 &2	S.1 &3	S. 1 & 4	S.2 &3	S. 2 & 4	S.3 &4
Mean number of alleles						
Mean genic diversity						
Mean size variance	X	X	X	X	X	X
Fst						
Classification Index						
Shared allleles distance						
dmu2 distance	X	X	X	X	X	X

Chapter 3

Growth-form evolution and hybridization in *Senecio* (Asteraceae) from the high equatorial Andes

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Abstract

Aim
Changes in growth forms frequently accompany plant adaptive radiations, including in páramo – a high-elevation treeless habitat type of the northern Andes. We tested whether diverse group of *Senecio* inhabiting montane forests and páramo represented such growth form changes. We also investigated the role of Andean geography and environment in structuring genetic variation of this group.
Location
Northern Andes of South America.
Methods
We sampled 108 populations and 28 species of *Senecio* (focusing on species from former genera *Lasiocephalus* and *Culcitium*) and analyzed their genetic relationships and patterns of intraspecific variation using DNA fingerprinting (AFLPs) and nuclear DNA sequences (ITS). We partitioned genetic variation into environmental and geographical components.
Results
ITS-based phylogeny supported monophyly of a *Lasiocephalus*-*Culcitium* clade. A grade of herbaceous alpine *Senecio* species subtended the *Lasiocephalus*-*Culcitium* clade suggesting a change from the herbaceous to the woody growth form. Both ITS sequences and the AFLPs separated a group composed of the majority of páramo subshrubs from other group(s) comprising both forest and páramo species of various growth forms. These morphologically variable group(s) further split into clades

encompassing both the páramo subshrubs and forest lianas, indicating independent switches among the growth forms and habitats. The finest AFLP genetic structure corresponded to morphologically delimited species except in two independent cases in which patterns of genetic variation instead reflected geography. Several morphologically variable species were genetically admixed, which suggests possible hybrid origins. Latitude and longitude accounted for 5–8% of genetic variation in each of three AFLP groups, while the proportion of variation attributed to environment varied between 8% and 31% among them.

Main conclusions

A change from the herbaceous to the woody growth form is suggested for species of high-elevation Andean *Senecio*. Independent switches between habitats and growth forms likely occurred within the group. Hybridization likely played an important role in species diversification.

Keywords: Adaptive radiation; Andes; *Culcitium*; Growth forms; Hybridization; *Lasiocephalus*; Neotropical montane forest; Páramo; *Senecio*.

Introduction

The uplift of Andean cordilleras played a major role in promoting diversification of the Neotropical flora (Antonelli et al., 2009; Hoorn et al., 2010; Hughes et al., 2013). The equatorial Andes in particular host a very diverse flora spanning a great variety of habitats between the montane forest and the alpine páramo belts (Churchill et al., 1995; Luteyn, 1999; Myers et al., 2000). Páramo habitats became available by the end of the north-Andean orogeny ca. 3–5 Ma (van der Hammen & Cleef, 1986). In spite of its relative youth, the páramo flora is especially rich in groups which underwent radiations (Sklenář et al., 2011; Madriñan et al., 2013). Elevational shifts of vegetation belts during the Pleistocene, which repeatedly fragmented and reunited plant populations, was coupled with the final uplift of the Andes, which created new ecological opportunities and promoted diversification of the flora (Hughes & Eastwood, 2006; Jabaily & Sytsma, 2012; Madriñan et al., 2013; Luebert & Weigend, 2014).

Adaptation to newly emerging supra-forest habitats has been an important source of functional diversity in the páramo flora (Sklenář et al., 2011). DNA-based phylogenetic studies indicate that the north-Andean genus *Espeletia* Mutis ex Bonpl. (Asteraceae) evolved distinct growth forms upon colonizing the páramo (Cuatrecasas, 1986; Panero et al., 1999; Rauscher, 2002). In *Aragoa* Kunth (Plantaginaceae), another genus endemic to the northern Andes, arborescent plants of the montane forest evolved into páramo shrubs (Fernández-Alonso, 1995; Bello et al., 2002), and growth form changes have been found in other Andean genera such as *Lupinus* L., *Hinterhubera* Sch. Bip. ex Wedd., *Laestadia* Kunth ex Less., and *Westoniella* Cuatrec. (Hughes & Eastwood, 2006; Karaman-Castro & Urbatsch, 2009). However, deep insights into growth-form evolution among north-Andean plant groups, based upon genetic markers with sufficiently detailed resolution have been rare (Jabaily & Sytsma, 2012; Uribe-Convers et al., 2016).

Species of the genus *Senecio* L. (Asteraceae), which were traditionally placed in the genus *Lasiocephalus* Willd. ex Schltdl. (Cuatrecasas, 1978), comprise a morphologically and ecologically diverse plant group in the northern and central Andes. About 25 species are distributed from Venezuela to Bolivia, with the highest richness in Ecuador (Cuatrecasas, 1978; Calvo & Freire, 2016). Two main growth forms are recognized. Broad-leaved lianas (Figure 1g, h) inhabit montane forests and secondary thickets usually between 2800–3800 m, although some species also occur in the forest–páramo shrubby ecotone called subpáramo (usually at 3800 m). The other growth form is ascending or erect, narrow-leaved subshrub (Figure 1a–c, e) that occur in the páramo dominated by tussock grasses (3800–4300 m) and in the uppermost belt of patchy vegetation called superpáramo (up to 4800–5000 m). One species, *Senecio mojandensis* Hieron. (Figure 1d), a basal rosette herb of wet páramo habitats cannot be satisfactorily placed in either of these categories. Most species are morphologically distinct and readily identifiable, although some are variable in leaf size and shape, such as *S. otophorus* Wedd.

Figure 1. Growth form variation among the investigated *Senecio* species from the high Andes: (a) *S. lingulatus*, Ecuador, páramo; (b) *S. longepenicillatus,* Venezuela, superpáramo; (c) high-elevation form of *S. otophorus,* Colombia, superpáramo; (d) *S. mojandensis*, Ecuador, páramo; (e) *S. superandinus*, Ecuador, superpáramo; (f) *Senecio nivalis*, Ecuador, superpáramo, (g) *S. pindilicensis*, Ecuador, montane forest; (h) *S. patens,* Ecuador, montane forest. Symbols are coloured according to species assignment to the main Structure clusters (see Figure 2); symbol shape indicates the growth form, i.e., square – basal rosette herb, circle – narrow-leaved subshrub, triangle – broad-leaved liana.

Phylogenetic molecular studies of the tribe *Senecioneae* suggest that the traditionally recognized Andean genera *Lasiocephalus* and *Culcitium* Bonpl. (scapose herbs forming basal leaf rosettes) belong to *Senecio* (Pelser et al., 2007, 2010). Our previous study of 13 *Senecio* species from the former *Lasiocephalus*, which all were diploid, based on nuclear DNA sequences (ITS region) and nuclear genome size data (Dušková et al., 2010), identified two major clades that largely correspond to the two habitat types, i.e., montane forest and páramo. The results also suggested that *Senecio* (*Culcitium*) *nivalis* (Kunth) Cuatrec. (Figure 1f) was closer to species of former *Lasiocephalus* than to other taxa of former *Culcitium*.

Given its likely origin within ca. the last 2 Myr (Pelser et al., 2010) and occurrence in the montane-alpine habitats, the former *Lasiocephalus* exemplifies recent plant radiation in the (sub)tropical Andes. Based on extensive population sampling throughout the northern Andes and using an extended sample of ITS sequences complemented with highly variable AFLP (amplified fragment length polymorphism) markers, here we present deeper insights into the relationships among the Andean species of *Senecio* formerly classified in *Lasiocephalus*. Specifically, we examine a hypothesis put forward by Dušková et al. (2010) that independent transitions between the montane forest and páramo habitats occurred that were accompanied by growth form changes. We further examine patterns of genetic diversity within the group, and particularly their correlation with environmental factors and Andean geography.

Materials and Methods

Plant material

Samples of species from the former *Lasiocephalus* and former *Culcitium*, along with co-occurring species of *Senecio*, were collected during 2006–2010 in Bolivia, Ecuador, Venezuela, and Colombia (Appendix S1). Due to the sampling gap in the central Andes, we lacked the single Peruvian species of former *Lasiocephalus*, a broad-leaved liana *S. loeseneri* Hieron. This species is, nevertheless, sometimes considered conspecific with *S. campanulatus* Sch. Bip. ex Klatt from Bolivia (Calvo & Freire, 2016), which was included in our study. Multiple populations were sampled for most of the species throughout their distribution ranges (Figure 2b). At each locality, geographical coordinates and elevation were recorded. Young, intact leaves were collected and desiccated in silica gel; vouchers were deposited in COL, PRC, QCA, QCNE, and VEN.

AFLP fingerprinting and DNA sequencing

In total, 356 accessions of 18 *Senecio* species formerly classified as *Lasiocephalus* and 18 accessions of *Senecio nivalis* were genotyped using AFLP fingerprinting (Vos et al., 1995) (see Appendix S2 for details on the protocol). Fragments were manually scored with genemarker version 1.80 (SoftGenetics). Only unambiguous fragments in the range of 60-500 bp. were scored, regardless of their intensity (Tribsch et al., 2002). For 5% of the samples, the whole AFLP protocol was repeated from the isolated DNA onwards to test the reproducibility of the method (Bonin et al., 2004). Internal transcribed spacer (ITS) regions were directly sequenced using the primers ITS4 and ITS5 (White et al., 1990) for 50 individuals of Andean *Senecio* (i.e., 44 of former *Lasiocephalus*, two of former *Culcitium*, four other members of *Senecio*). We selected the individuals in order to representatively cover all species of the former *Lasiocephalus* as well as all clusters and subgroups identified by AFLPs.

Clustering of AFLP data

Genetic structure was inferred using a Bayesian clustering method implemented in structure 2.2.3 (Falush et al., 2007) employing a recessive allele model with admixture, assuming independent allele frequencies with 1100000 MCMC (Markov chain Monte Carlo) generations, and discarding the first 100000 generations as burn-in. We limited the number of clusters (K) to 1 to 10, each K was replicated with ten runs, and we further assessed stability of the results by calculating similarity coefficients between the replicate runs (Nordborg et al., 2005) and delta K (Evanno et al., 2005), both calculated using the R-script Structure-sum-2009 (Ehrich, 2006). The Ks with consistent results over ten repeats were considered to be plausible and further examined. Since the analysis of the entire dataset showed that only runs with K = 3 converged to a consistent solution in ten repeats, subsequent, separate structure analyses of each of these three partitions (hereafter named clusters A, B, and C) were conducted using the same parameters. Only individuals assigned to a particular cluster with posterior probability > 0.9 in the initial analysis were included in these subsequent analyses. Major trends in the AFLP variation were visualized using principal coordinate analyses (PCoA) based on Jaccard inter-individual distances computed using famd 1.31 (Schlüter & Harris, 2006).

We further investigated the relationships among the major clusters based on a reduced subset of 266 individuals that were identified as non-admixed (i.e., with posterior probabilities of membership to both major clusters and subgroups > 0.9) in the structure analyses. We reconstructed phylogenetic relationships using a likelihood model for binary restriction site data implemented in MrBayes v 3.2.5 (Ronquist & Huelsenbeck, 2003). This model approximates the gain and loss of fragments by setting a condition that the characters that are absent (i.e., 0) in all individuals cannot be observed. We performed two independent runs of 5000000 generations each using the default prior settings, setting the restriction site model (lset nst=1 coding=noabsencesites) and discarding the first 25% generations as burn-in.

DNA sequence analyses

Sequences of the ITS region were aligned by Mafft 7 (Katoh & Standley, 2013) and edited using AliView (Larsson, 2014). In addition, we included in the final matrix previously published ITS sequences of: (i) 11 directly sequenced accessions of other Andean *Senecio* (Pelser et al., 2007) and (ii) 26 cloned individuals from the former *Lasiocephalus* (10–12 colonies per accessions were cloned; putative PCR errors and potential chimaeric sequences were removed previously by Dušková et al., 2010; no excessively long branches indicating non-functional copies were found). To reduce the number of phylogenetically uninformative tip branches (and number of pseudoreplicates for trait mapping analyses), we collapsed the cloned sequences from each individual to a consensus and intra-individual polymorphisms were coded using IUPAC (International Union of Pure and Applied Chemistry) ambiguity codes in cases where clones from single species formed a monophyletic cluster or fell within an unresolved polytomy. The single exception with highly divergent haplotypes was 88_Pi, in which the two divergent haplotypes were retained as separate accessions when constructing the tree. We performed phylogenetic analysis on the resulting matrix of 630 characters and 87 individuals using both maximum parsimony (in paup v4.0b10; Swofford, 2002, treating gaps as characters) and Bayesian analyses (in MrBayes v3.2.2; Ronquist & Huelsenbeck, 2003). The most parsimonious trees were searched heuristically with 1,000 replicates of random

sequence addition, tree bisection reconnection swapping and MulTrees on and the dataset was bootstrapped using 1000 replicates. In Bayesian analyses, we applied the generalised time reversible (GTR) substitution model (as selected by the Bayesian Information Criterion in JModeltest 2; Darriba et al., 2012) with gamma distribution of rate heterogeneity and simultaneously ran two MCMCMC runs with four chains each for 2000000 generations, sampling every 1000th generation using the default priors. The posterior probability of the phylogeny and its branches was determined from the combined set of trees, discarding the first 25% of trees as burn-in.

Growth form evolution

Character state reconstructions of the growth forms were performed employing a maximum likelihood approach implemented in the function rayDISC, part of the package corHMM (Beaulieu et al., 2013) in R (Ihaka & Gentleman, 1996). This method allows for reconstructions of multistate characters, unresolved nodes, and ambiguities (polymorphic taxa or missing data). Three models of character evolution were evaluated: equal rates (ER), symmetrical (SYM), and all rates different (ARD), and an Akaike information criterion corrected for sample size (AICc) was used to select the best fitting model. Association of growth forms and phylogeny was tested by computing Pagel´s lambda (Freckelton et al., 2002) using the function fitDiscrete in the package geiger (Pennel et al., 2014) in R (Ihaka & Gentleman, 1996). Statistical significance of estimated lambda was tested by computing likelihood ratio test (LRT) against lambda=0 model.

Geographical analyses of AFLP data

Geographical correlates of the genetic (AFLP) variation were examined after the admixed (i.e., posterior probability < 0.9) and Bolivian samples were excluded to avoid bias due to unclear cluster assignment and sampling gap, respectively. We tested for a significant correlation between matrices of genetic and geographical distances among populations (isolation by distance) using a Mantel test in *adegenet*. Among-population genetic chord distances derived from AFLP fragment frequencies were inferred using a Bayesian method with non-uniform priors (Zhivotovsky, 1999) as implemented in famd 1.31 (Schlüter & Harris, 2006).

Climatic data describing mean annual temperature, daily and annual temperature ranges, annual rainfall and its inter-annual variation expressed as coefficient of variation for each collection site were extracted from the WorldClim database (Hijmans et al., 2005). Those data together with elevation formed a group of environmental variables, while site latitude and longitude represented geographical variables. Variance of the AFLP data matrix was partitioned into environmental and geographical components (and their interaction) by a series of redundancy analyses (RDA) and partial RDA ordinations (Borcard et al., 1992) employing Canoco for Windows 4.5 (ter Braak & Šmilauer, 1998). Since the RDA employs Euclidean distance to measure dissimilarity between pairs of samples (Šmilauer & Lepš, 2014), this makes it analogous to analysis of molecular variance (AMOVA; Excoffier et al., 1992) but provides an opportunity to make partial tests to discriminate between pure effects of explanatory variables and their interaction.

Results

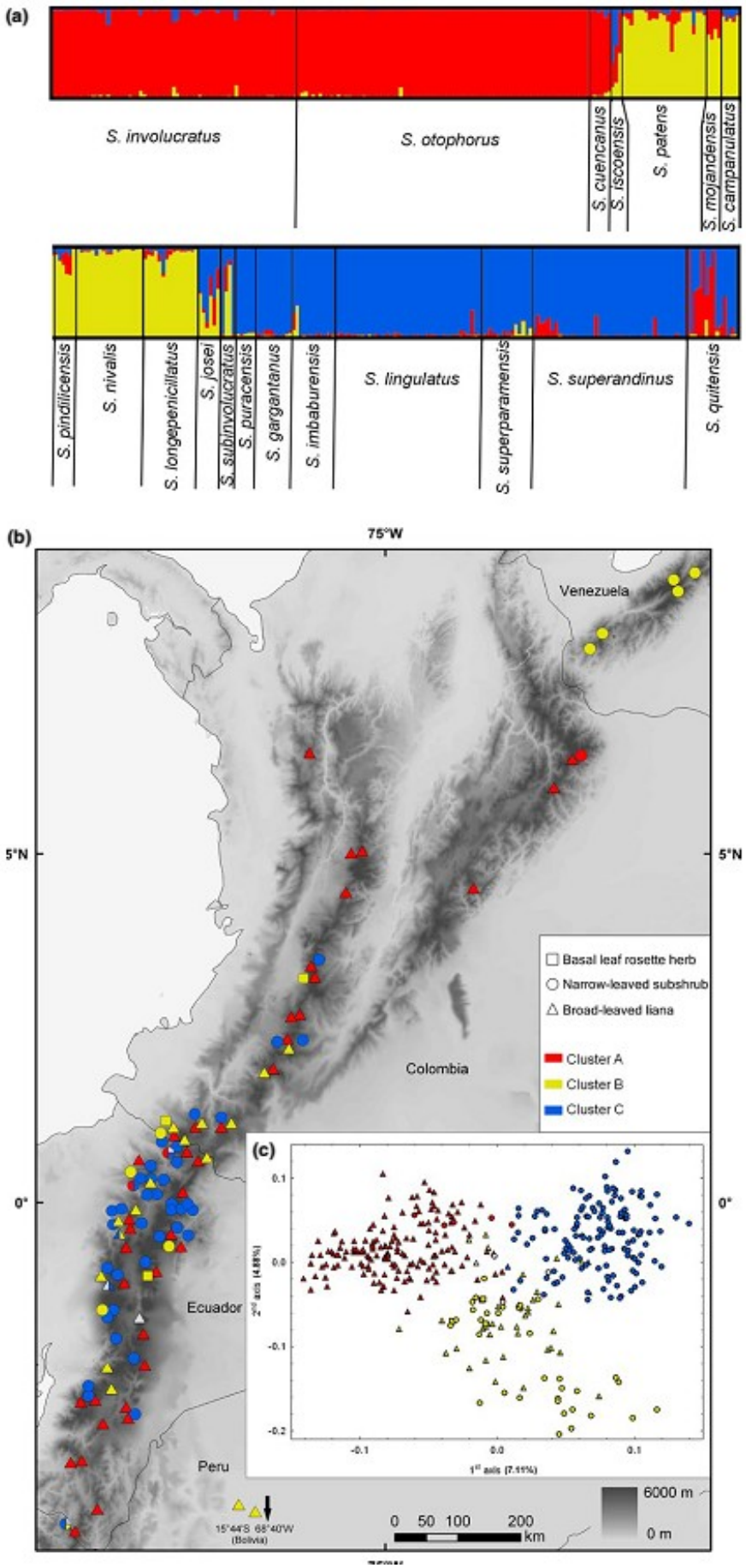
AFLP fingerprinting

AFLP analysis of 374 accessions resulted in 269 reliable fragments, of which 264 (98%) were polymorphic. The overall reproducibility of the dataset was 95%.

Main grouping within the entire dataset

Bayesian clustering of the entire dataset yielded the highest values of ΔK and among-replicate similarity (1.0) for partition into three clusters A, B, and C (Figure 2a, Appendix S3A). Cluster A contained mostly lianas of montane forest and forest–páramo ecotone, one of which, however, also formed a morphologically distinct subshrub-like high-elevation population (pop. 51). Cluster B was morphologically variable, encompassing montane forest lianas, narrow-leaved páramo subshrubs, and a basal rosette herb. Cluster C contained exclusively narrow-leaved (super)páramo subshrubs (Figure 2b, Table 1). Four species comprised mostly individuals that were admixed either between all three clusters (*S. josei* Sklenář, *S. iscoensis* Hieron.), between clusters A and C (*S.* aff. *quitensis*), or between clusters B and C (*S. subinvolucratus* Cuatrec.), although certain admixture was also found in some individuals of *S. patens* (Kunth) DC., *S. mojandensis*, *S. pindilicensis* Hieron., *S. longepenicillatus* Sch. Bip. ex Sandwith, *S. imbaburensis* Sklenář & Marhold, *S. lingulatus* (Schltdl.) Cuatrec., and *S. superandinus* Cuatrec.. The Bayesian clustering was also reflected in PCoA ordination, separating the three clusters along the first (cluster A vs. C) and second (cluster B vs. A+C) axes (Figure 2c).

Figure 2. (a) Assignment of 374 individuals (entire dataset) of high-elevation Andean *Senecio* into three main AFLP clusters inferred in STRUCTURE; (b) geographical locations of populations with growth form and STRUCTURE cluster assignment indicated; (c) Ordination of AFLP phenotypes by use of principal coordinate analysis (PCoA) based on Jaccard distances. The symbol colouration reflects the assignment of the individuals to the main STRUCTURE clusters (white – admixed individuals with assignment probability below 0.5); symbol shape indicates the growth form, i.e., square – basal rosette herb, circle – narrow-leaved subshrub, triangle – broad-leaved liana.


(a)
S. involucratus
S. otophorus
S. cuencanus
S. iscoensis
S. patens
S. mojandensis
S. campanulatus
S. pindilicensis
S. nivalis
S. longepenicillatus
S. josei
S. subinvolucratus
S. puracensis
S. gargantanus
S. imbaburensis
S. lingulatus
S. superparamensis
S. superandinus
S. quitensis
(b)
75°W
5°N
0°
Venezuela
Colombia
Ecuador
Peru
Basal leaf rosette herb
Narrow-leaved subshrub
Broad-leaved liana
Cluster A
Cluster B
Cluster C
(c)
15°44'S 68°40'W
(Bolivia)
0 50 100 200
km
6000 m
0 m

Finer structure within the main clusters

Separate Bayesian clustering of the accessions assigned to cluster A revealed that K = 2 and K = 3 exhibited high similarity among independent runs (> 0.998 in both partitions, although the former had higher ΔK; Appendix S3A), and the finer structuring was plotted onto the map (Figure 3b). With K = 2, *S. involucratus* and *S. cuencanus* Hieron. were classified in the first subgroup, although most of their accessions were admixed, while most accessions of *S. otophorus* (excluding those from southern Ecuador) fell into the second subgroup (Appendix S3B). With K = 3, two subgroups partly corresponded to species limits, namely (i) a subgroup of Colombian and north Ecuadorian populations of *S. involucratus* (Kunth) DC. (along with two *S.* aff. *quitensis* Cuatrec. accessions) and (ii) a subgroup of Colombian to central Ecuadorian accessions of *S. otophorus*. The third subgroup comprised all populations from southern Ecuador disregarding species identity (*S. involucratus, S. cuencanus*, and *S. otophorus*) along with populations of *S. involucratus* from northern and central Ecuador (Figure 3a, b). The PCoA of cluster A confirmed the Bayesian grouping, separating Colombian to central Ecuadorian populations of *S. otophorus* along the first axis and Colombian to north Ecuadorian populations of *S. involucratus* along the second axis (Figure 3c).

Separate analysis of cluster B yielded the same similarity coefficient, 1.0, for K = 2, 3, 4, and 5, although K = 3 had the highest ΔK (Appendix S3A). The finest partitioning (K = 5) separated all five species with almost no admixture (Figure 4a), whereas *S. patens* and *S. pindilicensis* merged at K = 4, and *S. campanulatus* and *S. longepenicillatus* joined this subgroup at K = 3 and K = 2, respectively, leaving *Senecio nivalis* apart from all other species at K = 2 (Appendix S3C). The PCoA of cluster B (Figure 4c) separated *S. nivalis* and *S. patens* along the first two ordination axes, whereas the third and fourth axes separated *S. campanulatus* and *S. pindilicensis*, respectively.

Figure 3. Genetic structure and geographical distribution of 149 individuals of high-elevation Andean *Senecio* from cluster A. (a) Posterior probabilities for membership of each individual in the three resulting subgroups (designated by different colours) as identified in a separate STRUCTURE analysis of cluster A members. (b) Geographical distribution of the analyzed populations. (c) Ordination of AFLP phenotypes (PCoA); symbol colour refers to the STRUCTURE subgroups (>0.5 posterior probability), symbol shape indicates species.

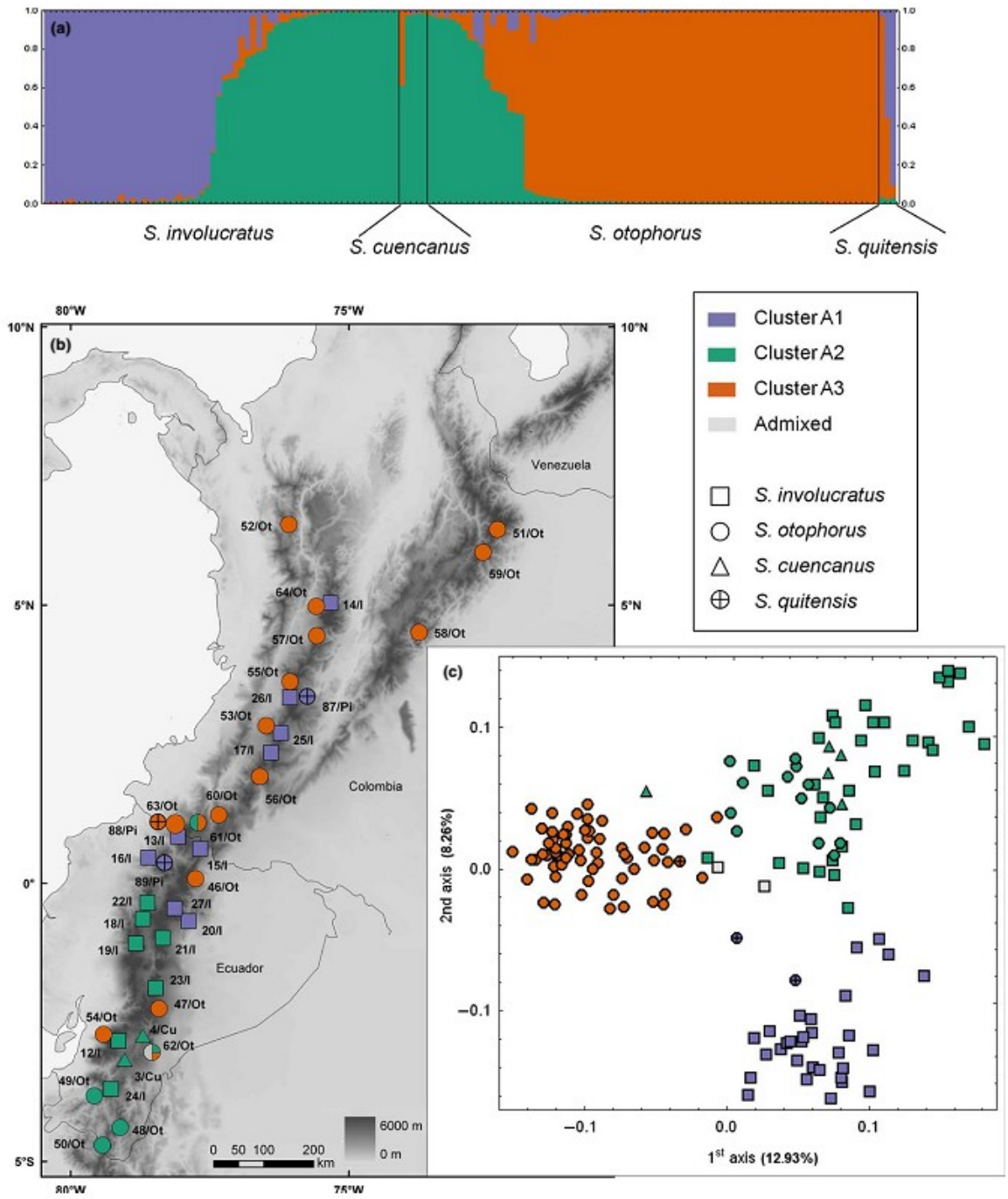
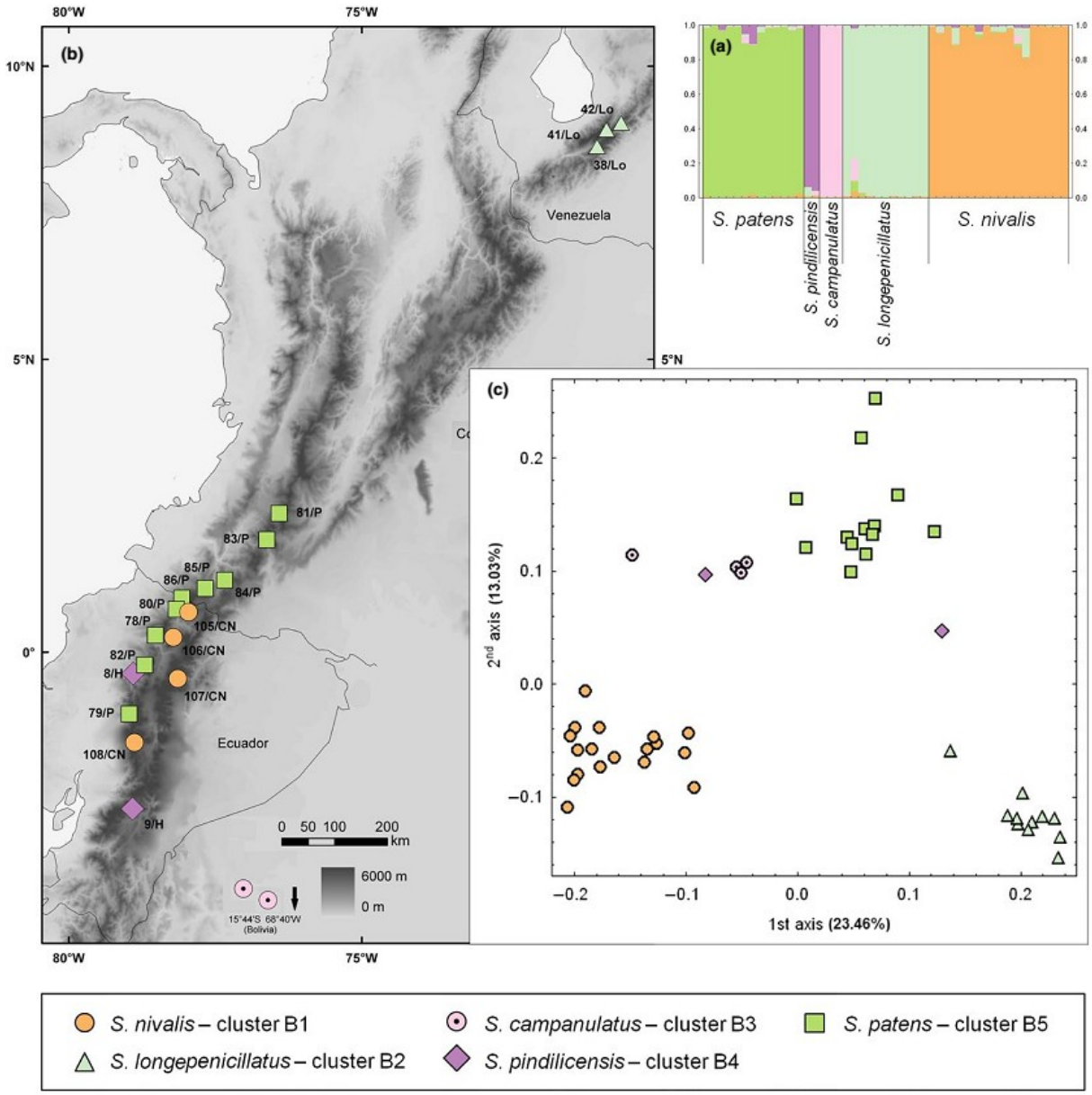
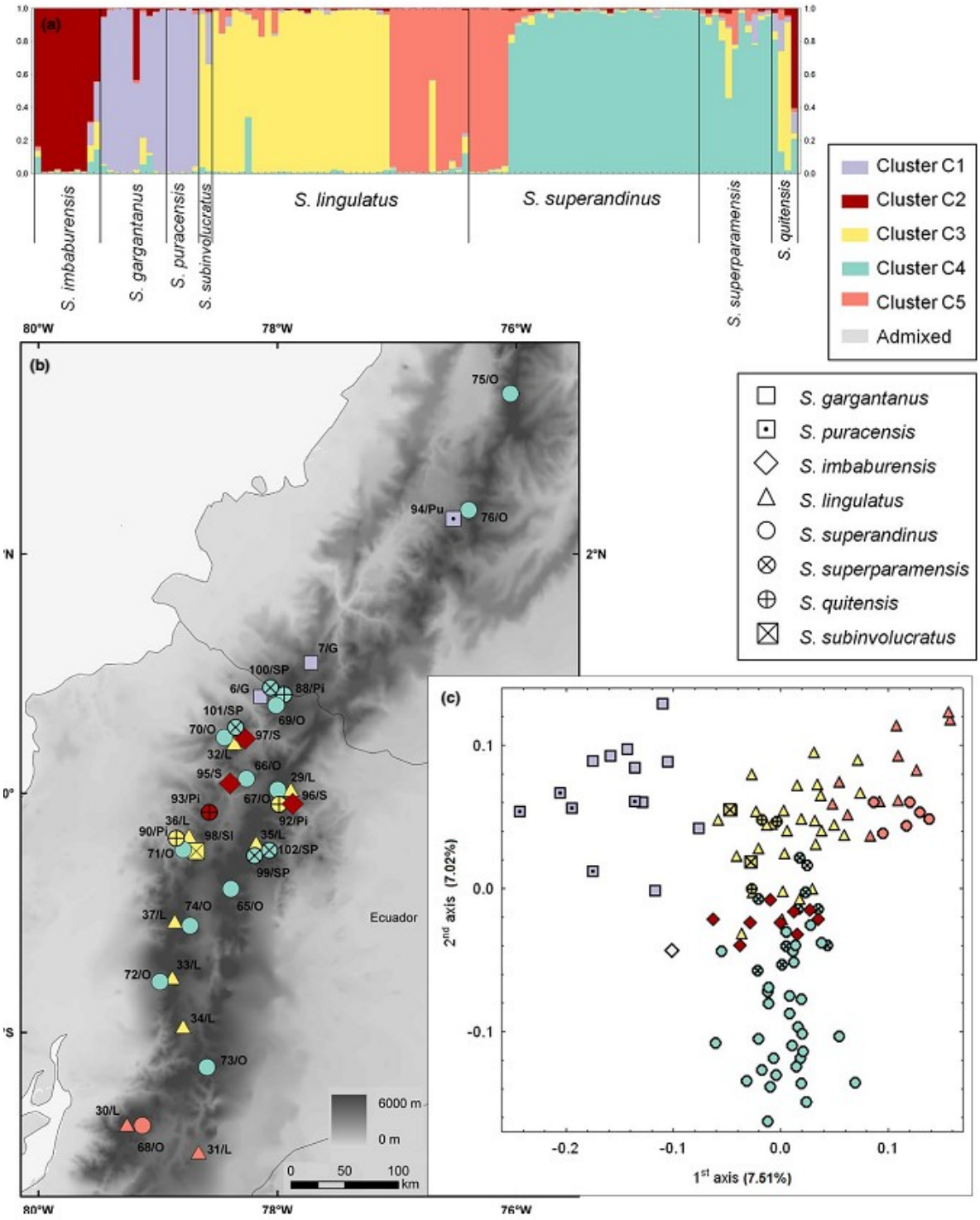


Figure 4. Genetic structure and geographical distribution of 47 individuals of high-elevation Andean *Senecio* from cluster B. (a) Posterior probabilities for membership of each individual in the five resulting subgroups (designated by different colours) as identified in a separate STRUCTURE analysis of cluster B members. (b) Geographical distribution of the analyzed populations. (c) Ordination of AFLP phenotypes (PCoA); symbol colour refers to the STRUCTURE subgroups (>0.5 posterior probability), symbol shape indicates species.



Analysis of cluster C showed the highest ΔK and similarity coefficient (0.995) to be yielded by K = 5 (Appendix S3A). The five subgroups comprised: (i) *S. puracensis* (Cuatrec.) Cuatrec. + *S. gargantanus* (Cuatrec.) Cuatrec.; (ii) *S. imbaburensis*; (iii) *S. lingulatus* (except for southern Ecuador) + *S. subinvolucratus*; (iv) *S. superandinus* (except for southern Ecuador) + *S. superparamensis* Sklenář, (v) southern Ecuadorian populations of *S. superandinus* and *S. lingulatus* (Figure 5a, b). *Senecio* aff. *quitensis*, was highly admixed and was scattered among three subgroups. The PCoA ordination diagram showed incomplete discrimination of the species (Figure 5c), but its first and second axes, respectively, suggested separation of the *S. gargantanus* + *S. puracensis* group and supported the distinct position of the southern Ecuadorian populations of *S. superandinus* and *S. lingulatus*.

Figure 5. Genetic structure and geographical distribution of 116 individuals of high-elevation Andean *Senecio* from cluster C. (a) Posterior probabilities for membership of each individual in the five resulting subgroups (designated by different colours) as identified in a separate structure analysis of cluster C members. (b) Geographical distribution of the analyzed populations. (c) Ordination of AFLP phenotypes (PCoA); symbol colour refers to the structure subgroups (>0.5 posterior probability), symbol shape indicates species.



ITS and AFLP phylogeny

Bayesian analysis of ITS sequences showed monophyly of a clade comprising all accessions of the former *Lasiocephalus* and former *Culcitium* (together with *Senecio chionogeton*), although it did not support separation of the two former genera (Figure 6a). Instead, along with several unresolved former *Culcitium* accessions, we identified two clades, corresponding to 'páramo' and 'forest' clades of Dušková et al. (2010), which with a few exceptions corresponded to major AFLP clusters C and A+B, respectively (Figure 6a, b, Table 1). The 'forest clade' further split into several well supported subclades (with uncertain relationships among them) which mostly corresponded to AFLP subgroups (namely B2, B3+B4, B5, A1+A3, A2+A3 subgroups). While the 'páramo clade' comprised narrow-leaved subshrubs and one basal rosette herb, the 'forest clade' contained representatives of all three growth forms. Broad-leaved liana is reconstructed as the ancestral state within the 'forest clade', which contains one subclade (f4) formed by lianas only, three subclades (f1, f5, f6) comprising a liana and a narrow-leaved subshrub, one subclade (f3) with a narrow-leaved subshrub (*S. longepenicillatus*), and one subclade (f2) comprising a narrow-leaved subshrub and a basal rosette herb (Table 1).

There were several remarkable incongruences among ITS and AFLP data. In particular, *Senecio nivalis* (cluster B1) shared the same ITS haplotypes with *S. superparamensis* (cluster C4) and both species formed a supported lineage (p1) within the 'páramo' clade (Figure 6a). AFLP cluster C1 was split into both major ITS clades, with the Colombian and Ecuadorian accessions being parts of the 'forest' and 'páramo' clades, respectively. ITS clones isolated from a single accession of *S.* aff. *quitensis* (pop. 88_Pi) fell into both major ITS clades. Finally, *S. puracensis* from 'páramo' cluster C appears nested within the ITS 'forest clade'.

Bayesian phylogenetic analysis of AFLP phenotypes of non-admixed individuals confirmed monophyly (> 90% posterior probability) of most of the AFLP subgroups. However, it failed to provide support for relationships among the AFLP subgroups, except for supported monophyly of cluster A (Figure 6b, Appendix S3D).

Growth form evolution

Equal rates (ER) model had the lowest AICc (94.24), compared to SYM (97.77) and ARD (107.13) models. The ER model reconstructed the basal rosette herb as the ancestral growth form for the *Lasiocephalus*-*Culcitium* species group (Figure 6a, Appendix S3E). The herbs switched to broad-leaved lianas in the 'forest clade', with further changes to narrow-leaved subshrub (*S. longepenicillatus*, *S. puracensis*) and a reversal to a basal rosette herb (*S. mojandensis*). The growth form of narrow-leaved subshrub is present in all species of the 'páramo clade' except for *S. cocuyanus* (basal rosette herb) and *S.* aff. *quitensis* (varying between broad-leaved liana and narrow-leaved subshrub). The growth form was strongly associated with phylogeny (Pagel´s lambda = 0.89, ln(lambda) = –75.63, ln(lambda=0) = –150.38, LRT p-value < 0.001).

Figure 6. (a) Phylogenetic reconstruction of 87 accessions of northern Andean *Senecio* based on sequences of ITS region of ribosomal DNA. Bayesian 50% majority rule consensus tree with posterior probabilities > 0.90 and bootstrap values > 50 % inferred with maximum parsimony are indicated, respectively, before and after the slash above each supported branch. Supported subclades of the 'forest' and 'páramo' clades are marked as f1–f6 and p1–p2, respectively. Growth form of each accession is marked by a symbol, membership in the AFLP subgroups (if applicable) is denoted by corresponding letters (A1–C5), accessions with ambiguous structure assignment are marked 'MIX'. Presence of highly divergent ITS sequences in the same individual of *S.* aff. *quitensis* is marked by an arrow. *Senecio doryphyllus*, *S. decipiens* and *S. alatopetiolatus*, although belonging to the former *Lasiocephalus*, were not analyzed using the AFLPs. Reconstruction of the growth form evolution according to the equal rates (ER) model has been superimposed onto the ITS tree (see Appendix S3E for original). (b) Relationships among AFLP phenotypes of 266 non-admixed (see Methods) individuals of former *Lasiocephalus* and *Senecio nivalis* reconstructed in Bayesian framework. Cluster codes correspond with Figures 3–5; branches with posterior probabilities >0.95 are marked with dots.

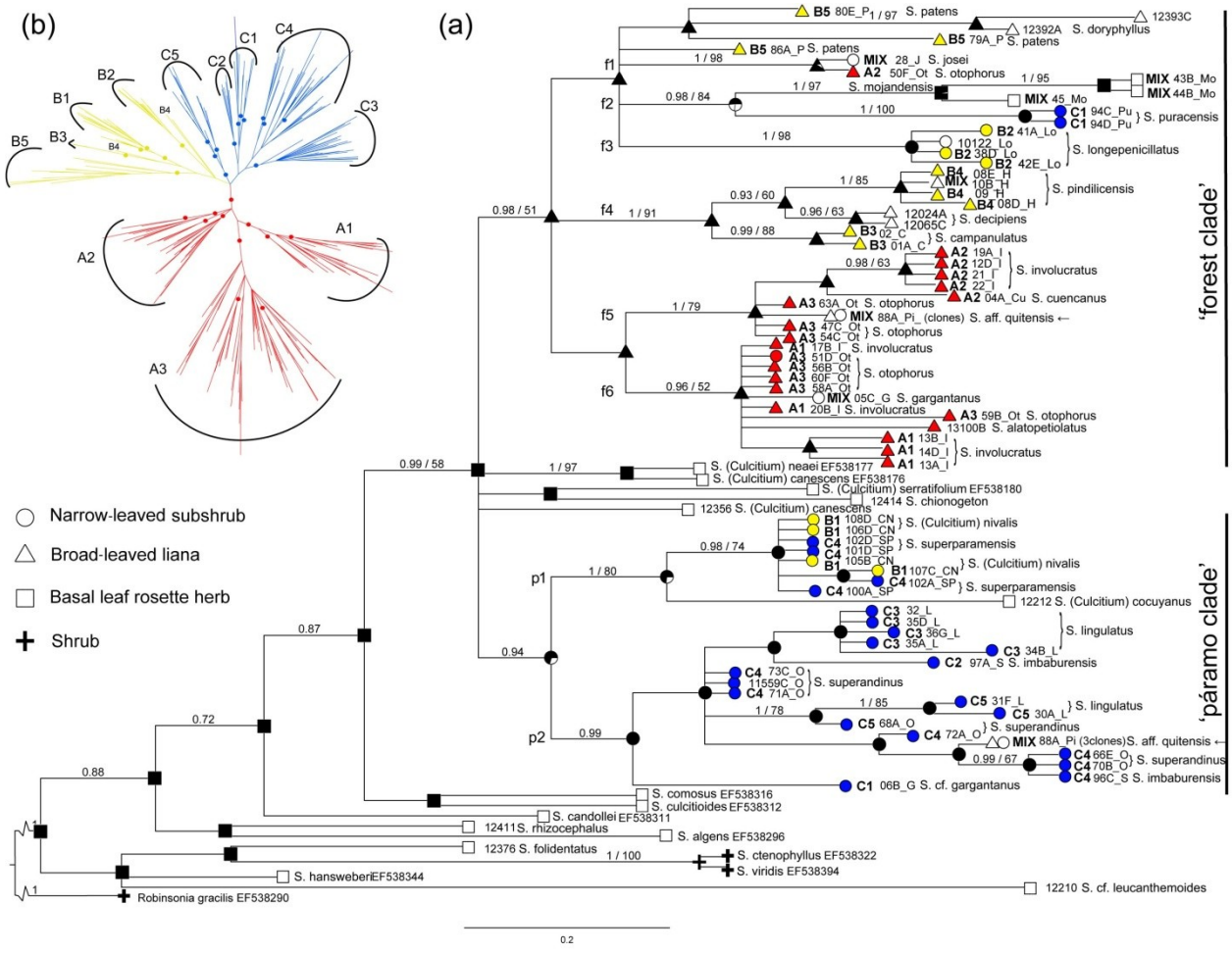


Table 1. Summary of the associations between growth forms (uppercase letters) and genetic relationships reconstructed by the two sets of molecular markers (AFLPs, ITS sequences) in high-elevation north Andean *Senecio* (see Figures 2–5 for AFLP (sub)groups and Figure 6a for ITS (sub)clades delimitation). B – basal rosette herb, mostly from lower páramo; L – broad-leaved liana, from montane forest and forest-páramo ecotone; N – narrow-leaved subshrub, from páramo to superpáramo. Others refer to admixed samples with equivocal assignment to AFLP (sub)groups and accessions not genotyped by AFLPs (see Appendix S1).

	Páramo ITS clade		Forest ITS clade					
	p1	p2	f1	f2	f3	f4	f5	f6
AFLP cluster A								
A1								L
A2							L	
A3							L	N, L
AFLP cluster B								
B1	N							
B2					N			
B3						L		
B4						L		
B5			L					
AFLP cluster C								
C1		N		N				
C2		N						
C3	N	N						
C4	N	N						
C5		N						
Others	B	N, L		B		L	N, L	N

Geographical and environmental analyses of AFLP data

Senecio populations as a whole and members of cluster A showed a very weak correlation between genetic and geographical distances (isolation by distance, IBD), whereas this relationship was non-significant in the other two clusters (Table 2). Subgroups with sufficient numbers of populations were available only in cluster A; here we observed significant correlations in both Ecuadorian-Colombian subgroups A1 and A2 but a lack of correlation in the southern Ecuadorian subgroup A2.

Less than 10% of total variance in the entire AFLP dataset was accounted for by the effects of either environmental (rainfall, temperature, elevation; altogether 6% of variability) geographical (latitude, longitude; 2% of variability) components or their interaction (1.5% of variability) (Table 2). When the three AFLP clusters were analyzed separately, the geographical component accounted for a similar (5–8%) proportion of the total variation, whereas the environmental component accounted for almost a third of variation in cluster B but only 8–9% in clusters A and C. Moreover, there was a distinct interaction (5%) between the two sets of variables in cluster A, whereas the interaction was very low or lacking in the two other clusters.

Table 2. Eco-geographical covariates of genetic variation of north-Andean Senecio. Variance partitioning (by means of RDA) of AFLP genetic variation into environmental (rainfall, temperature, elevation) and geographical (latitude, longitude) components and correlation between genetic and geographical distances (by means of Mantel test and quantified by correlation coefficient r_M). The partitioning was based on characteristics of the original collection sites after genetically admixed individuals and spatially remote Bolivian samples were excluded; all RDA ordinations were significant at $p = 0.002$ under 499 Monte Carlo permutations.

	Entire dataset	Cluster A	Cluster B	Cluster C
Variance partitioning (RDA)				
Environment	5.8%	7.8%	31.2%	8.8%
Environment[a] geography	1.5%	5.2%	0%	1.1%
Geography	2.2%	6%	7.7%	5.2%
Residual	90.5%	81%	61.1%	84.9%
Isolation by distance (Mantel test)	$r_M = .12$, $p = .04$	$r_M = .12$, $p = .04$[a]	n.s.	n.s.

[a]Subgroups, A1: $r_M = .44$, $p = .02$; A2: n.s.; A3: $r_M = .31$, $p = .03$.

Discussion

Lasiocephalus-Culcitium species group

Pelser et al. (2007, 2010) and Dušková et al. (2010) pointed to close relationships between the former genera of *Lasiocephalus* and *Culcitium*. The present study, using ITS sequences and an extended list of species, suggests monophyly of the *Lasiocephalus*-*Culcitium* species group but with neither of the two former genera monophyletic. Although relationships within the group are only partly resolved in both the ITS and AFLP datasets, suggesting a recent diversification (Turner et al., 2013), there is a partial congruence between the two markers, since AFLP cluster C corresponds to the ITS 'páramo clade' (except for *Senecio nivalis*) and AFLP clusters A and B correspond to the ITS 'forest clade' (except for *S. puracensis*) (Figure 6, Table 1); incongruences will be discussed below.

Growth form changes and habitat shifts

Species of different growth forms and preferences for páramo or montane forest fell within several different AFLP (sub)groups and ITS (sub)clades, suggesting that independent shifts in ecology were accompanied by changes in morphology. Both AFLP and ITS data indicate that at least two distinct genetic entities occur in the páramo, representatives of which demonstrate convergence in such traits as growth form, size and number of capitula, and leaf morphology (Figure 1). The first entity is the páramo-dwelling AFLP cluster C (largely corresponding to the ITS 'páramo clade'), species of which occur throughout most of Ecuador and southern Colombia. The second entity is represented by Venezuelan *S. longepenicillatus* (AFLP cluster B, f3 subclade within the ITS 'forest clade'), which is a narrow-leaved páramo subshrub but

sporadically also appears in a broad-leaved form at the tree-line ecotone. In addition, *S. otophorus* (AFLP cluster A, f6 subclade within the ITS 'forest clade'), which grows as a slender liana twining in subpáramo thickets, also occurs as a narrow-leaved subshrub in the superpáramo of Colombian Cordillera Oriental (Figure 1c), and similar habit variation is demonstrated by montane forest and superpáramo plants of *S. doryphyllus* Cuatrec. from Sierra Nevada de Santa Marta (northern Colombia). These findings suggest that convergent growth form evolved independently in various parts of the northern Andes, although the variation in *S. otophorus* and *S. doryphyllus* may only represent phenotypic plasticity.

Transitions in growth form associated with habitats at different elevations have been presented for various Andean plant groups. Whereas Lobeliaceae (Knox et al., 2008), *Huperzia* Bernh. (Wilkström et al., 1999), *Chusquea* Kunth (Fisher et al., 2009), and *Disterigma* (Klotzsch) Nied. (Pedraza-Peñalosa, 2009) apparently colonized alpine habitats from the montane forest, for *Chaetanthera* Ruiz & Pav. and *Puya* Molina migration was suggested in the opposite or both directions, respectively (Hershkovitz et al., 2006; Jabaily & Sytsma, 2012). Since a grade of herbaceous *Senecio* species from alpine habitats subtends (although the support is weak) the *Lasiocephalus*-*Culcitium* clade (Figure 6a), the ITS phylogeny is consistent with an alpine-to-forest transition for the evolution of the *Lasiocephalus*-*Culcitium* group as a whole. If such a relationship is confirmed, a change from the herbaceous (basal leaf rosette) to the woody (liana, ascending subshrub, shrub) state is implied, similar to, e.g., Andean *Valeriana* L., *Gentianella* Moench, and *Loricaria* Wedd. (Sklenář et. al., 2011; Kolář et. al., 2016). The polytomy consisting of the 'páramo clade', 'forest clade', and several basal rosette herbs does not permit interpretation of the growth form transitions within the *Lasiocephalus*-*Culcitium* clade to evaluate Cuatrecasas' (1978) idea that the páramo growth form of former *Lasiocephalus* species evolved from the growth form of their montane forest ancestor(s). However, Cuatrecasas' view could be valid for some páramo subshrubs found within the 'forest clade' (e.g., *S. longepenicillatus*). Moreover, the ITS phylogeny suggests another transition in this clade, i.e., to a basal rosette herb in *S. mojandensis*.

Role of hybridization

Gene flow is known to occur among closely related alpine species (Wagstaff & Garnock-Jones, 2000; Vargas, 2003; Winkworth et al., 2005), yet the role of hybridization in the evolution of the Andean flora has not been documented, except in the cases of *Polylepis* Rioz & Pav. (Schmidt-Lebuhn et al., 2006), *Hypochaeris* (Tremetsberger et al., 2006), and *Puya* (Jabaily & Sytsma, 2012). In agreement with frequent hybridization in the *Senecioneae* (Hodálová & Marhold, 1996; Lowe & Abbott, 2000; Kirk et al., 2004; Osborne et al., 2016), our molecular data, morphological observations, and previously published genome size values (Dušková et al., 2010) indicate that homoploid hybridization likely occurred among multiple species of former *Lasiocephalus*.

Traces of hybridization are indicated by consistently admixed AFLP profiles across multiple populations of several species (Figure 2a). This is especially apparent for *Senecio* aff. *quitensis*, a taxon with morphology varying between subshrubs and lianas and whose accessions variously combine AFLP profiles of clusters A (forest lianas) and C (páramo subshrubs). Furthermore, ITS sequences of this species (including divergent ITS copy types

from a single individual) are placed in the divergent 'páramo' and 'forest' clades, and its genome size is intermediate between them (Dušková et al., 2010).

Incongruence between the AFLP and ITS datasets suggests that hybridization might also have been involved in the origin of other Andean *Senecio* species. This was particularly documented for *Senecio superparamensis*, a species of intermediate morphology and genome size (Dušková et al., 2010; as "*L.* sp. 4" there) between *S. superandinus*, with which it was assigned to AFLP subgroup C4, and *S. nivalis*, with which it shares ITS sequences (Figure 6a). Such conflict may be explained by (past) gene flow of *S. nivalis* ITS haplotypes that was followed by rapid homogenization of the ITS sequences (Alvarez & Wendel, 2003) towards *S. nivalis*-like paralogues. A similar process might have led to 'deeper' incongruences in other species (*S. nivalis* and *S. puracensis*; Figure 6a) that also exhibit conflicts among the three major AFLP clusters vs. the two main ITS clades. Such indications of past hybridization events, however, should be interpreted with caution as AFLP data do not allow distinguishing admixture from incomplete lineage sorting.

Geographical and ecological correlates of genetic variation

Geographical barriers along with ecological differentiation promote species diversification in mountains (Kolář et al., 2016; Luo et al., 2016). Since a geographical signal was comparably strong in the three main AFLP clusters, geography may structure genetic variation in a similar way in both the Andean montane forest and the páramo. In support of this, the AFLP data reveal two strikingly similar cases of genetic separation corresponding to geography which are incongruent with morphology-based species limits. Two páramo species, *Senecio lingulatus* and *S. superandinus* (cluster C), are readily distinguished morphologically (Figure 1a, e) and are genetically distinct throughout most of Ecuador. Their populations in southern Ecuador, however, merge and form another distinct genetic subgroup (Figure 5a, b). Similarly, two morphologically distinct species from the montane forest-páramo ecotone, *S. involucratus* and *S. otophorus* (cluster A), appear as distinct AFLP entities in northern-central Ecuador and Colombia, but the markers fail to discriminate between them in southern Ecuador (Figure 3a, b). There, the species form a separate subgroup together with another morphologically distinct montane forest liana, *S. cuencanus*.

Since morphologically intermediate plants between *S. superandinus* and *S. lingulatus* occur in southern Ecuador, gene flow due to hybridization might have generated the observed pattern. In contrast, we did not observe any putative hybrids between *S. involucratus* and *S. otophorus*, nor did we find any consistent morphological distinction in plants of either species from southern Ecuador. Therefore, we hypothesize that southern Ecuador represents an ancestral area of cluster A where high levels of ancestral polymorphisms have been retained, preventing the genetic discrimination of species by means of the AFLPs. Both species might have independently migrated northwards, leaving a footprint of gradual genetic differentiation which is documented by a significant isolation by distance relationship observed in A1 and A3 subgroups. Such northward migration would be consistent with the biogeographical reconstructions of Andean plant groups such as *Azorella*, *Oreobolus* R. Br., and *Puya* (Andersson et al., 2006; Chacón et al., 2006; Jabaily & Sytsma, 2012). The northern and central Ecuadorian Andes experienced a different Quaternary history from the south of the country, namely in having volcanism and glaciation (Jørgensen & Ulloa, 1994). Glaciation

events and volcanism may have structured the genetic patterns of the species through the effects of repeated bottlenecks and founder events (Luo et al., 2016; Vásquez et al., 2016).

The genetic structure of species from the montane forest-páramo ecotone (cluster A) and páramo (cluster C) showed little association with environmental variables, which we acknowledge might be at least partly due to the lack of precision of extrapolated climatic variables for high mountains (Hijmans et al., 2005; Kirchheimer et al., 2016). However, in cluster B, the high proportion of genetic variation associated with environmental factors is consistent with the variety of habitats occupied by its species. The ecological differentiation coupled with high AFLP and morphological diversification may suggest a relatively long divergence time and/or efficient isolation (Kolář et al., 2016). The very small or entirely lacking association between environment and geography in clusters C and B, respectively, suggests that two distinct and (largely) independent signals are involved. However, the stronger association in cluster A suggests that migration along the cordilleras was coupled with a shift in species ecology, such as the entry of *S. otophorus* in the superpáramo in Colombia.

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Appendix S1. List of species with the most commonly used synonyms in the former genera *Lasiocephalus* and *Culcitium* and the localities with details and numbers of individuals analyzed using AFLPs and ITS; BL refers to broad-leaved, NL refers to narrow-leaved.

Senecio alatopetiolatus J. Calvo, E. Freire & Sklenář
Senecio campanulatus Sch. Bip. ex Klatt [*Lasiocephalus campanulatus (Sch. Bip. ex Klatt) Cuatrec.*]
Senecio canescens (Bonpl.) Cuatrec. [Culcitium canescens Bonpl.]
Senecio cocuyanus (Cuatrec.) Cuatrec [Culcitium cocuyanum Cuatrec.]
Senecio cuencanus Hieron. [*Lasiocephalus cuencanus (Hieron.) Cuatrec.*]
Senecio decipiens Benoist [Lasiocephalus decipiens (Benoist) Cuatrec.]
Senecio doryphyllus Cuatrec. [Lasiocephalus doryphyllus (Cuatrec.) Cuatrec.]
Senecio gargantanus (Cuatrec.) Cuatrec. [*Lasiocephalus gargantanus (Cuatrec.) Cuatrec.*]
Senecio imbaburensis Sklenář & Marhold [*Lasiocephalus sodiroi (Hieron.) Cuatrec.*]
Senecio involucratus (Kunth) DC. [*Lasiocephalus involucratus (Kunth) Cuatrec.*]
Senecio iscoensis Hieron.
Senecio josei Sklenář
Senecio lingulatus (Schltdl.) Cuatrec. [*Lasiocephalus lingulatus Schltdl.*]
Senecio longepenicillatus Schultz-Bip. ex Sandw. [*Lasiocephalus longipenicillatus (Schultz-Bip. ex Sandw.) Cuatrec.*]
Senecio mojandensis Hieron. [*Lasiocephalus mojandensis (Hieron.) Cuatrec.*]
Senecio nivalis Kunth [*Culcitium nivale* (Kunth) Cuatrec.]
Senecio otophorus Wedd. [*Lasiocephalus otophorus (Wedd.) Cuatrec.*]
Senecio patens (Kunth) DC. [*Lasiocephalus patens (Kunth) Cuatrec.*]
Senecio pindilicensis Hieron. [*Lasiocephalus heterophyllus (Turcz.) Cuatrec.*]
Senecio puracensis (Cuatrec.) Cuatrec. [*Lasiocephalus puracensis (Cuatrec.) Cuatrec.*]
Senecio quitensis Cuatrec. [*Lasiocephalus pichinchensis (Cuatrec.) Cuatrec.*]
Senecio subinvolucratus Cuatrec. [*Lasiocephalus subinvolucratus (Cuatrec.) Cuatrec.*]
Senecio superandinus Cuatrec. [*Lasiocephalus ovatus Schltdl.*]
Senecio superparamensis Sklenář

Species	Population code	Growth form	No. of AFLP samples	ITS sample	Locality	Latitude	Longitude	Collector(s)	Voucher number
S. campanulatus	1_C	BL liana	1	1	Bolivia: La Paz, east slopes of Sorata (3 300 m a.s.l.)	S 15.7703	W 68.6305	J. Macek	LC 1
S. campanulatus	2_C	BL liana	4	1	Bolivia: La Paz, north slopes of Sorata (3 300 m a.s.l.)	S 15.745	W 68.6703	J. Macek	LC3
S. cuencanus	3_Cu	BL liana	5	—	Ecuador: Azuay, along the road from Cuenca to the Tinajillas pass (3 400 m a.s.l.)	S 3.1711	W 79.0341	P. Sklenář & J. Karbulková	11128

S. cuencanus	4_Cu	BL liana	1	1	Ecuador: Azuay, along the road Gualaceo-Limon, montane forest and shrubby thickets (3 000 m a.s.l.)	S 2.9333	W 78.7001	P. Sklenář & J. Karbulková	11167
S. gargantanus	5_G	NL ascending subshrub	2	1	Colombia: Nariño, Volcan Galeras, road from Pasto to the crater (3 860 m a.s.l.)	N 1.2214	W 77.3447	P. Sklenář & E. Dušková	12391
S. cf. *gargantanus*	6_G	NL ascending subshrub	5	1	Ecuador: Carchi, Volcan Chiles, margin of the road Tufino - Maldonado, ca 100 m E of Laguna Verde (4 030 m a.s.l.)	N 0.8006	W 77.9358	P. Sklenář, E. Rejzková, F. Kolář	11511
S. gargantanus	7_G	NL ascending subshrub	5	—	Colombia: Nariño, Volcan Azufral, along the path from Tuquerres to Laguna Verde, ca 300 m E of the laguna (3 970 m a.s.l.)	N 1.0913	W 77.7154	F. Kolář	54
S. pindilicensis	8_H	BL liana	2	2	Ecuador: Pichincha, along the road Quito-Nono (3 250 m a.s.l.)	S 0.0963	W 78.5562	P. Sklenář & A. Kučerová	11101
S. pindilicensis	9_H	BL liana	1	1	Ecuador: Azuay, thickets along the road between Canad and Azogues (2 890 m a.s.l.)	S 2.6708	W 78.9061	P. Sklenář & A. Kučerová	11115
S. pindilicensis	10_H	BL liana	3	1	Ecuador: Chimborazo, along the road between Chunchi and Zhud, near Sta. Rosa (2 840 m a.s.l.)	S 2.3631	W 78.97	P. Sklenář & J. Karbulková	12001
S. involucratus	11_I	BL liana	2	—	Ecuador: Pichincha, along the road from San Juan towards the antennas de Atacazo (3 440 m a.s.l.)	S 0.2885	W 78.6247	P. Sklenář, A. Kučerová & P. Macek	11065
S. involucratus	12_I	BL liana	6	1	Ecuador: Azuay, along the road from Cuenca to Molleturo, in forest thickets (3 200 m a.s.l.)	S 2.8289	W 79.1363	P. Sklenář & J. Karbulková	11116
S. involucratus	13_I	BL liana	5	1	Ecuador: Carchi, Volcan Chiles, margin of the road Tufino - Maldonado, ca 1.5 km ESE of Laguna Verde (3 910 m a.s.l.)	N 0.7994	W 77.9153	P. Sklenář, E. Rejzková, F. Kolář	11504
S. involucratus	14_I	BL liana	3	2	Colombia: Caldas-Tolima, Los Nevados, paramo las Letras, along the road from Honda to Manizales, ca 2 km NE of Letras (3660 m a.s.l.)	N 5.038	W 75.3342	Fabio, P. Sklenář et al.	FAA 628
S. involucratus	15_I	BL liana	5	—	Ecuador: Sucumbios, Páramo El Mirador, ca 6 km to the east of Huaca (3700 m a.s.l.)	N 0.6163	W 77.6717	P. Sklenář, E. Rejzková, F. Kolář	11533

S. involucratus	16_l	BL liana	1	—	Ecuador: Imbabura, Volcan Cotacachi, left of the trail from the TV antennas towards the summit, near rocky outcrops (4 190 m a.s.l.)	N 0.3455	W 78.3442	P. Sklenář, E. Rejzková, F. Kolář	11539
S. involucratus	17_l	BL liana	5	1	Colombia: Cauca, Paramo de Purace, along the footpath from Pilimbala to Volcan Purace (3 817 m a.s.l.)	N 2.3474	W 76.3993	E. Rejzková, F. Kolář & D. Vasquez	39
S. involucratus	18_l	BL liana	5	—	Ecuador: Pichincha - Cotopaxi, Páramo de Iliniza, along the trail from the parking place towads the Ilinizas (4 290 m a.s.l.)	S 0.6428	W 78.6963	P. Sklenář, E. Rejzková, F. Kolář	11555
S. involucratus	19_l	BL liana	5	1	Ecuador: Cotopaxi, Páramo de Quispicacha, on both sides of the ridge of Puncungusacha, ca 10 km to the west of Quindigua (4 380 m a.s.l.)	S 1.0786	W 78.8317	P. Sklenář, E. Rejzková, F. Kolář	11566
S. involucratus	20_l	BL liana	2	1	Ecuador: Carchi, road from Laguna Voladero towards Tulcan (3 700 m a.s.l.)	S 0.6833	W 77.8833	P. Sklenář & J. Karbulková	11114
S. involucratus	21_l	BL liana	5	1	Ecuador: Napo, Road San Miguel de Salcedo-Tena (3 490 m a.s.l.)	S 0.9834	W 78.3397	P. Sklenář & A. Kučerová	11031
S. involucratus	22_l	BL liana	6	1	Ecuacor: Pichincha, Along the trail from the antennas towards the summit of Atacazo (4 190 m a.s.l.)	S 0.3513	W 78.6178	P. Sklenář, A. Kučerová & P. Macek	11067
S. involucratus	23_l	BL liana	2	—	Ecuador: Chimborazo, Volcan Altar, around Cerro Quilimas, margin of the road from Alao to the valley of Rio Alao, ca 6 km NE of Alao (3 260 m a.s.l.)	S 1.8848	W 78.4688	P Sklenář & E Rejzková	11595
S. involucratus	24_l	BL liana	4	—	Ecuador: Loja, Paramo de Fierro Urco, to the southwest of Saraguro (3 680 m a.s.l.)	S 3.695	W 79.3486	P. Sklenář, J. Macková & P. Macek	12015
S. involucratus	25_l	BL liana	4	—	Colombia: Cauca, Paramo de Moras road from Silvia to Mosoco, ridges between the road and Cerro de Penas Blancas (3 500 m a.s.l.)	N 2.71	W 76.2167	P. Sklenář	12266
S. involucratus	26_l	BL liana	2	—	Colombia: Valle de Cauca, Paramo de Tinajas, Cordillera Central, road from Florida towards the mountain pass (3 790 m a.s.l.)	N 3.3393	W 76.0636	P. Sklenář & D. Vasquez	12271
S. involucratus	27_l	BL liana	5	—	Ecuador: Napo, Paramo de Antisana, NE side of the mountain (4	S 0.4517	W 78.1256	P. Sklenář	10014

					150 m a.s.l.)				
S. josei	28_J	NL ascending subshrub	6	1	Ecuador: Loja, Cordillera las Lagunillas (de Sabanilla), Páramo de las Lagunas Negras (3 330 m a.s.l.)	S 4.7106	W 79.4367	P. Sklenář, J. Macková & P. Macek	12027
S. lingulatus	29_L	NL ascending subshrub	2	—	Ecuador: Pichincha, grass páramo on the southern side of Cayambe, along the road towards the refugio (3 900 m a.s.l.)	S 0.0242	W 78.0519	P. Sklenář & A. Kučerová	11080
S. lingulatus	30_L	NL ascending subshrub	6	1	Ecuador: Azuay, superpáramo vegetation to the N from the pass of the road Cuenca-Molleturo (4 300 m a.s.l.)	S 2.7696	W 79.2433	P. Sklenář & J. Karbulková	11121
S. lingulatus	31_L	NL ascending subshrub	6	1	Ecuador: Morona Santiago, Mountain pass of the road Gualaceo-Limon, along the way from the pass towards the antennas (3 470 m a.s.l.)	S 3.0033	W 78.6614	P. Sklenář & J. Karbulková	11162
S. lingulatus	32_L	NL ascending subshrub	3	1	Ecuador: Imbabura, Volcan Cotacachi, margin of the 4 WD road from Laguna Cuicocha north to the TV antennas, near the antennas (4 010 m a.s.l.)	N 0.3323	W 78.3389	P. Sklenář, E. Rejzková, F. Kolář	11538
S. lingulatus	33_L	NL ascending subshrub	5	—	Ecuador: Chimborazo, páramo to the south of Chimborazo (4 270 m a.s.l.)	S 1.5355	W 78.8809	P. Sklenář & A. Kučerová	11035
S. lingulatus	34_L	NL ascending subshrub	5	1	Ecuador: Chimborazo, Páramo Chanlor, to the west of Guamote (4 030 m a.s.l.)	S 1.9534	W 78.7933	P. Sklenář & A. Kučerová	11038
S. lingulatus	35_L	NL ascending subshrub	4	2	Ecuador: Napo, páramo on the western side of Antisana (4 500 m a.s.l.)	S 0.4667	W 78.1667	P. Sklenář	11077
S. lingulatus	36_L	NL ascending subshrub	4	1	Ecuador: Pichincha, around the upper antennas of Atacazo (4 160 m a.s.l.)	S 0.3464	W 78.6158	P. Sklenář, A. Kučerová & P. Macek	11074
S. lingulatus	37_L	NL ascending subshrub	5	—	Ecuador: Cotopaxi, páramo de Quispicacha, ca 0.5 km below the pass to Quebrada Tauricucho (4 150 m a.s.l.)	S 1.072	W 78.8573	P. Sklenář, E. Rejzková, F. Kolář	11569
S. longepenicill	38_L	NL asce	4	1	Venezuela: Mérida, páramo de Mucubají, trail from Laguna de	N 8.7802	W	P. Sklenář,	10205

atus	o	nding subs hrub			Mucubají to the Cascadas and Laguna Negra (3 640 m a.s.l.)		70.8212	P. Ubiergo et al.	
S. longepenicillatus	39_Lo	NL ascending subshrub	2	—	Venezuela: Táchira: páramo Colorado, near the road from Queniquea to El Cobre (3 080 m a.s.l.)	N 7.9406	W 72.0794	P. Sklenář, P. Ubiergo et al.	10377
S. longepenicillatus	40_Lo	NL ascending subshrub	1	—	Venezuela: Táchira: northern reaches of the Páramo del Batallon, to the east from La Grita (3 310 m a.s.l.)	N 8.1619	W 71.8989	P. Sklenář, P. Ubiergo et al.	10417
S. longepenicillatus	41_Lo	NL ascending subshrub	3	1	Venezuela: Mérida, Páramo de Aguila, along the road from Mucuchies towards the pass de Aguila, northern side of Cerro El Balcón (3 980 m a.s.l.)	N 8.8364	W 70.8321	P. Sklenář, P. Ubiergo et al.	10120
S. longepenicillatus	42_Lo	NL ascending subshrub	5	1	Venezuela: Mérida/Trujillo, Alto del Arenal, páramo south-east of Tuname along the road to Santo Domingo (3 730 m a.s.l.)	N 9.0294	W 70.5844	P. Sklenář, P. Ubiergo et al.	10279
S. longepenicillatus		NL ascending subshrub	—	1	Venezuela: Mérida, Páramo de Mucuchíes, road pass Aguila to Pinango, near the antennas (4240 m a.s.l.)	N8.8584	W70.8261	P. Sklenář, P. Ubiergo et al.	10122
S. mojandensis	43_Mo	BL rosette herb	2	1	Ecuador: Carchi, Volcan Chiles, margin of the road Tufino - Maldonado, ca 1.5 km ESE of Laguna Verde (3 910 m a.s.l.)	N 0.7994	W 77.9153	P. Sklenář, E. Rejzková, F. Kolář	11507
S. mojandensis	44_Mo	BL rosette herb	1	1	Ecuador: Cotopaxi, Páramo de Lagunas de Anteojos, to the south of Latacunga (3 930 m a.s.l.)	S 0.9781	W 78.3893	P. Sklenář & A. Kučerová	11028
S. mojandensis	45_Mo	BL rosette herb	1	1	Colombia: Valle de Cauca, Paramo de Tinajas, road from Florida towards the mountain pass (3 790 m a.s.l.)	N 3.3393	W 76.0636	P. Sklenář & D. Vasquez	12272
S. otophorus	46_Ot	NL liana	4	—	Ecuador: Pichincha, northern side of Nevado Cayambe, along the road from Laguna San Marcos towards antennas (3750 m a.s.l.)	N 0.1011	W 77.9772	P. Sklenář & E. Rejzková	10713
S. otophorus	47_Ot	NL liana	6	1	Ecuador: Chimborazo, Volcan Altar, around Cerro Quilimas, along the trail Alao-Huamboya, ca 1 km N of the bridge across Rio Alao (3 630 m a.s.l.)	S 2.3342	W 78.4458	P. Sklenář, E. Rejzková, F. Kolář	11599
S. otophorus	48_Ot	NL liana	5	—	Ecuador: Loja, Parque Nacional Podocarpus, paramo near the summit of Cerro Toledo (3 400 m a.s.l.)	S 4.3917	W 79.1125	P. Sklenář, J. Macková & P.	12072

								Macek	
S. otophorus	49_Ot	NL liana	2	—	Ecuador: Loja, Paramo de Fierro Urco, to the southwest of Saraguro (3 720 m a.s.l.)	S 3.6908	W 79.3525	P. Sklenář, J. Macková & P. Macek	12007
S. otophorus	50_Ot	NL liana	5	1	Ecuador: Loja, Cordillera las Lagunillas (de Sabanilla), páramo de las Lagunas Negras (3 400 m a.s.l.)	S 4.7111	W 79.4308	P. Sklenář, J. Macková & P. Macek	12045
S. otophorus	51_Ot	NL ascending subshrub	4	1	Colombia: Boyacá, Sierra Nevada del Cocuy, valley of the Rio Lagunillas, shrubby vegetation around lagunas (3 940 m a.s.l.)	N 6.3644	W 72.3331	P. Sklenář, E. Dušková et al.	12211
S. otophorus	52_Ot	NL liana	3	—	Colombia: Antioquia, paramo Frontino, trail towards Alto de Burros (3 580 m a.s.l.)	N 6.4475	W 76.0845	P. Sklenář, E. Dušková et al.	12240
S. otophorus	53_Ot	NL liana	4	—	Colombia: Cauca, paramo de Moras road from Silvia to Mosoco, ridges between the road and Cerro de Penas Blancas (3 600 m a.s.l.)	N 2.71	W 76.2167	P. Sklenář	12267
S. otophorus	54_Ot	NL liana	3	1	Ecuador: Azuay, superparamo vegetation to the N from the pass of the road Cuenca-Molleturo, mountain ridge towards Cerro Amarillo (4 300 m a.s.l.)	S 2.7696	W 79.2433	P. Sklenář & J. Karbulková	11117
S. otophorus	55_Ot	NL liana	2	—	Colombia: Valle de Cauca, Paramo de Tinajas, grass paramo above the Laguna Guayabal (3 790 m a.s.l.)	N 3.3393	W 76.0636	P. Sklenář & D. Vasquez	12276
S. otophorus	56_Ot	NL liana	5	1	Colombia: Cauca, paramo del Letrero, trail from Valencia towards Laguna Santiago and Laguna Suramerica (3 700 m a.s.l.)	N 1.9206	W 76.5956	P. Sklenář, E. Dušková et al.	12337
S. otophorus	57_Ot	BL liana	5	—	Colombia: Quindio-Tolima, Cerro Campanario, on the paved road leading to the military anntenas at the mountain ridge (3 590 m a.s.l.)	N 4.4503	W 75.5773	P. Sklenář, E. Dušková et al.	12343
S. otophorus	58_Ot	BL liana	4	1	Colombia: Cundinamarca, paramo de Chingaza, trail from Laguna Chingaza to Laguna de Media (3500 m a.s.l.)	N 4.5098	W 73.743	P. Sklenář & F. Kolář	12349
S. otophorus	59_Ot	BL liana	5	1	Colombia: Boyacá, paramo de Pisba, shrubby vegetation on slopes to the west of Rio Arzobispo (3 420 m a.s.l.)	N 5.9503	W 72.5915	P. Sklenář & E. Dušková	12373

S. otophorus	60_Ot	NL liana	6	1	Colombia: Nariño, Volcan Galeras, road from Pasto to the crater (3 520 m a.s.l.)	N 1.2292	W 77.3392	P. Sklenář & E. Dušková	12382
S. otophorus	61_Ot	NL liana	2	—	Colombia: Nariño, Volcan Azufral, along the path from Tuquerres to Laguna Verde, ca 1 km ENE of the laguna (3 890 m a.s.l.)	N 1.092	W 77.7085	F. Kolář	53
S. otophorus	62_Ot	NL liana	4	—	Ecuador: Morona Santiago, along the road Gualaceo-Limon, humid bamboo subparamo with scattered shrubs (3 320 m a.s.l.)	S 3.0005	W 78.665	P. Sklenář & J. Karbulková	11163
S. otophorus	63_Ot	NL liana	6	1	Ecuador: Carchi, Volcan Chiles, margin of the road Tufino - Maldonado, ca 1.5 km ESE of Laguna Verde (3 910 m a.s.l.)	N 0.7994	W 77.9153	P. Sklenář, E. Rejzková, F. Kolář	11508
S. otophorus	64_Ot	NL liana	5	—	Colombia: Caldas, Los Nevados, shrubs along the road from Termales to Nevado El Ruiz (3 860 m a.s.l.)	N 4.9582	W 75.3579	Fabio, P. Sklenář et al.	FAA 631
S. superandinus	65_O	NL erect subshrub	1	—	Ecuador: Cotopaxi, paramo to the west of Quilindana (4 120 m a.s.l.)	S 0.8008	W 78.3884	P. Sklenář	10045
S. superandinus	66_O	NL erect subshrub	4	1	Ecuador: Imbabura, páramo de Mojanda, on the SW slope of the peak Nudo de Mojanda (4 130 m a.s.l.)	N 0.1186	W 78.26	P. Sklenář & E. Rejzková	10744
S. superandinus	67_O	NL erect subshrub	5	—	Ecuador: Pichincha, páramo on the southern side of Cayambe, along the road towards the refugio, bellow the steep rock walls (4 430 m a.s.l.)	S 0.0246	W 78.0501	P. Sklenář & A. Kučerová	11092
S. superandinus	68_O	NL erect subshrub	6	1	Ecuador: Azuay, superparamo vegetation to the N from the pass of the road Cuenca-Molleturo, mountain ridge towards Cerro Amarillo (4 300 m a.s.l.)	S 2.7696	W 79.2433	P. Sklenář & J. Karbulková	11118
S. superandinus	69_O	NL erect subshrub	2	—	Ecuador: Carchi, Volcan Chiles, ca 0.5 km N of the antennas, ca 1 km N of the pass with the road Tufino - Maldonado (4 160 m a.s.l.)	N 0.8063	W 77.9422	P. Sklenář, E. Rejzková, F. Kolář	11518
S. superandinus	70_O	NL erect subshrub	1	1	Ecuador: Imbabura, Volcan Cotacachi, along the trail from the TV antennas towards the summit (4 130 m a.s.l.)	N 0.3437	W 78.3431	P. Sklenář, E. Rejzková, F. Kolář	11540
S. superandinus	71_O	NL erect subshrub	5	1	Ecuador: Pichincha, along the trail from the antennas towards the summit of Atacazo (4160 m a.s.l.)	S 0.3513	W 78.6178	P. Sklenář, A. Kučerová & P. Macek	11071

S. superandinus	72_O	NL erect subshrub	4	1	Ecuador: Cotopaxi, Páramo de Lagunas de Anteojos, to the south of Latacunga (3 930 m a.s.l.)	S 1.5355	W 78.8809	P. Sklenář & A. Kučerová	11032
S. superandinus	73_O	NL erect subshrub	2	1	Ecuador: Chimborazo, páramo de Osogochi, grass páramo on the slopes above Laguna Cubillín (3 970 m a.s.l.)	S 2.287	W 78.5863	P. Sklenář & J. Karbulková	11188
S. superandinus	74_O	NL erect subshrub	5	—	Ecuador: Imbabura, páramo de Quispicacha, pass between valley of Rio Pigua and Quebrada Tauricucho, ca 5 km ESE of Chinipamba (4 130 m a.s.l.)	S 1.0788	W 78.8474	P. Sklenář, E. Rejzková, F. Kolář	11568
S. superandinus	75_O	NL erect subshrub	6	—	Colombia: Valle de Cauca, paramo de Tinajas, Cordillera Central, rocky summit of the mountain ridge above Laguna Guayabal (4 160 m a.s.l.)	N 3.3445	W 76.0541	P. Sklenář & D. Vasquez	12288
S. superandinus	76_O	NL erect subshrub	1	—	Colombia: Cauca, Volcan Purace, the trail from the sulphur mine to the northern side of the crater (4 160 m a.s.l.)	N 2.34	W 76.4037	P. Sklenář, E. Dušková et al.	12316
S. superandinus		NL erect subshrub	—	1	Ecuador: Pichincha, Páramo de Iliniza, along the trail from the parking place towads the Ilinizas (4294 m a.s.l.)	S0.6428	W78.6963	P. Sklenář, E. Rejzková, F. Kolář	11559
S. patens	77_P	BL liana	1	—	Ecuador: Carchi - Sucumbios, Páramo El Mirador, ca 6 km to the east of Huaca (3 230 m a.s.l.)	N 0.647	W 77.674	P. Sklenář, E. Rejzková, F. Kolář	11530
S. patens	78_P	BL liana	2	—	Ecuador: Imbabura, Volcan Cotacachi, margin of the 4 WD road from Laguna Cuicocha north to the TV antennas (3 390 m a.s.l.)	N 0.313	W 78.3526	P. Sklenář, E. Rejzková, F. Kolář	11536
S. patens	79_P	BL liana	2	1	Ecuador: Cotopaxi, páramo Quispicacha (3 730 m a.s.l.)	S 1.0453	W 78.9673	P. Sklenář, E. Rejzková, F. Kolář	11565
S. patens	80_P	BL liana	3	1	Ecuador: Carchi, road from El Angel towards Laguna Voladero (3 260 m a.s.l.)	N 0.8283	W 77.9001	P. Sklenář & J. Karbulková	11113
S. patens	81_P	BL liana	3	—	Colombia: Cauca, Purace, margin of the road Popayan-La Plata, 3 km ESE of municipio Purace (3 290 m a.s.l.)	N 2.374	W 76.4061	E. Rejzková, F. Kolář, D. Vasquez	35
S. patens	82_P	BL liana	1	—	Ecuador: Pichincha, northern side of Pichincha, along the road to the forest reserve Jocotoco (3 650 m	S 0.115	W 78.5742	P. Sklenář & A. Soukup	12097

					a.s.l.)				
S. patens	83_P	BL liana	6	—	Colombia: Cauca, paramo del Letrero, trail from Valencia towards Laguna Santiago and Laguna Suramerica (3 230 m a.s.l.)	N 1.9157	W 76.6228	P. Sklenář, E. Dušková et al.	12333
S. patens	84_P	BL liana	2	—	Colombia: Nariño, Volcan Galeras, road from Pasto to the crater (3 490 m a.s.l.)	N 1.2265	W 77.3374	P. Sklenář & E. Dušková	12377
S. patens	85_P	BL liana	2	—	Colombia: Nariño, Volcan Azufral, margin of the road from Tuquerres to Laguna Verde, ca 6 km W of Tuquerres (3 500 m a.s.l.)	N 1.0925	W 77.6698	F. Kolář	51
S. patens	86_P	BL liana	1	1	Ecuador: Carchi, Volcan Chiles, margin of the road Tufino - Maldonado, ca 1.5 km ESE of Laguna Verde (3 910 m a.s.l.)	N 0.7889	W 77.8798	P. Sklenář, E. Rejzková, F. Kolář	11501
S. aff. quitensis	87_Pi	NL/BL subshrub	2	—	Colombia: Valle de Cauca, paramo de Tinajas, Cordillera Central, rocky summit of the mountain ridge above Laguna Guayabal (4 160 m a.s.l.)	N 3.3445	W 76.0541	P. Sklenář & D. Vasquez	12289
S. aff. quitensis	88_Pi	NL/BL subshrub	4	1	Ecuador: Carchi, Volcan Chiles, margin of the road Tufino - Maldonado, ca 1.5 km ESE of Laguna Verde (3 910 m a.s.l.)	N 0.7994	W 77.9153	P. Sklenář, E. Rejzková, F. Kolář	11509
S. aff. quitensis	89_Pi	NL/BL subshrub	2	—	Ecuador: Imbabura, Volcan Cotacachi, southern slopes of the mountain (4 370 m a.s.l.)	N 0.3536	W 78.3494	P. Sklenář, E. Rejzková, F. Kolář	11546
S. aff. quitensis	90_Pi	NL/BL subshrub	2	—	Ecuador: Pichincha, around the upper antennas of Atacazo, lower superpáramo vegetation (4 160 m a.s.l.)	S 0.3464	W 78.6158	P. Sklenář, A. Kučerová & P. Macek	11075
S. aff. quitensis	91_Pi	NL/BL subshrub	1	—	Ecuador: Pichincha, páramo on the southern side of Cayambe, along the road towards the refugio, bellow the steep rock walls (4 250 m a.s.l.)	S 0.0167	W 78.05	P. Sklenář & A. Kučerová	11084
S. aff. quitensis	92_Pi	NL/BL subshrub	1	—	Ecuador: Pichincha, páramo on the southern side of Cayambe, along the road towards the refugio, bellow the steep rock walls (4 430 m a.s.l.)	S 0.0246	W 78.0501	P. Sklenář & A. Kučerová	11091
S. aff. quitensis	93_Pi	NL/BL subshrub	2	—	Ecuador: Pichincha, Rucu Pichincha, along the trail from the Teleferico to the summit (4 550 m a.s.l.)	S 0.1611	W 78.5653	P. Sklenář & J. Karbulková	11191
S. puracensis	94_Pu	NL ascending subs	5	2	Colombia: Cauca, Volcan Purace, the trail from the sulphur mine to the northern side of the crater (4 110 m	N 2.3324	W 76.3943	P. Sklenář, E. Dušková	12311

		hrub			a.s.l.)			et al.	
S. imbaburensis	95_S	NL ascending subshrub	3	—	Ecuador: Imbabura, paramo de Mojanda, on the SW slope of the peak Nudo de Mojanda (4 020 m a.s.l.)	N 0.1147	W 78.2633	P. Sklenář & E. Rejzková	10752
S. imbaburensis	96_S	NL ascending subshrub	5	1	Ecuador: Pichincha, páramo on the southern side of Cayambe, along the road towards the refugio, bellow the steep rock walls (4 430 m a.s.l.)	S 0.0246	W 78.0501	P. Sklenář & A. Kučerová	11093
S. imbaburensis	97_S	NL ascending subshrub	2	1	Ecuador: Imbabura, Volcan Cotacachi, margin of the 4 WD road from Laguna Cuicocha north to the TV antennas, near the antennas (4010 m a.s.l.)	N 0.3323	W 78.3389	P. Sklenář, E. Rejzková, F. Kolář	11547
S. subinvolucratus	98_Sl	BL liana	4	—	Ecuador: Pichincha, páramo on the NW side of Atacazo (4 160 m a.s.l.)	S 0.3867	W 78.6002	P. Sklenář, A. Kučerová & P. Macek	11076
S. superparamensis	99_SP	NL ascending subshrub	2	—	Ecuador: Napo, superparamo vegetation on the western side of Antisana (4 600 m a.s.l.)	S 0.4722	W 78.1653	P. Sklenář & D. Carrate	10739
S. superparamensis	100_SP	NL ascending subshrub	3	1	Ecuador: Carchi, Volcan Chiles, margin of the road Tufino - Maldonado, around Laguna Verde (4 030 m a.s.l.)	N 0.8006	W 77.9358	P. Sklenář, E. Rejzková, F. Kolář	11510
S. superparamensis	101_SP	NL ascending subshrub	4	1	Ecuador: Imbabura, Volcan Cotacachi, southern slopes of the mountain (4 370 m a.s.l.)	N 0.3536	W 78.3494	P. Sklenář, E. Rejzková, F. Kolář	11544
S. superparamensis	102_SP	NL ascending subshrub	5	2	Ecuador: Napo, Páramo of Antisana, NE side of the mountain (4 400 m a.s.l.)	S 0.45	W 78.1333	P. Sklenář	11079
S. iscoensis	103_3	BL (sub) shrub	2	—	Ecuador: Cotopaxi, Loma Ingapirca, right turn-of from the road Zumbagua - Angamarca, ca 5 km NNW of Quindigua (3 730 m a.s.l.)	S 1.0453	W 78.9673	P. Sklenář, E. Rejzková, F. Kolář	11567
S. iscoensis	104_3	BL (sub) shrub	1	—	Ecuador: Chimborazo, Candelaria, road from the village towards lagunas (3 240 m a.s.l.)	S 1.6486	W 78.5058	P. Sklenář, J. Macková & P. Macek	12094
S. nivalis	105_	NL herb	4	1	Ecuador: Carchi, Volcan Chiles, ca 0.5 km N of the antennas, ca 1 km N	N 0.8006	W	P. Sklenář,	11515

	CN	to subs hrub			of the pass with the road Tufino - Maldonado (4 030 m a.s.l.)		77.9422	E. Rejzková, F. Kolář	
S. nivalis	106_ CN	NL herb to subs hrub	4	1	Ecuador: Imbabura, Volcan Cotacachi, southern slopes of the mountain (4 370 m a.s.l.)	N 0.3536	W 78.3494	P. Sklenář, E. Rejzková, F. Kolář	11543
S. nivalis	107_ CN	NL herb to subs hrub	5	1	Ecuador: Napo, Volcan Antisana, N side of the mountain, towards the Quebrada of Río Blanco (4 350 m a.s.l.)	S 0.4501	W 78.1364	P. Sklenář & F. Kolář	11580
S. nivalis	108_ CN	NL herb to subs hrub	5	1	Ecuador: Tungurahua - Chimborazo - Bolivar, páramo to the SW of Chimborazo, ca 2 km of the road Cruz del Arenal-San Juan (4 270 m a.s.l.)	S 1.5353	W 78.8808	P. Sklenář, E. Rejzková, F. Kolář	11586
S. alatopetiolatus		BL liana	—	1	Ecuador: Tungurahua, Parque Nacional Llanganatis, near laguna at the western side of Cerro Hermoso (3870 m a.s.l.)	S1.2308	W78.3012	P. Sklenář	13100
S. canescens		BL rosette herb	—	1	Colombia: Boyaca, Paramo around the Laguna Alcohol (3900 m a.s.l.)	S1.1354	W78.3694	P. Sklenář, E. Dušková	12356
S. chionogeton		NL rosette herb	—	1	Ecudor: Tungurahua, Parque Nacional Llanganatis, grass paramo above the Laguna Pisayambo (3910 m a.s.l.)	S1.1354	W78.3694	P. Sklenář	12414
S. cocuyensis		BL rosette herb	—	1	Colombia: Boyaca, Sierra Nevada del Cocuy, valley of the Rio Lagunillas, shrubby vegetation around lagunas (3940 m a.s.l)	N6.0814	W72.937	P. Sklenář, E. Dušková, F. Kolář, D. Vásquez	12212
S. decipiens		BL liana	—	1	Ecuador: Loja/Zamora-Chinchipe, Road from Jimbura to Las Cienegas (2550 m a.s.l.)	S4.6786	W79.4517	P. Sklenář, J. Macková, P. Macek	12024
S. decipiens		BL liana	—	1	Ecuador: Loja, Via antigua from Loja to Catamayo, western outskirts of Loja (2380 m a.s.l.)	S4.0164	W79.2464	P. Sklenář, J. Macková, P. Macek	12065
S. rhizocephalus		Acaulescent herb	—	1	Ecudor: Tungurahua, Parque Nacional Llanganatis, grass paramo above the Laguna Pisayambo (3910 m a.s.l.)	N6.3644	W72.3331	P. Sklenář	12411
S. doryphyllus		BL liana	—	1	Colombia: Cesar, Cerro del Avion, rocks on the top of the mountain, approx 10 km ESE of Manaure	—	—	F. Kolář, D. Vásquez	12392

S. doryphyllus		BL liana	—	1	Colombia: Cesar, Páramo de Cerro del Avion, forest close to ruines of the house, SSE of Cerro Pintado, approx 8 km ESE of Manaure	—	—	F. Kolář, D. Vásquez	12393
S. sp.1		NL herb	—	1	Colombia: Boyaca, Paramo de Pisba, shrubby vegetation on slopes to the west of Rio Arzobispo (3420 m a.s.l)	N5.9503	W72.5915	P. Sklenář, E. Dušková	12376
S. sp. 2		NL herb		1	Colombia: Boyaca, Sierra Nevada del Cocuy, valley of the Rio Lagunillas, paramo vegetation along the trail towards the Laguna Cuadrada (4120 m a.s.l)	N6.3536	W72.3269	P. Sklenář, E. Dušková, F. Kolář, D. Vásquez	12210

Appendix S2. Supplementary molecular protocols

DNA extraction

Lab work was done mostly at the DNA laboratory, Department of Botany, Charles University, Prague, Czech Republic, with some of the extractions done at the Botany and Systematics Lab, Universidad de los Andes, Bogota, Colombia. Total genomic DNA was extracted from silica-dried material using the Invisorb Spin Plant Mini Kit (Invitek) according to the manufacturer's instructions.

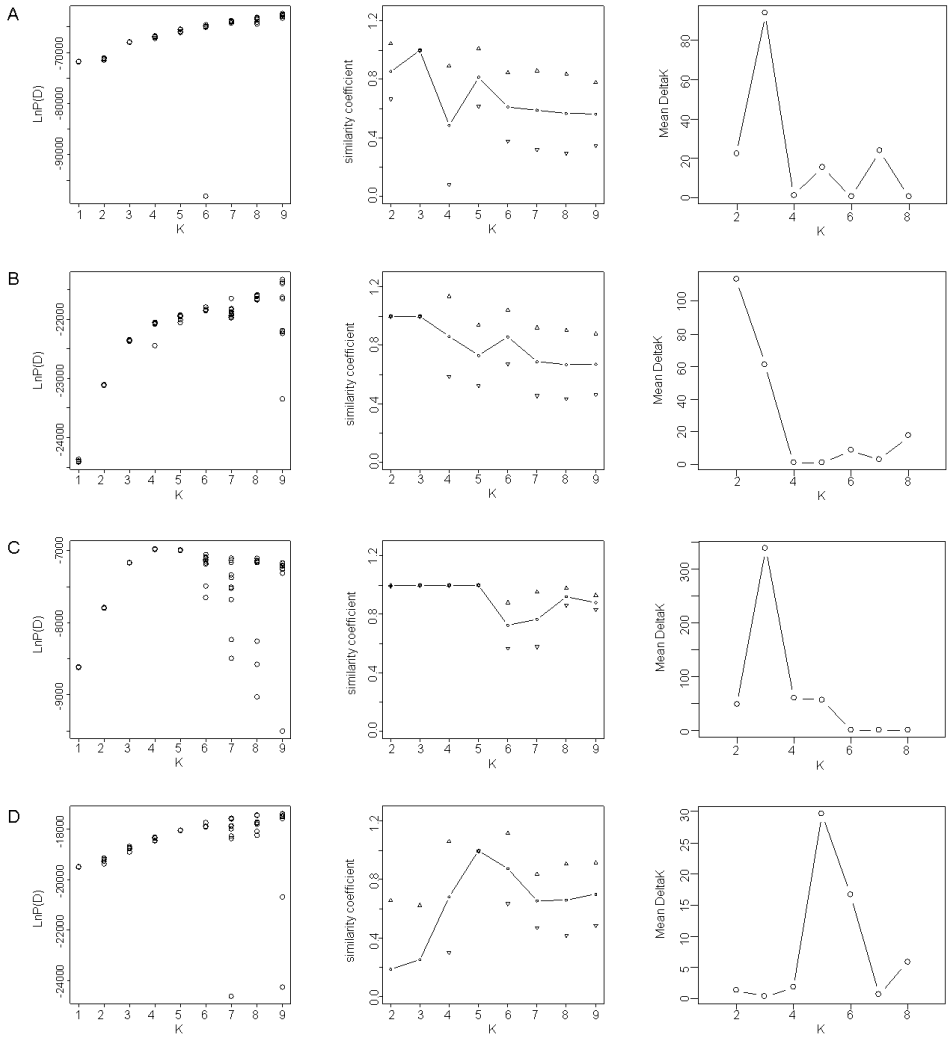
AFLP protocol

We used AFLP Core Reagent Kit I (Invitrogen) and AFLP Pre-Amp Primer Mix I (Invitrogen) following manufacturer instructions with several minor modifications, including using five times smaller reaction volumes. Fifteen to 50 ng of genomic DNA was digested for 7.5 hr. at 37°C with 0.5 U of *Eco*RI/*Mse*I (Invitrogen), 1 µl 5 × reaction buffer (Invitrogen), and 2.5 µl ddH_2O. Final incubation for 15 min. at 70°C was done immediately after restriction. Adapters were ligated to the digested fragments by adding 4.8 µl Adapter/Ligation Solution (Invitrogen) and 0.2 U T4 DNA Ligase (Invitrogen) and incubated for 12-16 hr. at 37°C. Pre-amplification reaction was performed with AFLP Pre-Amp Primer Mix I (Invitrogen). For each sample, a pre-amplification mixture containing 0.5 µl DNA from the ligation reaction, 4.0 µl PA mix (Invitrogen), 0.5 µl 10× Buffer for JumpStartRedTaq (Sigma), and 0.1U JumpStartRedTaq DNA Polymerase (Sigma) was placed in a Mastercycler ep S thermal cycler (Eppendorf). Reaction conditions consisted of an initial step of 2 min. at 94°C, then 2 min. at 72°C followed by 20 cycles of 1 sec. at 94°C, 30 sec. at 56°C, and 2 min. at 72°C, with a final extension of 30 min. at 60°C. Once the pre-amplification was complete, selective amplification was performed using 2.5 µl of 5× diluted pre-amplification product as a template, 1 µl 10× Buffer for JumpStartRedTaq (Sigma), 0.2 mM dNTP, 0.5 pmol *Eco*RI-selective fluorescence-labeled primer, 2.5 pmol *Mse*I-selective primer, 0.2 U JumpStartRedTaq DNA Polymerase (Sigma), and 5.1 µl ddH_2O. Three primer combinations were used for selective amplification: *Eco*RI-ACT (6-FAM labeled) + *Mse*I-CTA, *Eco*RI-ATC (6-FAM labeled) + *Mse*I-CAT, and *Eco*RI-AGG (HEX labeled) + *Mse*I-CAT. For all samples, the reactions were done in a Mastercycler ep S thermal cycler (Eppendorf). Reaction conditions consisted of an initial step of 2 min. at 94°C, then 2 min. at 72°C followed by 8 cycles of 1 sec. at 94°C, 30 sec. at 64°C (reduced by 1°C per cycle), and 2 min. at 72°C, followed by 23 cycles of 1 sec. at 94°C, 30 sec. at 56°C, and 2 min. at 72°C, with a final extension time of 30 min. at 60°C. For each sample, 1 µl of each selective PCR product was combined with 0.25 µl of ROX 500 size standard (AppliedBio). Fragments were resolved on an ABI3130 Avant Genetic Analyzer (AppliedBio).

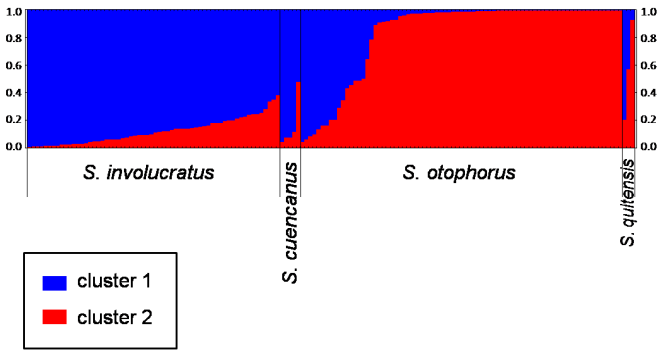
ITS sequencing

PCR amplifications were done in XX µl reaction containing 0.18 mM of each dNTP (Fermentas), 0.23 mM of each primer (Sigma), 0.5 unit of JumpStart REDTaq polymerase (Sigma), 1 × PCR buffer for JumpStart REDTaq (Sigma) and 5 ng of genomic DNA. An initial denaturation step at 94°C for 1 min was followed by 35 cycles of denaturation (94°C for 45 s), annealing (49-52°C for 45 s) and extension (72°C for 1 min) steps, and a final extension at 72°C for 10 min. PCR products were purified using JETQUICK PCR Product Purification Spin Kit (Genomed) and subsequently sequenced (Macrogen, lll).

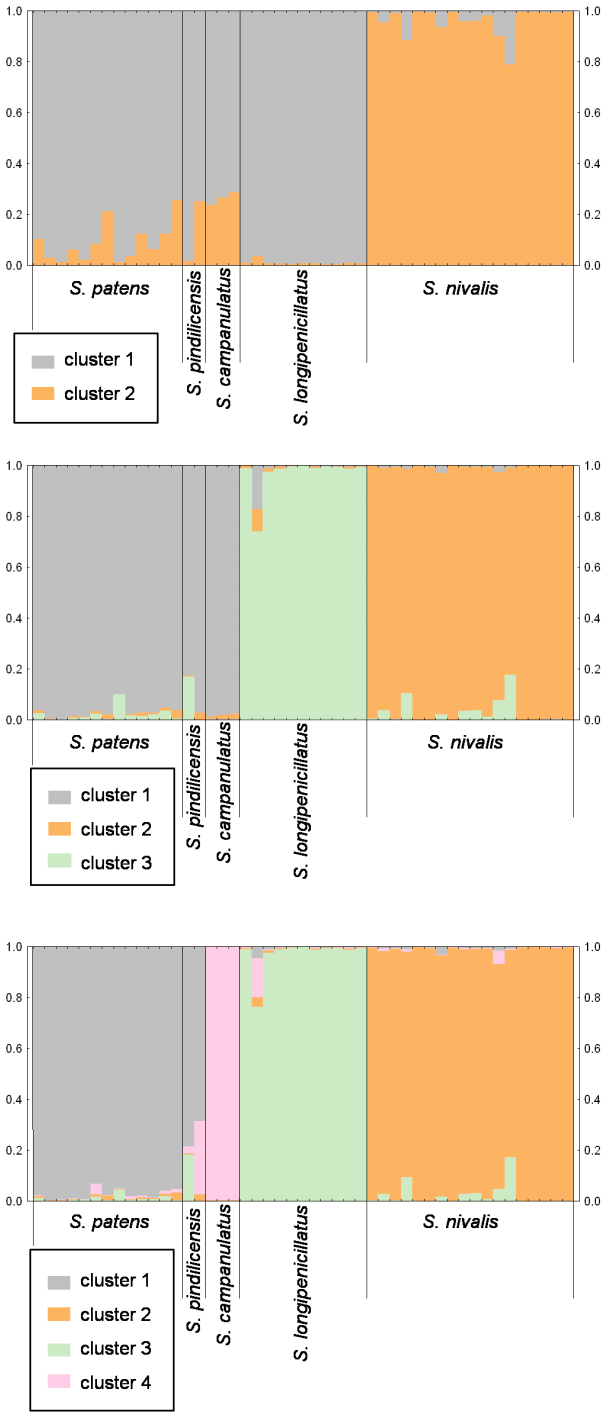
Appendix S3A. Comparison of the results of different STRUCTURE runs with increasing K (from K1 to K10, ten replicates each, see Materials and Methods for details). Analysis of entire dataset (A) and separate analyses of individuals assigned only to cluster A (B); cluster B (C); and cluster C (D).



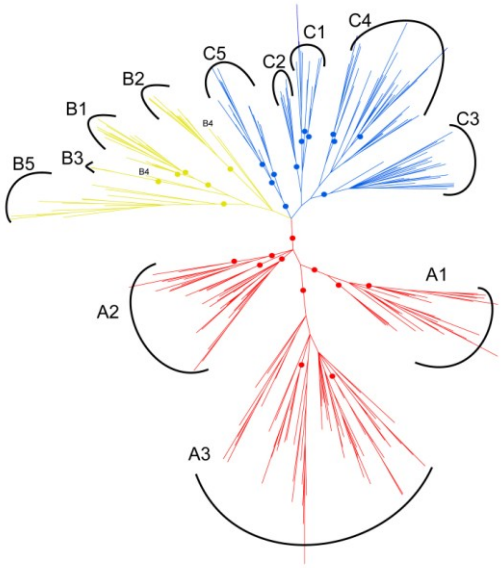
Appendix S3B. Results of STRUCTURE analysis of 149 individuals of high-elevation Andean *Senecio* assigned to cluster A for K = 2. Colors indicate posterior probabilities for membership of each individual in the two resulting subgroups.



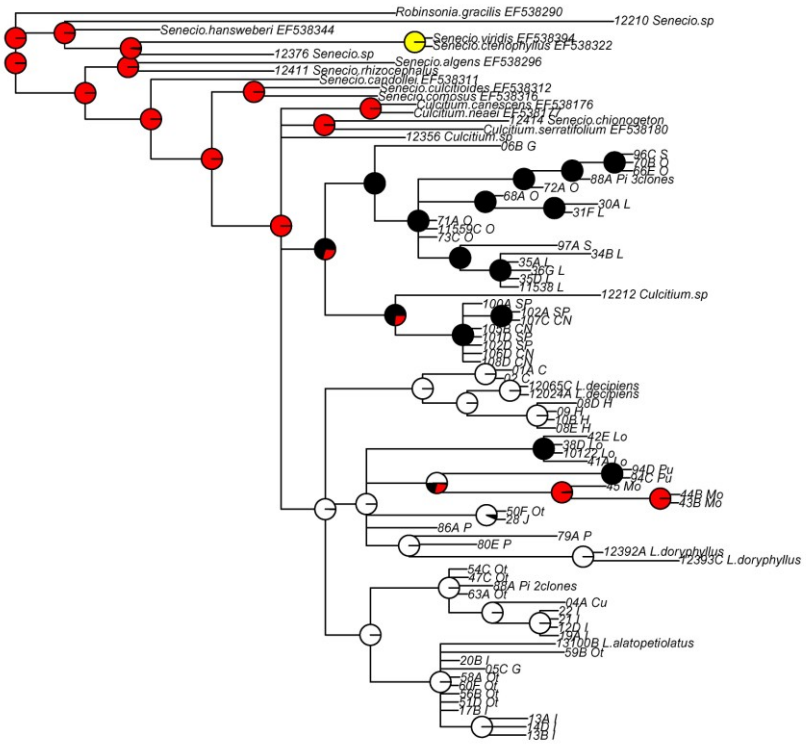
Appendix S3C. Results of STRUCTURE analysis of 47 individuals of high-elevation Andean *Senecio* assigned to cluster B for (A) K = 2, (B) K = 3, (C) K = 4. Colors indicate posterior probabilities for membership of each individual in the resulting sub-groups.



Appendix S3D. Relationships among AFLP phenotypes of 266 non-admixed (see Methods) individuals of former *Lasiocephalus* and *Senecio nivalis* reconstructed in Bayesian framework. Cluster codes correspond with Figs. 3–5; branches with posterior probabilities > 0.95 are marked with dots.



Appendix S3E. Equal rates (ER) model reconstruction of the growth form evolution in the Senecio-*Lasiocephalus*-*Culcitium* species group; basal leaf rosette herb – red, narrow leaved subshrub – black, broad leaved liana – white, shrub – yellow.



Chapter 4

Senecio sangayensis (Asteraceae, Senecioneae): a striking new species from the Ecuadorian highlands

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Abstract

Senecio sangayensis, a putative endemic species from the eastern Ecuadorian Cordillera, is described as a new species. It is chiefly distinguished by its habit, striking racemiform synflorescence composed of dense cymose-corymbs subtended by triangular-ovate bracts, and life-history strategy. This new species is known from the páramo of the Sangay National Park. Its IUCN conservation status is preliminarily assessed as Endangered (EN).

Resumen

Senecio sangayensis, especie probablemente endémica de la Cordillera Oriental Ecuatoriana, se describe como una especie nueva. Se distingue principalmente por su hábito, su llamativa sinflorescencia racemiforme formada por corimbos cimosos, densos, sostenidos por brácteas triangular-ovadas, y por su estrategia de vida. Esta nueva especie se conoce de los páramos del Parque Nacional Sangay. Se propone preliminarmente el estado de conservación En peligro (EN) según IUCN.

Keywords: Compositae, Ecuador, páramo, semelparous species.

Introduction

The genus *Senecio* Linnaeus (1753: 866) (Senecioneae), with approximately 1000 species, is one of the most species-rich genera of flowering plants (Nordenstam et al, 2009). It exhibits a wide range of life forms and ecological preferences, and is distributed across all major biomes with its primary centers of diversity in southern Africa and South America (Pelser et al., 2007; Nordenstam et al., 2009). The genus is particularly rich in species and endemics along the highlands of the northern Andes (Smith & Cleef 1988; Sklenář et al., 2011), which are known as the páramo (Cuatrecasas, 1968). A new delimitation of the genus based on molecular data (Pelser et al., 2007) includes the Andean genera *Lasiocephalus* Willdenow ex Schlechtendal (1818: 308), *Aetheolaena* Cassini (1827: 453), and *Culcitium* Bonpland in Humboldt & Bonpland (1808: 1).

According to this new delimitation, about 77 native species of *Senecio* occur in the páramo of Colombia and Ecuador (Ávila et al.; in Bernal et al., 2015; Calvo & Freire, 2016), 28 of which are found in the Ecuadorian Andes (Calvo, 2015). These species are adapted to the Andean high-elevation conditions. They are perennial herbs, suffrutescent herbs or scandent subshrubs, with heterogamous-radiate or homogamous-discoid capitula, nodding or erect. The ray florets, when present, usually are yellow, although species with purple ray florets are also found, i.e. *S. formosus* Kunth in Humboldt *et al.* (1818: 138). The capitula are arranged in cymose-corymbose synflorescences or are solitary, the involucres usually bear supplementary bracts at the base (calyculate), and the style branches are truncate or penicillate. The leaves are simple, alternate, usually decreasing in size up the stem. Some species display basal leaves well developed or in a dense rosette when the species are scapiform or acaulescent.

The new species described herein markedly differs from all other Andean *Senecio* species growing in the páramo and the montane forest mainly in its habit, racemiform synflorescence composed of dense cymose-corymbs subtended by triangular-ovate bracts, and life-history strategy. It is only known from three localities in the Sangay National Park, Ecuador.

Taxonomy

Senecio sangayensis D.L.A. Vásquez & J. Calvo, **sp. nov.** (Figure 1, Figure 2)

Type:—ECUADOR. Chimborazo: near Atillo lakes, 02º13’51” S 78º32’43” W, 4061 m, 10 October 2010, *D.L.A Vásquez 302* (holotype PRC, isotype MO).

Senecio sangayensis differs from all other Andean *Senecio* species in the combination of the following characters: robust habit, erect stem densely covered by coriaceous sessile leaves, striking racemiform synflorescence composed of dense cymose-corymbs subtended by triangular-ovate bracts, and semelparous life-history strategy. Perennial semelparous plant up to 1.7 m (in bloom). Stem erect, unbranched, up to 5 cm in diam., and densely leaved with no internode elongation. Before developing the stem, basal leaves arranged in a rosette and covered with a mucilaginous fluid. When the stem starts developing, the basal and lower cauline leaves wither and remain attached to the stem. Leaves 13–35 cm long and 5–8 cm wide, coriaceous, elliptic-lanceolate, acute, sessile, slightly decurrent, denticulate, glabrescent

above, densely arachnoid beneath especially when young. Synflorescence up to 50 cm long, 20 cm in diam., racemiform, composed of dense cymose-corymbs of ca. 6 capitula subtended by triangular-ovate bracts. Capitula radiate, ca. 5 cm in diam., becoming nodding as time passes. Involucre 10–13 mm in diam., 10–15 mm long, bell-shaped; involucral bracts 14–16, 10–12 mm long, 1.5–2.2 mm wide, without scarious margin, lanceolate, glabrous; supplementary bracts 10–14, 7–10 mm long, 1–1.2 mm wide, subulate, without scarious margin, almost as long as involucral bracts, glabrous. Ray florets 7–13, up to 25 mm long, yellow; disc florets 40–50, 6–10 mm long, yellow; style branches truncate. Achenes glabrous, 3–4.5 mm long; pappus whitish. Chromosome number: unknown.

Distribution, Habitat, and Ecology:—*Senecio sangayensis* is only known from three localities in the southern part of the Sangay National Park, which is located in the eastern Ecuadorian Cordillera (Figure 2B). Two localities are situated around 3700 m a.s.l. on the west-oriented slopes of the Cordillera; one locality between the community of Pomacocho and the Ozogoche lakes, and the other between the community Pillcopata and the Culebrillas lake. The third locality is situated at 4061 m a.s.l. between the Ozogoche and Atillo lakes on the east-oriented slopes of the Cordillera. This side of the Cordillera, unlike the west-oriented slopes, is covered by forests that extend from the tree-line to the Amazonian forests (Bader & Ruijten, 2008).

The species occurs above the tree-line in a locally very humid páramo. The vegetation is dominated by *Calamagrostis* sp. and *Neurolepis aristata*. The species is semelparous, having only one reproductive episode during its life cycle.

Etymology:—The epithet *sangayensis* refers to the name of the National Park, "Sangay", where the three localities known for the species were found. The National Park was named after one of its main features, the Sangay volcano.

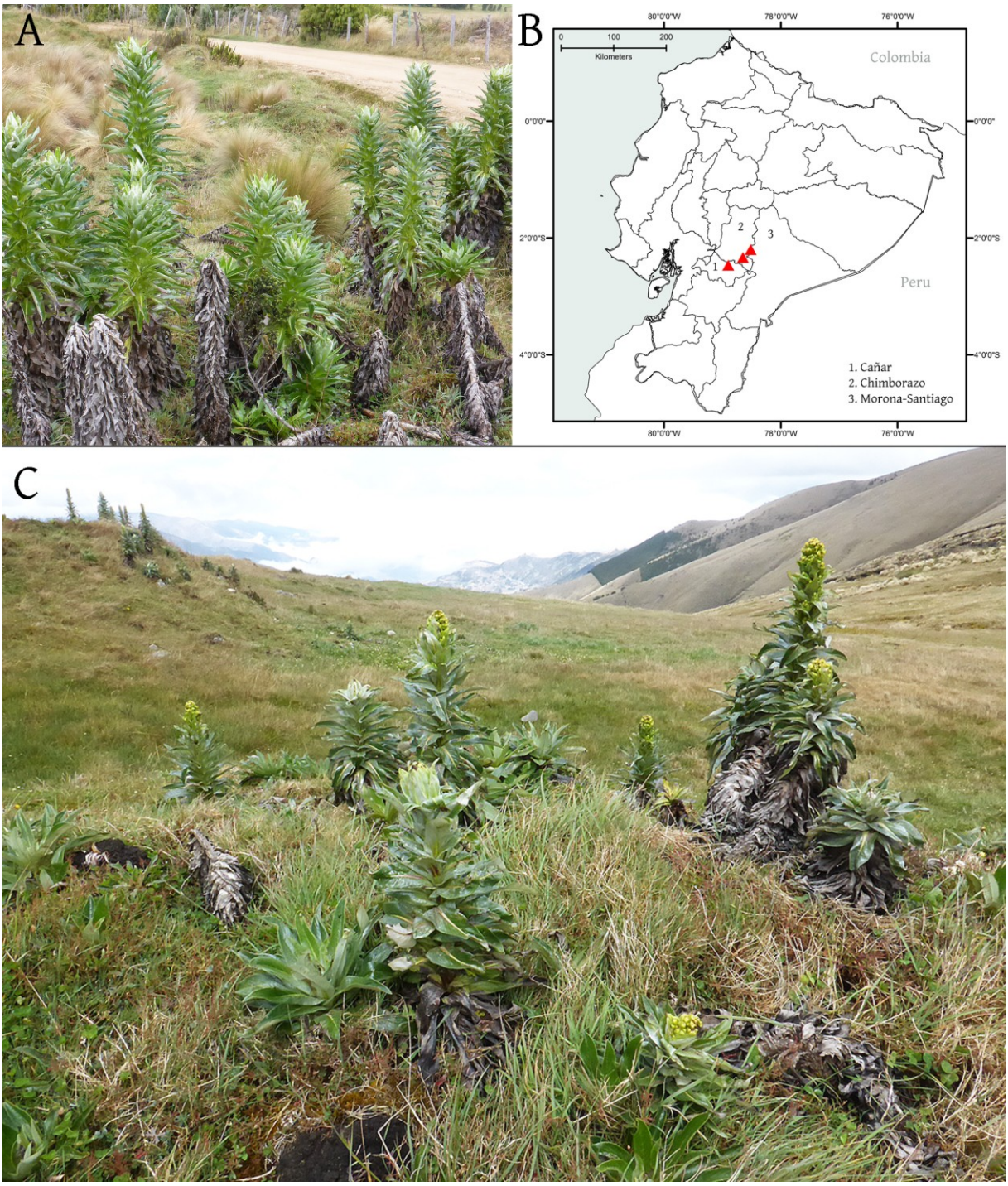
Additional specimens examined (paratypes):—ECUADOR. Chimborazo: Alausí, Achupallas, páramo de Pomacocho, 02º20' S 78º39' W, 3655 m, 17 June 2013, *J. Caranqui et al. 2306* (QCA-230005!, CHEP).

Discussion: —The racemiform synflorescence composed of dense cymose-corymbs subtended by triangular-ovate bracts, the coriaceous sessile leaves closely arranged along the stem, and the large radiate capitula make this species unique among the Andean *Senecio* species. Its robust habit (almost reaching 2 m tall) and the long-lived semelparous strategy are also unusual characters within Andean *Senecio*. Another robust species displaying large radiate yellow capitula is the Colombian endemic *S. niveoaureus* Cuatrecasas (1940: 6). However, this species has a laxer synflorescence, paniculiform instead of racemiform, narrower leaves, and the whole plant is covered with a white lanate indumentum. Any confusion is very unlikely.

The singular morphology of *S. sangayensis* resembles that of *Dendrosenecio keniensis* Baker (1894: 140) Mabberley (1986: 100) from both the lower and upper alpine zone of Mount Kenya (Kenya). The striking terminal racemiform synflorescence with large triangular-ovate bracts are pretty similar. The architecture of the capitulum is also similar, with a few subulate supplementary bracts almost as long as the involucral bracts and long yellow ray florets. The basal and lower cauline leaves of *D. keniensis* tend otherwise to be persistent at the flowering time, they are larger than in *S. sangayensis* and have a thick, whitish, lanate indumentum beneath. Unlike *S. sangayensis, D. keniensis* produces lateral branches near ground-level capable of rooting.

Figure 1. ***Senecio sangayensis***. A. Radiate capitulum. B. Involucre. C. Part of the racemiform synflorescence and detail of the synflorescence bracts. D. Cauline leaves. E. Detail of young rosette leaves with mucilage. F. Rosette. A–D, F: plant photographed near laguna Culebrillas, Cañar (J. Calvo, not collected). E: plant from the type locality (D.L.A. Vásquez).

Figure 2. ***Senecio sangayensis.*** A. Habit. B. Distribution map (▲). C. Habitat. Population near laguna Culebrillas, Cañar (J. Calvo).



Another singular trait of *S. sangayensis* is that the young rosette secretes a mucilaginous substance as recorded in the Andean species *Valeriana plantaginea* Kunth in Humboldt *et al.* (1819: 329) and *Oritrophium peruvianum* (Lamarck 1786: 316) Cuatrecasas (1961: 22), and in some Afro-alpine giant rosette plants, i.e. *Lobelia keniensis*, *Lobelia aberdarica*, *D. keniensis* (Young & Orden Robe, 1986; Beck et al., 1982). This mucilage was suggested to act as a thermal buffer that protects plant organs from freezing temperatures. Thermal

buffering requires the avoidance of intense supercooling. This is assured by the polysaccharide content of the mucilage that allows nucleation already upon slight supercooling (Beck, 1994). Further studies should be carried out to understand the significance and the role that this substance plays in *S. sangayensis*.

Conservation status:—This species is known from a restricted geographic area in the southern part of the Sangay National Park belonging to Chimborazo and Cañar Provinces (Ecuador). The size of the populations varied from 5 to 100 individuals and consisted mainly of adult individuals (flowering and senescent). Several communities are found in their vicinity (e.g. Atillo, Ozogoche, Achupallas, Pillcopata). The páramo where the species occurs is used for cattle grazing and potato cultivation. Based on this, the species is assigned a preliminary conservation status of Endangered (EN) using the IUCN red list criteria (IUCN, 2001).

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