Diversification and Speciation Patterns of Ground Beetles (Coleoptera, Carabidae) And Rove Beetles (Coleoptera, Staphylinidae, Pselaphinae) in the Highlands of Ecuador

Sofia Isabel Muñoz Tobar
Clemson University, smunoz@clemson.edu

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DIVERSIFICATION AND SPECIATION PATTERNS OF GROUND BEETLES
(COLEOPTERA, CARABIDAE) AND ROVE BEETLES (COLEOPTERA,
STAPHYLINIDAE, PSELAPHINAE) IN THE HIGHLANDS OF ECUADOR

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Entomology

by
Sofía Isabel Muñoz Tobar
August 2018

Accepted by:
Dr. Michael S. Caterino, Committee Chair
Dr. Peter Adler
Dr. Antonio Baeza
Dr. Sarah DeWalt
ABSTRACT

The tropical Andes are a biodiversity hotspot for numerous evolutionary lineages. Allopatric speciation and paleoclimatical events are the major drivers for species diversification in the tropics, especially for páramo species which show high diversity and endemcity. This dissertation elucidates speciation and diversification patterns of widely distributed species of beetles from isolated páramo patches across Ecuador, to provide insight into basic evolutionary processes for Andean insect species. Sampling targeted 17 sites in the páramo ecosystem (3500 – 4000 m), with pitfall trapping, hand collecting and leaf litter sampling. One nuclear and one mitochondrial marker were used to assess the genetic diversity of four beetle lineages within the ground beetles (Coleoptera, Carabidae) and ant–loving beetles (Coleoptera, Carabidae, Pselaphinae), through a combination of phylogenetics, divergence time estimates and population genetics. The analysis of beetle lineages from páramo reveals there is no general pattern of diversification in beetles from páramo. The effect of mountain isolation varies across ground beetles and ant–loving beetles, where the distribution of the genetic diversity for each beetle lineage appears to be influenced by several factors such as divergence time, range size, dispersal capability, and geological and paleoclimatical events that occurred in the Miocene–Pleistocene, as recorded for plant lineages from páramo.
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CHAPTER ONE
INTRODUCTION

Understanding common diversification patterns of different lineages is a key aim of evolutionary biology. The study of such patterns in hotspots, areas of high richness of species, can give us an insight into basic evolutionary processes and the distribution of genetic diversity at different spatial scales (Myers et al. 2000, Moritz 2002). The tropical Andes Mountains are considered a biodiversity hotspot for numerous evolutionary lineages (Myers et al. 2000). The páramo is a tropical alpine ecosystem between 2800–4700 m in the South American Andes Cordillera and is considered to be one the fastest evolving biodiversity hotspots in world (Madriñán et al. 2013). Many factors have played into shaping the current diversity in the páramo, such as allopatric speciation and climatic oscillation during glacial and interglacial periods (Neil 1999, Madriñán et al. 2013). Species in this ecosystem exhibit unique adaptations to extreme environmental changes and have evolved during the last 3–5 Ma (Pleistocene) when the Andes reached their current altitude (Somme and Block 2012, Madriñán et al. 2013). Consequently, the páramo represents an ideal model for studying diversification of species.

The páramo provides many ecosystem services such as hydrologic resources, biodiversity (i.e. genetic stock for food) and carbon sequestration. This ecosystem is threatened by agricultural expansion, livestock farming, mining activities and global warming (De Brievre and Calle 2011, Farley et al. 2011). The lack of studies related to diversification in the páramo has hindered the management of this endangered ecosystem.
Hence, the present research aims to determine topographic barriers involved in the diversification processes for beetle lineages by studying the diversification patterns of four widely distributed species of beetles in isolated páramo patches across the Ecuadorian Andes. Furthermore, I examine the relationship between species diversification with topographical barriers and paleoclimatic events such as the effect of the last glacial period, east and west vicariance (Balslev 1988, Society 2012), and the effect of dry valleys and rivers as barriers of species dispersal (Mira, Chimbo and Chanchan river and valley; Krabbe, 2008; Guayasamin et al., 2010). In addition, I test if certain areas of endemism, previously proposed for ground beetles, show genetic structuring among populations across páramo patches (Moret 2009). Lastly, Phylogenetic Species Concept (PSC) was employed in this study to distinguish species. This concept defines species “as the smallest diagnosable clusters of individual organism within which there is a parental pattern of ancestry and descent” as described by Cracraft, (1983).

My study illuminates patterns of diversification and should provide support for the importance of preserving the páramo as a hotspot for species diversification. My study specifically addresses the following questions:

1. How are genetic lineages of beetles distributed in Ecuadorian páramo?
2. Are the levels of genetic diversity similar on the eastern and western sides of the Ecuadorian Andes?
3. How have geological and paleoclimatic events affected beetle diversification and genetic structure for páramo beetle lineages?
4. How interconnected are beetle populations across páramo patches?
CHAPTER TWO

LITERATURE REVIEW

South American Biogeography

South America is a continent located in the Western Hemisphere that covers 12% of Earth’s land area, composed of tropical, subtropical and temperate regions (Veblen et al. 2007). This continent harbors the greatest biodiversity on Earth, containing five of the world’s biodiversity hot spots: Choco, Central Chile, Tropical Andes, Brazil’s Cerrado and Atlantic Forest (Myers et al. 2000). Five geotectonically units compose the South American continent, including kratons, epi–continental basins, neso–epikratons, peri–cratonic basins and the Andine geosyncline. The diversity in soil types is the result of the magmatic activity, stratigraphy, paleobiology and climate (Fittkau et al. 1969).

The biogeographical patterns in South America reflect the effect of fluctuating climate and biotic processes on slowly changing tectonic phases (Veblen et al. 2007). The origin of South American fauna appears to be a complex process. Species evolved from isolated Gondwanan roots, North American immigrants, temperate region species, as well as cosmopolitan species (Vuilleumier 1970, Veblen et al. 2007, Santos et al. 2009, Sklenář et al. 2011). Generally, South America is divided into three main ecological regions based on vegetation types: tropical, subtropical and temperate regions (Veblen et al. 2007). Still, biogeographical regionalization based on endemic taxa divides South American into 7 sub–regions: Pacific, Boreal Brazilian, South Brazilian, Southeastern Amazonian, Chacoan, Parana, and South America transition zones, with 41
floristic and faunistic provinces within those sub–regions (Morrone 2017). My study is focused on a temperate region of South America, the páramo province in high mountain peaks of the Andean Cordillera of Venezuela, Colombia and Ecuador (Morrone 2017). The Andes Cordillera has played an essential role in the ecological structure of South America by altering the landscape and climate (Fittkau et al. 1969).

**Geological origin of the Andes**

The Andes range is the longest mountain chain in the world, and it is located in the western margin of South America (Gregory–Wodzicki, 2000; Ramos & Aleman, 2000). The orogenic formation of the Andes started in the Mesozoic (late Cretaceous 100–66 Ma) and has peaked with massive uplift over the past 30 Ma (Orme 2007). The formation of the Andes involved five major sequences of events: plate subduction, magmatism, crustal shortening, terrain accretion and isostatic adjustments (Orme 2007).

The Ecuadorian Andes are mainly part of the Northern Andes range, which is divided into East and West Cordilleras, both separated by inter–Andean, high–altitude valleys (Neil 1999). The Eastern cordillera is composed of Paleozoic and Mesozoic–era metamorphic rocks, whereas the Western cordillera is composed of volcanic and pyroclastic rocks from the late Mesozoic era (Cretaceous; Neil, 1999; Orme, 2007). Volcanic activity during the Tertiary period (25–2.5 Ma) has greatly influenced the orogeny of the Andes. During this period, the Ecuadorian Andes experienced a major uplift due to volcanism. This volcanic activity extended into the Quaternary period (2.5 Ma), during which large amounts of volcanic ash has been deposited (Neil 1999).
Evolution of páramo species and orogeny

The northern Andes emerged beyond tree–line by the end of the Pliocene (3–5 Mya), which created the appropriate conditions for the development of páramo species (Sklenář et al. 2011). The páramo is a tropical alpine ecosystem located between the continuous forest line and perpetual snow line (3000–5000 m) in Central and South America (Ecuador, Venezuela, Colombia, Peru, Costa Rica and Panama; Luteyn, 1999; Josse, 2000). The páramo is characterized by the presence of tussock grasses, large rosette plants, shrubs and cushion plants (Luteyn 1999). The great majority of these plant species are endemic to this ecosystem, with close relatives in lowland–tropical, northern and southern hemisphere temperate regions (Luteyn 1999, Madriñán et al. 2013). Similar tropical alpine ecosystems occur around the world, such as the Zacatonales in Mexico and Guatemala volcanic highlands, the Jalca and Puna in northern Peru and drier areas of the Central Andes, and the Afroalpines and Moorlands in East Africa and Malaysia (Luteyn 1999).

The evolution of páramo species is linked to the Andean orogenic and paleoclimatical processes (Heine 2004, Sklenář et al. 2011). Quaternary glaciations (2.58 Ma–present; Gradstein et al., 2004) had an important impact in the contraction and expansion of protopáramo and páramo species (Sklenář et al. 2011). During the last glacial period (20 glacial cycles during the Pleistocene), vegetation zones were shifted upward by as much as 1,500–2,000 m, many of the isolated páramos merged, and the ecosystem had a greater area than in the present times (Sklenář et al. 2011). For
Ecuadorian Andes, the exact extent and advances of the Last Glacial Maximum (LGM; 21,000 years; Smith et al., 2008) are not well known and require more research (Heine 2004). Still, the mean lower limit of the glaciers for Ecuadorian Andes is estimated to be 4000–4100 m for the western cordillera, and 3500–3750 m for the eastern cordillera (M4 moraines during LGM; Heine, 2004; Smith et al., 2008). These paleoclimatological events have affected the distribution of páramo species through the contraction and expansion of this ecosystem (Hooghiemstra et al. 2006). Palynological records from the valley of Cajuma in southern of Ecuador support the hypothesis of the expansion of páramo to the inter–Andean valleys during glacial periods in the northern Andes, since herbaceous páramo was the main vegetation type in the Cajuma valley during the LGM (Villota and Behling 2014).

**Origin of páramo species**

Species that occur in the northern Andes are considered to be relatively young, and possibly are part of the Pleistocene diversifications (2–5 Ma; Hughes & Eastwood, 2006; Garzione et al., 2008; Madriñán et al., 2013). This is particularly understood for páramo plant lineages that comprise a combination of Neotropical, temperate (Austral–Antarctic and Holarctic) and cosmopolitan species (Cleef 1979). An important proportion of páramo plant species are of Neotropical origin–lowland species that gave rise to species adapted to high elevations through local adaptation (Cleef 1979, Sklenář et al. 2011). Still, species of temperate origin and cosmopolitan species have also contributed to the plant diversity seen in páramo (Cleef 1979, Sklenář et al. 2011). This trend is also
seen in vertebrate lineages (i.e. birds and amphibians), which are of South American and North American origin (Vuilleumier 1970, Santos et al. 2009).

The origin of tropical alpine insect faunas is thought to be a complex evolutionary process, which has not been studied in detail in the tropical Andes. Based on other tropical alpine systems, we understand that insect faunas in young tropical mountains comprise a mix of immigrant pre–adapted lineages to the alpine ecosystem (i.e. Holarctic species) and lineages from adjacent lowland areas that have been able to adapt to alpine ecosystems (i.e. subtropical and tropical areas; Merckx et al., 2015). This mix of lineages is found in vertebrates and plants in the tropical Andes (Vuilleumier 1970, Santos et al. 2009, Sklenář et al. 2011).

**Patterns of diversification for northern Andes species**

Factors that influence diversification rates among different species lineages can provide insight into macroevolutionary processes (Hughes and Eastwood 2006, Moyle et al. 2009). Tropical Andean mountains are hotspots of biodiversity, where allopatric speciation appears to be the main cause of rapid diversification (Madriñán et al., 2013; Merckx et al., 2015). Diversification patterns of plant lineages are well understood for plant lineages of the tropical Andes, where high rates of diversification were recorded during the Pleistocene (2–5 Ma; Sklenář et al., 2011). New species resulted from a combination of factors, such as ecological opportunities due to the uplift of the Andes, and isolation of species on the mountain tops promoted the diversity seen in páramo (Hughes and Eastwood 2006, Sklenář et al. 2011). In addition to geological events, the
repeated isolation and expansion of plant populations caused by Quaternary glaciations and habitat heterogeneity have been suggested as possible mechanisms that enhanced species evolution in páramo (Sklenář et al., 2011; Muñoz–Mendoza et al., 2017).

Different patterns of vicariance are recorded among Andean species. East and west vicariance appears to be the most common mechanism reported (Chaves et al. 2007, Guayasamin et al. 2010, Sklenář et al. 2011). This pattern has been described for several plant species in members of the genera *Brunfelsia* (Solanaceae) and *Phytelephas* (Arecales) from the Central and Northern Andes (Trenel et al. 2007, Filipowicz and Renner 2012, Luebert and Weigend 2014). Similarly, this pattern has been documented in vertebrate species. In the case of amphibians, it has been observed in members of the genera *Osornophryne* (Bufonidae) from the páramo of Colombia and Ecuador (Páez–Moscoso & Guayasamin, 2012) and *Atelopus* (Bufonidae) from the montane forest and páramo of Ecuador (Guayasamin et al., 2010). East and West vicariance has also been seen in bird species of the genera *Pionus* (Psittacidae) and *Adelomyia* (Trochilidae) from montane forest and páramo in the northern Andes (Chaves et al. 2007, Ribas et al. 2007). For insect lineages, the Andean uplift had an important role. East and west vicariance has been well documented for the clear wing butterflies (Nymphalidae, Oleriina), which diversified during the Pliocene and Pleistocene (2–5 Ma), where this pattern of vicariance is reported for species in the northern portion of the Andes (De–Silva et al., 2016). This pattern was also seen in species of the genus *Bembidion* (Carabidae) from the north of Ecuador (Maddison 2014), among many other examples.
Over a broader scale, north and south vicariance exists between northern and central Andes species. The Amotape–Huancabamba zone in the northern Peru appears to limit long–distance dispersal of species among these two regions of the Andes (Luebert and Weigend 2014, Morrone 2014). This vicariant pattern has been described for several plant species in the genera *Myrceugenia* (Myrtaeae; Murillo–A. et al., 2012), *Balsamocarpon* and *Zuccagnia* (Fabaceae; Ulibarri, 2005). This has also been recorded for vertebrate lineages (Morrone 2014) such as *Grallaria* birds (Grallariidae; Winger et al., 2015) from montane forest of the north and central Andes and lizards species of the genera *Pholidobolus* and *Macropholidus* (Gymnophthalmidae) from Ecuador and Peru (Torres–Carvajal & Mafla–Endara, 2013). North and south vicariance has also been reported for mammal lineages identified through the analysis of environmental resistance, species turnover and level of endemism (Ruggiero et al. 1998, Maestri and Patterson 2016). For insect lineages, this north and south vicariance has been observed in members of the tribe Troidini (Lepidoptera, Papilionidae; Condamine et al., 2012).

Although the north–south depression in the northern Andes of Peru is considered to be a major geographical barrier for the distribution of northern and central Andes lineages, some species have overcome this barrier (Luebert and Weigend 2014), and north–to–south interchanges were favored during Quaternary oscillations (5–7 Ma; Zachos et al., 2001; Luebert & Weigend, 2014). Based on floristic and paleoecological studies, there is enough evidence to suggest that the Andes were used as a dispersal corridor. Examples of the north–south interchange are seen in the plant genera *Azorella*, *Laretia* and *Mulimum* (Apiaceae), which originated in the southern portion of the Andes.
and are now present from Costa Rica to Patagonia (Nicolas et al. 2012). Similarly, this pattern is found in the genera *Oreobolus* (Cyperaceae; Chacón et al., 2006) and *Perezia* (Asteraceae; Simpson et al., 2009). Apart from plants, south and north vicariance has not been documented in other lineages. The Amotape–Huancabamba zone appears to be a significant barrier for mammals, birds and arthropods (Morrone 2014).

**Biogeographical patterns of species in the Ecuadorian Andes**

For species in the Ecuadorian Andes, the main phylogenetic disjunction is between species that live in the east and west cordillera. Several studies report east and west vicariance for lineages of amphibians (Guayasamin et al., 2010; Páez–Moscoso & Guayasamin, 2012), bats (Pinto, 2009), birds (Krabbe 2008) and insects (Maddison, 2014; De–Silva et al., 2016). Other important geographical barriers are dry valleys and rivers. Dry inter–Andean valleys have an important role in the delimitation of species distribution, which is particularly understood for bird and plant lineages in Ecuador (Krabbe 2008, Quintana 2010, Quintana et al. 2017). The Mira, Chimbo and Chanchan valley appear to mark the endpoints of the distribution for some high altitude species of birds and amphibians (Krabbe 2008, Guayasamin et al. 2010). The Mira, Chanchan and Pastaza rivers also reinforce the limits of species distribution (Krabbe 2008). The role of dry valleys and rivers has only been assessed through phylogenetic studies of the genus *Atelopus* (Bufonidae) from montane forests to páramo (300–4000 m) in Ecuador. Genetic clusters for this genus appear north and south of the Chimbo and Chanchan rivers and valleys, as well as north and south of Mt. Chimborazo (Guayasamin et al. 2010).
For páramo insect species, hypotheses of species distribution have only been proposed for ground beetles (Moret 2005, 2009). Moret (2009) proposed 11 areas of endemism along Ecuadorian páramo based on local surveys and diversity indexes that reflect the presence of endemic and non–endemic species. Moret's results (2005; 2009) provide evidence of continental insularity, which is fragmentation of an ecosystem which promotes high levels of endemism of species in the Andes (Anthelme et al. 2014). This pattern is partially supported by phylogenetic analyses done for the mayfly species *Andesiops peruvianus* (Ulmer, 1920) from the eastern cordillera of the Ecuadorian Andes. Finn et al. (2016) showed that *A. peruvianus* populations are isolated among mountains, where haplotypes were only shared between Cotopaxi and Antisana populations, but not with Chimborazo populations (Finn et al. 2016).

Basic evolutionary processes are not well understood for alpine insect lineages in the tropical Andes. General patterns of insect diversification and distribution of insect lineages have not been established using a phylogenetic approach. The effects of the hypothesized geographical barriers (east–west vicariance, dry valleys, rivers, continental insularity) and paleoclimatical events have not been tested for most páramo insect lineages. The study of populations of widely distributed species of beetles from the Ecuadorian páramo will improve the knowledge about evolutionary processes for terrestrial arthropods and will provide some insight into the main factors that explain general patterns, including rates and times of diversification. With a better understating of these evolutionary processes, we can point out areas of high genetic diversity, which should be taken into account for conservation purposes.
Keeping evolutionary potential is particularly important for hot spot areas, since the expansion of agriculture and climate change will have a profound impact on the distribution and abundance of insects living in alpine ecosystems (Cuervo and Restrepo 2007). Multiple altitudinal shifts in herbivorous insect species have recently been reported for several alpine insect around the world due to changes in temperature (Doucet et al. 2009, Robinet and Roques 2010). This is particularly important for insect species in the Andes due to the high levels of endemism (Dangles 2009, Fernanda and Donoso 2014), where the contraction of species ranges have been documented for some beetle species in the Ecuadorian Andes (Moret et al. 2016).
HYPOTHESES

Hypotheses in this study are divided into vicariance hypotheses and dispersal hypotheses. Vicariance hypotheses focus on aspects associated with the uplift of the Andes such as allopatric speciation, patterns or vicariance and divergence time estimates of beetle lineage. In comparison, dispersal hypotheses explore the role of the presence and absence of functional wings and their influence in the genetic structure beetle lineages from páramo. Also, maximum entropy models of species distribution can examined for correlation with patterns of genetic diversity of beetle lineages from the páramo, or contrasted when genetic patterns do not show correspondence with the projected distribution.

A. Vicariance hypotheses

Hypothesis 1: Páramo beetle lineages of wide distribution have similar genetic structuring.

With the analysis of four beetle lineages within the family Carabidae and Staphylinidae, I will be able to assess general evolutionary patterns for páramo beetle lineages. If similar patterns of genetic structuring are shared across beetle lineages, this could indicate that the same factors (abiotic/biotic) have helped shaped the current diversity in páramo (i.e. uplift of the Andes and climate oscillation), and might be consistently affecting genetic structure of beetle lineages from páramo. If each beetle species shows different genetic clustering patterns, this might be evidence of more complex evolutionary processes, such as different diversification rates, dissimilar levels
of connectivity across populations and even local adaptation (to microclimate and to resources). To understand differences in patterns, knowing more natural history traits, geographic distribution and the origin of lineages is essential.

**Hypothesis 2:** Beetle lineages that live in the páramo diversified during the Pliocene and Pleistocene epochs.

Páramo diversification rates are well understood for vascular plants. Recent phylogenetic studies have suggested that the flora of the páramo originated during the last 2–5 Ma (Hughes & Eastwood, 2006; Garzione *et al*., 2008; Madriñan *et al*., 2013). For beetle lineages, I expect to find similar divergence dates as seen in plants. The same evolutionary forces might have affected the rates of diversification in beetle lineages. The origin of an alpine insect fauna is a complex process because species in tropical mountains derive both from lowland lineages that have adapted to high elevation and from lineages arriving from temperate regions (Luteyn 1999, Merckx *et al*. 2015). In the context of Andean radiations, most species appear to have diverged starting in the Miocene and continued throughout Pliocene and Pleistocene (Elias *et al*., 2009; Luebert & Weigend, 2014; Muñoz–Mendoza *et al*., 2017).

**Hypothesis 3:** Beetle species of wide distribution in the Ecuadorian páramo are currently differentiating following east and west vicariance.

East and west vicariance is commonly seen among Andean lineages, and has been reported for plants, birds, mammals, amphibians and insect species (Sklenar *et al*., 2011,
Páez–Mosoco & Guaysamin, 2014; Maddison, 2014). Because high altitude alpine insects appear to be restricted to mountain tops, the interchange of genetic flow between populations on each side of the Andes could be infrequent. This hypothesis follows from the fact that many alpine species present a reduction or atrophy of wings, which will reduce flight activities (Hodkinson, 2005; Somme & Block, 2012). Still, not all beetle lineages might be following an east–west vicariance pattern. This could be an indication of high rate of dispersal between páramo patches (passive/active transportation); or that species have not diverged since the last glacial maximum when páramo was present in the inter–Andean valleys and beetles experienced panmixia; in this case, the genetic signal of east–west vicariance would still be low.

**Hypothesis 4:** Beetle species of wide distribution in the Ecuadorian páramo ecosystem are differentiating from north to south of dry valleys and rivers.

Dry valleys and flowing rivers appear to limit species distributions in the Ecuadorian Andes (Krabbe, 2008; Guaysamin *et al*., 2011). This pattern has been described through the phylogenetic studies of the genus *Atelopus* (Bufonidae), where species appear to have genetic clusters north and south of each dry valley and major rivers that crosses the Andes (Guayasamin *et al*., 2011). Beetle lineages might be following similar patterns, assuming that they have low dispersal capabilities and low thermal tolerances to other climates that prevent them from dispersing across inter–Andean valleys. On the other hand, not all beetle lineages might be following these patterns. Dry valleys and rivers might not be limiting species distribution, in particular
for beetle lineages that have better dispersal capabilities (e.g. wings), that maintain some level of migration between populations. Another possibility might be that there has not been enough evolutionary time since the last major climatological and/or geological event to have produced genetic structure in beetle populations.

B. Dispersal hypotheses

Hypothesis 5: Higher phylogeographic structure is observed in flightless beetle lineages when performing comparative analyses of winged and flightless species of beetles from paramo.

The secondary loss of wings in high elevation beetle lineages can affect the dispersal ability of flightless beetles and restrict the geographical areas they inhabit (Gutierrez and Menendez 1997, Ikeda et al. 2012). In contrast, macropterous (winged) species appear to present wider distributional ranges when compared to flightless species (Gutierrez and Menendez 1997, Ikeda et al. 2012). I expect to see higher genetic variation among populations of flightless beetle lineages compared to macropterous species, due to poor dispersal capability of beetles with reduction and absence of wings (Moret 2005). A lack of high genetic structure in flightless beetle lineages might be associated with the presence of gene flow between populations.

Hypotheses 6: Beetle lineages with wide distributional ranges show higher levels of genetic connectivity among populations.
Models of species geographic species distribution have an important role in ecological, biogeographical and conservation studies. Maximum entropy models generate predictive models of species geographical distributions (Phillips et al. 2006), which can be used to correlate with the genetic heterogeneity across the distribution of a lineage. I predict species with wider predicted distributions have higher levels of genetic connectivity across sites, while species with smaller predicted ranges might possess higher genetic structure. The lack of correspondence between distribution and patterns of genetic diversity, could be associated with more complex processes, such as dispersal ability of each beetle lineage and the presence of biogeographical barriers that limit species distributions.

In Chapter 3, which is focused in the widely distributed species *Pelmatellus columbianus* (Reiche, 1843) I test hypotheses 2, 3, 4 and 6 by using niche modelling in combination with phylogenetic and population genetics methods. In Chapter 4, which explores multiple flightless ground beetle lineages, I test hypotheses 1, 2, 3, 4, 5 and 6 by comparing intra- and interspecific genetic diversity across ground beetle lineages through phylogenetic analyses, population genetics indexes and niche modelling. Lastly, in Chapter 5, which estimates the number of species in the rove beetle lineage *Panabachia* through species delimitation analyses, I test hypothesis 1, 2, 3 and 4 through phylogenetic and population genetic methods.
CHAPTER THREE

WEAK GENETIC DIFFERENTIATION AMONG POPULATIONS OF THE ANDEAN GROUND BEETLE *PELMATELLUS COLUMBIANUS* (REICHE, 1843)  
(COLEOPTERA: CARABIDAE)

INTRODUCTION

The tropical Andes are a biodiversity hotspot for numerous evolutionary lineages (Myers et al. 2000, Veblen et al. 2007). Ranging from 500 to over 4800 meters, the tropical Andes comprise a great variety of ecosystems, from low land forest in the Amazon, to mid–elevation wet and dry forests, through cloud forest, to alpine grasslands, and permanently glaciated areas (Josse et al. 2009). Many factors shaped the existing diversity, although most tropical Andes models of diversification hypothesize vicariance as the primary driver of speciation (Brumfield and Edwards 2007, Guarnizo et al. 2009, Penz et al. 2015). Lower elevation forests such as the tropical and cloud forest, exhibit some of the highest biodiversity anywhere on earth (Terborgh 1977, Gentry 1988, Hoorn et al. 2010), and have received substantial attention for their species–rich ecosystems. Nevertheless, at higher elevations, alpine ecosystems such as páramo exhibit unique biota (Madriñán et al. 2013) that have received much less attention.

Páramo is a tropical alpine ecosystem located between the continuous forest line and perpetual snow line (2800–5000 m) in Central and South America (Luteyn, 1999; Josse, 2000). Plant species in this ecosystem are relatively young (2–5 Mya) and highly endemic (Hughes & Eastwood, 2006; Garzione et al., 2008; Madriñán et al., 2013). The
high number of endemic species and high diversification rates for páramo plants are attributed to the complex orogeny due to the uplift in the northern Andes (30–2 Mya), and paleoclimatical events during the Pleistocene (Gregory-Wodzicki, 2000; Hughes & Eastwood, 2006; Madriñán et al., 2013). Diversification seemingly resulted from a combination of factors including, the creation of new niches with the uplift of the Andes, and the isolation of high elevation–adapted populations from their lower–elevation ancestors (Hughes and Eastwood 2006, Sklenář et al. 2011). In addition to geological events, the repeated isolation and expansion of plant populations caused by Quaternary glaciations and habitat heterogeneity have been suggested as possible mechanisms that accelerated the evolution of páramo species (Sklenář et al., 2011; Muñoz–Mendoza et al., 2017).

Most páramo species are thought to have originated from lowland tropical lineages, but temperate (Austral–Antarctic and Holarctic) and cosmopolitan lineages have also contributed to páramo diversity (Cleef 1979, Sklenář et al. 2011). Currently, we have a good understanding about the diversification rates of vascular plants, which have led to the suggestion that páramo is one of the fastest evolving hotspots in the world (Madriñán et al. 2013). For other lineages, studies that include páramo species are focused in bigger phylogeographical patterns in South America (i.e. amphibians & reptiles; Santos et al., 2009; Goicoechea et al., 2012), where allopatry is reported between highland and lowland species. In cases of insect lineages, isolation of species of butterflies and mayflies have been reported between mountains in the Northern Andes (Finn et al. 2016, Pyrcz et al. 2017); and east and west vicariance has been observed in phylogenetic
analyses of highland species of ground beetles (Maddison 2014). Still, not much is known about diversification rates or patterns of genetic diversity of insect lineages in páramo.

Ground beetle species from the Ecuadorian páramo are taxonomically well known, where ground beetle communities are composed mainly of micro and mesoendemic species, with only a few putatively widespread species (Moret 2005, 2009). Recent studies in the Ecuadorian Andes have reported shifts in elevational range for several carabid species due to climate change, including widely distributed species (Moret et al. 2016). Having a better understanding about the genetic diversity and connectivity levels of a beetle lineages in a highly fragmented ecosystem such as páramo will aid conservation efforts for Andean species, clarifying where páramo and adjacent ecosystems are critical for maintaining large–scale connectivity among regions. Conversely, more accurate delimitation of endemic species is crucial for the conservation of species that have restricted ranges in the Ecuadorian Andes (Cuesta, Peralvo, et al. 2017).

*Pelmatellus* Bates 1882 is a new world genus of carabid beetles in Nearctic and Neotropical regions (Bousquet 2012). This genus belongs to the Harpalinae subfamily, a species–rich clade of carabids characterized by diverse morphological forms and ecological interactions (Ober and Heider 2010). A total of 28 species have been described (2 Nearctic, 26 Neotropical; Bousquet, 2012), including local endemics and other more widespread species. *Pelmatellus* species are present in a variety of ecosystems from oak–pine forest to tropical cloud forest (Goulet 1974, Moret 2005). This clade originated in
the mid Cretaceous and underwent rapid diversification in the late Cretaceous to early Paleogene (Ober and Heider 2010).

*Pelmatellus columbianus* (Reiche 1843) is a macropterous species of wide distribution, present at high elevations in Venezuela, Colombia, Ecuador and Peru (Perrault 1994, Moret 2005, Kroschel and Cañedo 2009). Populations of *P. columbianus* are found between 2100 and 4150 m, in a vast diversity of vegetation types, from montane forest, inter-Andean valleys and páramo (Moret 2005). Currently, little is known about this species’ biology. Studies conducted in potato growing areas of Peru report *P. columbianus* as a beneficial insect in agricultural systems (Kroschel and Cañedo 2009). This ground beetle feeds on seeds and preys on phytophagous insects that feed on potatoes (Kroschel and Cañedo 2009, Cañedo and Kroschel 2012). Its preferences in natural systems are unknown, and its wide distribution in an area of otherwise high endemism is unusual.

As a species of wide distribution, *P. columbianus* is an ideal candidate for testing biogeographic hypotheses. The analyses of genetic diversity across a wide geographic range can reveal if the apparent wide distribution reflects an actual range of distribution or inadequate taxonomic resolution, which may reflect the presence of cryptic species. Understanding the distribution of genetic lineages of a widely distributed species can also show areas of high gene flow between beetle populations, as well as areas of high genetic diversity, revealing historical patterns of range expansion. In contrast, areas with high connectivity levels may reveal corridors that can be used as an aid in the effort to conserve highland species. The aim of this study is to assess the distribution of genetic
diversity for \textit{P. columbianus}, through analyses of one mitochondrial and one nuclear gene. By analyzing the genetic diversity of this beetle, I test if this beetle lineage constitutes the same biological unit across the Ecuadorian páramo, or if potential geographical barriers (i.e. east and west cordillera, dry valleys and rivers) are shaping the intraspecific diversity along the Ecuadorian Andes. Also, I estimate the levels of genetic connectivity between beetle populations through the analyses of F\textsubscript{ST} values, which could reveal the role of dispersal for maintaining gene flow between páramo patches. Because populations of \textit{P. columbianus} are not restricted to páramo but are present at lower elevation in the inter–Andean valleys, we might expect that adjacent sites would have a higher level of genetic connectivity, and distant populations could show higher differentiation levels. I also estimate the age of the \textit{P. columbianus} clade to understand if this species is contemporary with most species in the northern Andes (2–5 mya). This will allow us to compare the rates of diversification of beetle and plant lineages, from which we will better understand the drivers of species evolution, and determine if this beetle lineage was affected by the same paleoclimatrical and orographic events. Lastly, I estimate the present and past distribution of \textit{P. columbianus}, using bioclimatrical variables through a maximum entropy model to have a better understanding of the present distribution and potential changes since the last glacial maximum (LGM).
METHODS

Sample collection

Samples were collected at 8 sites along the Ecuadorian Andes between 3100 and 4000 m in national parks during two field seasons in the summer 2015 and 2016 (Figure 3.1, Table 3.1). The localities were chosen based on conservation status and patterns of vicariance observed in other highland lineages, where geographical barriers such as the east and west mountain ranges (Chaves et al. 2007, Guayasamin et al. 2010, Sklenář et al. 2011); dry valleys (Mira, Chimbo and Chanchan) and rivers (Mira, Chanchan and Pastaza (Krabbe 2008, Guayasamin et al. 2010, Quintana et al. 2017) are shaping the distribution of plant and vertebrate species.

Permissions necessary for collection and exportation of samples were previously obtained (MAE–DNG–ARGG–CM–2014–004). Sampling employed manual collecting (underneath rocks and vegetation) and pitfall trapping using two 100 m transects (1 trap every 10 m) where traps ran for 2–3 days. Adult beetles were collected in 100% EtOH for DNA preservation. The specimens were identified using Moret’s (2005) taxonomic keys. Voucher specimens will be deposited in the QCAZ museum (Quito, Ecuador) once the study is completed.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from the pronotum muscles using GeneJet Genomic DNA Purification Kit (Thermo Fisher Scientific, Vilnius, Lithuania). For the
population genetic analyses I chose one mitochondrial (COI) and one nuclear protein-coding gene (CAD), using 10 individuals of each species per site.

Samples were amplified in 25 μl reactions, containing 17.5 μl water, 2.5 μl 10x buffer, 0.5 μl dNTPs, 0.75 μl MgCl₂, 0.1 μl AmpliTaq® DNA polymerase (Thermo Fisher Scientific) and 1 μl of each primer (10nm). Amplification cycles were performed in a Mastercycler® nexus (Eppendorf), using COI primers C1–J–2183 (5’–CAACATTTATTTTGGATTTTTTG–3’) and TL2–N–3014 (5’–TCCAATGCACTAATCTGCCATATTA–3’, Simon et al., 1994), using the amplification profile described by Caterino & Tishechkin (2014). CAD was amplified using the primers CD439F (5’–TTCAGTGACARTTYCAYCCHGARCAYAC–3’) and CD688R (5’–TGTATACCTAGAGGATCDACRTTYTCCATRTTRCA–3’, Wild & Maddison, 2008) following the amplification profile described by Wild & Maddison (2008). PCR products were purified using ExoSAP–IT (USB/Affymetric, CA, U.S.A), and sequencing was done commercially by Macrogen USA, Inc. (Rockville, MD, U.S.A).

**Data Analysis**

All DNA sequences were edited in Geneious R8 (Biomattters Ltd., Auckland NZ) and aligned using Mafft v.7 (http://mafft.cbrc.jp/alignment/server/). Models of molecular evolution for each marker were assessed using JModeltest 2.0 (Darriba et al. 2012), and GTR+I+G model was the best fit for COI and CAD data.

To determine the distribution of genetic lineages of *P. columbianus* across the highlands of Ecuador, phylogenetic analyses were performed to determine relationships
among individuals of each species using Mr. Bayes 3.2 (Huelsenbeck and Ronquist 2001) and RAxML v.8.0.0 (Stamatakis 2014). This was complemented with the construction of haplotype networks for each species using TCSv1.21 (Clement et al. 2000) and DnaSp (Rozas et al. 2003), which provided a perspective on the relationships among individuals, and quantified intraspecific variation for each species across its range of distribution.

To assess each population’s structure, \( F_{ST} \) were calculated in Arlequin 3.5.2.2 (Excoffier & Lischer, 2010). The analysis of the molecular variance (AMOVA) was performed to test the influence of geographical barriers, by testing different groupings: 1) east and west vicariance, 2) northern and southern populations, and 3) species grouping according to geographical barriers such as rivers and dry valleys. A correlation between intraspecific diversity and geographical isolation was calculated using Arlequin 3.5.2.2 (Excoffier & Lischer, 2010), with 1000 randomizations. The number of populations along the species distribution was determined by calculating the K in the population using Structure 2.3.4. (Pritchard et al. 2000). Lastly, the number of migrants per generation was estimated using DnaSP (Rozas et al. 2003).

To understand the timing and rate of diversification in these highland ecosystems, divergence times were estimated using an uncorrelated relaxed clock in BEAST 2.0 (Bouckaert et al. 2014) for the COI and CAD data matrixes. Three points of calibration all designating minimum node ages within Harpalini were used: one Harpalini fossil from the early Paleocene (61.0 – 65.0 Mya) from the Staratschin cap (Heer, 1870); a second fossil in the genus *Bradycellus* from the middle Eocene (44.1 Mya) of Baltic origin
(Wichard et al. 2002); and a fossil in the genus *Stenolophus* from the late Eocene (34.9–38 Mya) from Florissant, Colorado (Cockerell 1913).

**Maximum entropy models for species distribution predictions**

To predict present and past distributions of *P. columbianus*, the ecological niche was modeled to identify potential distribution areas using present and past (21,000 y) bioclimatic variables at 2.5’ spatial resolution (WorldClim v.1.4; Fick & Hijmans, 2017) in MAXENT 3.3.3 (Phillips et al. 2006). Geographical information from this study, as well as georeferenced localities reported in the literature (Moret, 2005), were gathered with a total of 51 occurrence records. The model of distribution was based on five bioclimatic variables: BIO1 = Annual Mean Temperature, BIO2 = Mean Diurnal Range (Mean of monthly (max temp – min temp)), BIO5 = Max Temperature of Warmest Month, BIO6 = Min Temperature of Coldest Month, BIO12 = Annual Precipitation, BIO13 = Precipitation of Wettest Month and BIO14 = Precipitation of Driest Month. Default settings were used for the convergence threshold (10^{-5}), with 500 iterations and 10 replicates using a 10% training presence. Output from the average run was reclassified using ArcMap 10.4.1 (ESRI, 2016) with a 10% threshold. Model performance was evaluated using the area under the curve (AUC) calculated by MAXENT, values between 0.7 and 0.9 indicated a good discrimination (Swets 1988). Lastly, the raster file of each replicate was used to compare past and present distribution with a t–test.
RESULTS

Sampling and data collection

Sampling effort resulted in the collection of 164 adult *P. columbianus* from 8 localities (Figure 3.1). For the population genetic analyses, I used 10 individuals per site (Table 3.1). The portion of the amplified COI gene was 820 bp long with 52 parsimony informative sites, yielding 9 haplotypes (Figure 3.2) from 80 individuals, with one haplotype present throughout the species distribution (GenBank accessions MG792176 to MG792184). The nuclear CAD had 631 bp, with 37 parsimony informative sites and 22 alleles from 73 individuals sequenced (GenBank accessions MG792185 to MG792206). Seven heterozygotes were observed for CAD locus, which were inferred with DnaSP (Rozas et al. 2003).

Data analyses

MtDNA based analysis in Structure shows one main population (K=1). The 9 haplotypes identified for COI exhibited low levels of overall diversity ($\pi=0.03737$). Most individuals analyzed belonged to haplotype 1 (N=51). Nevertheless, unique haplotypes were observed for three localities in the East Cordillera: Huagraguasi, Releche and Culebrillas, as well as for three northern localities: Cayambe, Pichincha and El Angel (Figure 3.3A). These results are consistent with the neutrality test ($D=-2.10276$; Table 3.3), where rare alleles appear at high frequency, and might denote a rapid population
expansion. Still, the Mantel test performed with $F_{ST}$’s and geographic distances show no
significant correlation ($p=0.18$, $R^2=0.004$), between genetic and geographical distances.

Results from the structure analyses of the nuclear coding gene showed four
clusters ($K=4$). A total of 22 alleles were identified using TCS and DNAsp. However, the
genetic diversity for this marker was low ($\pi=0.00429$, Table 3.3). Half of the individuals
analyzed belonged to haplotype 2 (Figure 3.3B), with some unique haplotypes in most
localities sampled, especially for the northern portion of the distribution. The Tajimas D’s
for the CAD data ($D=-1.82500$, Table 3.3) also indicated population expansion and a
high number of rare alleles in the population. The Mantel test also showed no correlation
between genetic and geographic distances ($p=0.8$, $R^2=0.04$).

The AMOVA was used to test different hypotheses, including east and west
vicariance, north and south vicariance and potential geographical barriers (Table 3.2).
Results from both molecular markers showed that most of the genetic variation was
found within populations. Based on COI data, a higher variation among groups was
observed when two eastern localities, Cayambe and Releche, were considered as separate
groups (28.30%; Table 3.2). CAD showed a higher variation among groups when the
locality of Releche was treated as a separate group (21.21%; Table 3.2). Overall $F_{ST}$
values exhibited low genetic differentiation between populations (COI $F_{ST}=0.3$; CAD
$F_{ST}=0.23$). Based on COI data, there is high genetic differentiation between the
northeasternmost locality of Cayambe and the southwesternmost locality of el Cajas
($F_{ST}=0.60$). Some level of genetic differentiation was recorded between the eastern
localities of Cayambe and Huagrahuasi ($F_{ST}=0.50$). For the CAD data set, $F_{ST}$ values
revealed some level of differentiation between Cayambe and Huagrahuasi ($F_{ST}=0.52$), as well as for Pichincha and Huagrahuasi ($F_{ST}=0.50$). The overall number of migrants per generation calculated with both genes showed an average of one migrant per generation ($N_m=1–1.5$; Table 3.3). When comparing the number of migrants population by population, there were a higher number of migrants between Culebrillas and el Cajas ($n_m=23$), located at opposite sides in the southern Ecuadorian Andes. Similarly, a high number of migrants was recorded for two adjacent sites Releche and Huagrahuasi ($n_m=3.4$), located in the northeast of the Ecuadorian Andes.

**Phylogenetic relationships and divergence time estimation**

Phylogenetic relationships of *P. columbianus* for COI sequences using Bayesian and Maximum Likelihood (ML) yielded trees with similar support (Figure 3.2A). Most of the specimens sampled were in the *P. columbianus* clade. However, haplotype 6 might be a new undescribed species for the genus *Pelmatellus*, from the east mountain range locality of Releche (Figure 3.2A), appearing as sister to the outgroup *Pelmatellus leucopus*. These results were consistent with the TCS analysis (Figure 3.3A), which yielded two separate networks. The analyses among COI haplotypes showed low levels of divergence, with only three haplotypes (H2, H7, H9) representing two distinct geographical areas of distribution. Haplotype 2 (Pichincha and Cayambe) and haplotype 7 (Pichincha) represent northern localities, while haplotype 9 (Cashca Totoras, Releche and Culebrillas) represents southern localities. COI data show that most areas sampled have a dominant haplotype (H1), or a closely related haplotype, haplotype 4. Low
haplotypic diversity was especially marked for specimens from el Cajas, with only haplotype 1 recorded.

Phylogenetic relationships using CAD showed a higher number of haplotypes (N=22). Similarly, with COI results, most individuals sampled belonged to the *P. columbianus* clade, with good clade support (Figure 3.2B), with the exclusion of haplotype 16, which appears to be a new species (Figure 3.2B, 3.3B). Unique haplotypes were recorded for all sampled sites, with the exception of the locality of Pichincha, which showed 4 haplotypes. Low levels of divergence were also observed for this nuclear gene, where haplotype 2 is the dominant haplotype across the distribution of the species. Closely related haplotypes 4 and 9 were also present at high frequency across sites. No clear pattern of vicariance was observed, though Cayambe and Huagrahuasi differ from the rest of the clade with strong posterior probability support (1–0.60; Figure 3.2B).

A relaxed molecular clock was imposed on the combined COI and CAD dataset. These analyses support the origin of the *P. columbianus* clade in the Miocene, 11.19 Mya (6.5–22 Mya). The timing of splitting–events for the *Pelmatellus* lineage (Figure 3.4) shows that this lineage is older than most páramo plant species (2–5 Mya; Madriñán et al., 2013), but contemporary with the evolution of highland species in the northern Andes mountain chain which arose during the Miocene (Weir 2006, Hines 2008).
Maximum entropy models for species distribution predictions

The AUC values for *P. columbianus* for present and past (21,000 y) distributions showed a high predictive ability of the ecological niche model under a random model, with AUC values of 0.997 for both scenarios. Populations of this ground beetle are restricted to highland areas of the Ecuadorian northern Andes (Figure 3.5), but are also present throughout the Andes into Colombia and Peru. There was a significant difference between present and past distributions (p > 0.001), and an increase of 20% of the species distribution was recorded since the LGM (Figure 3.5B), which suggests an expansion of the species' range.

DISCUSSION

Genetic diversity and structure

The tropical Andes are an area of high diversity of species, where some of the highest rates of diversification have been recorded in plant lineages (Vuilleumier 1970, Madriñán et al. 2013, Smith et al. 2014). Phylogeographic studies for South American species are still scarce, with more studies for plant and vertebrate lineages (Turchetto–Zolet et al., 2013). Prominent contributions have been made for insect species from lowland areas in the Neotropics (e.g., *Triatoma* and *Anopheles*; Turchetto–Zolet et al., 2013). The few studies of highland insects, and general patterns observed in other organisms, indicate that the uplift of the Andes and paleoclimatical events have played an important role in the diversification of highland species of the Andes. The role of
allopatry and paleoclimatical events has not been evaluated for most alpine insect species in the Ecuadorian Andes.

Results from this study suggest that the ability of individual species to disperse between fragmented ecosystems plays an important role in the pattern of genetic diversity (Anthelme et al. 2014). The genetic variation in populations of *P. columbianus* shows high levels of genetic connectivity across páramo sites, with the presence of dominant haplotypes recorded throughout the sampled sites (Figure 3.3), for both nuclear and mitochondrial markers (Figure 3.3–3.4). A higher number of haplotypes was found in the nuclear coding gene, which did not provide higher resolution for the patterns of genetic diversity. Most of these nuclear haplotypes appeared to be adjacent haplotypes (differing by single mutations) to the dominant haplotypes. Additional analyses, including overall F$_{ST}$ values (F$_{ST}$=0.23–0.3) and the hierarchical AMOVA (Table 2), support the idea that populations of *P. columbianus* had high levels of genetic connectivity between populations in the recent past (Miocene–Pliocene).

Species with low genetic divergence are the result of either a recent speciation process or high levels of genetic exchange (Petit & Excoffier, 2009). In the case of *P. columbianus*, high levels of gene flow are evident based on standard genetic diversity indexes. With the use of the divergence time estimation, we infer that this ground beetle originated in the Miocene (~11.15 Mya), which is contemporary with the evolution of several highland species in the northern Andes (Weir 2006, Hines 2008, Hoorn et al. 2010). Similarly, highland species of *Bombus* (Hymenoptera, Apidae) and *Ithomia* (Lepidoptera, Nymphalidae) diverged in the Miocene 6–8 Mya (Hines 2008, Elias et al. 2010).
2009). This is contemporary with the uplift of the northern Andes (Hoorn et al. 2010) but older than most vascular plants in the páramo ecosystem (2–5 Mya), which exhibit accelerated rates of speciation (Madriñán et al. 2013). Populations of *P. columbianus*, therefore, probably were present through several cycles of glaciation that occurred during the Quaternary (Gradstein et al. 2004). Paleoclimatological events in combination with orogenic changes are important mechanisms for the origin of new species in the Andes (Sklenář et al., 2011; Muñoz–Mendoza et al., 2017). Still, *P. columbianus* has maintained its species integrity across the Ecuadorian Andes (~600 km). The movement of highland vegetation zones to lower elevations during glaciation periods, possibly favored gene flow between páramo patches, and might be a key factor to understanding the genetic diversity of this ground beetle. Other patterns of population structure might be observed with the addition of samples from other parts of the species range including Peru across the Huancabamba depression, which is a major biogeographical barrier for species in South America (Duellman 1979, Weigend 2004). Further collections are needed to test this hypothesis.

Even though this ground beetle species has low levels of genetic diversity across the geographic range I sampled, unique haplotypes were reported for most sampled sites, with a higher number of single haplotypes for the northern localities (i.e. Cayambe and El Angel). Individual *F*ₚₜ values show genetic differentiation between some (mostly non–neighboring) populations (Table 4). For example, individuals from the Cayambe (NE) population show a high *F*ₚₜ value (*F*ₚₜ = 0.60) when compared to individuals from El Cajas (SW), displaying some genetic differentiation between east and west populations.
Moderate levels of genetic differentiation were observed between neighboring Pichincha (NW) and Huagrahuasi (NE) populations, as well as between Cayambe (NE) and Huagrahuasi (NE). We might have expected to see a higher number of unique haplotypes at higher elevation sites, assuming some local adaptation, but this was not the case. At El Cajas, where fieldwork was done at ~3900 m above sea level, I recorded only 4 haplotypes (COI =1, CAD=3), making this site the least diverse population, while populations from Cayambe, El Angel and Releche, sampled at lower elevations (3100–3700 m) exhibited 10–12 haplotypes per site, more than double that recorded for el Cajas. The lower number of haplotypes observed in el Cajas could also reflect cycles of local extinction and recolonization by founder individuals that reset individual populations each time with less genetic diversity, where smaller populations show less genetic diversity (Frankham 1996). Without a systematic sampling across el Cajas and adjacent areas, I cannot be certain which of these processes are happening with *P. columbianus* populations in this area.

**Maximum entropy models and species distribution**

Determining this species distribution was key to understanding the effect of potential geographical barriers (i.e. dry valleys and rivers; Krabbe, 2008; Guayasamin et al., 2010; Quintana et al., 2017). Niche modeling analyses using present and past distributions (LGM – 21,000 y) corroborate the hypothesis that *P. columbianus* is distributed mainly in highland areas, including páramo (3000–5000 m) and inter–Andean valleys (above 2000), and probably extending into high montane forest. Yet, there is
higher prevalence of the species at higher elevation. The t–test between past and present distributions shows they are significantly different (p < 0.001); current populations of *P. columbianus* are expanding toward lower areas (20% expansion). This is partially supported by the negative value of Tajima’s D (Table 3), which reveals that populations are increasing in size, and going through an expansion process. Even though there is low haplotypic diversity for both molecular markers, a recent bottleneck was discarded as a potential answer for the negative value of Tajima’s D. In case of a bottleneck, rare alleles are the first alleles to be lost in the population, and populations of *P. columbianus* have a high frequency of rare alleles across populations. The haplotypic diversity for this beetle species might be the result of sweeping selection, a reduction of allelic diversity due to the occurrence of beneficial mutations, and this event might be taking place in *P. columbianus* populations given the haplotypic diversity and other statistical indices calculated.

Overall, the Inter–Andean valleys in the Ecuadorian Andes appear to be a key component for maintaining genetic flow for this ground beetle’s populations. Other life history traits, such as fully developed wings for dispersal and an omnivore diets (seeds and insects; Moret, 2005; Kroschel & Cañedo, 2009), have favored this ground beetle’s wide distribution. The analyses of its genetic diversity revealed that it is a good disperser, present through highly fragmented habitats (páramo and inter–Andean valleys) in the Ecuadorian Andes. Still, most highland species in the Northern Andes are affected by habitat transformation, which includes expansion of agriculture and other forms of human
impact since most of the major cities in the Andes are in inter–Andean valleys (De Brievre and Calle 2011).

**Management implications**

The future of highland species will be affected by conservation policies. The use of biological corridors appears to be particularly important for species, such as *P. columbianus*, that are present at different elevational zones through fragmented ecosystems, where the conservation of adjacent habitats will help to maintain gene flow between populations. Research on implementation of corridors has been partially conducted in continental Ecuador, with the focus on 9 areas between reserves (Cuesta, Peralvo, et al. 2017), including two biological corridors: Illinizas–Mindo–Nambillo mountain range and Cajas Masiff, which include páramo sites. Results from the present study reveal that on average there is 1 migrant per generation between the sampled sites (nm=1–1.5; Table 3). But when compared population by population, the highest number of migrants per generation was between El Cajas and Culebrillas (nm=23), followed by Huagrahuasi and El Releche (nm=3.4). Adjacent areas that appear to have high connectivity levels could be considered as potential new corridors that can be used as an aid to conserve highland species, especially for meso and macro–endemic species that are not restricted to a particular ecosystem.

Contrary to what was initially expected, a higher population structure across a complex landscape such as the Andes, *P. columbianus* populations show the importance of understanding factors that affect the distribution of a species, particularly their
dispersal ability and distributional ranges. We still lack relevant data on the natural
history and physiological mechanisms to cope to with changes in the abiotic factors
between ecotopes for this species. These results raise the question about the patterns of
genetic diversity for other highland lineages, given that the northern Andes are a highly
diverse area of the world (Smith et al. 2014). Lineages with smaller elevational ranges
and decreased dispersal ability might show a higher population structure across the
northern Andes, as seen in other terrestrial arthropods in other mountain systems in the
world (Hodkinson 2005).
Table 3.1. Geographical coordinates and elevation for 10 sampling sites for *P. columbianus* from Ecuadorian Andes. Data set compiled from 2015 to 2016.

<table>
<thead>
<tr>
<th>Geographical area</th>
<th>No.</th>
<th>Location</th>
<th>Latitude</th>
<th>Longitude</th>
<th>N</th>
<th>Altitude (m)</th>
<th>Col. Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Andes</td>
<td>1</td>
<td>El Angel</td>
<td>N00°42.3521'</td>
<td>W77°57.985'</td>
<td>11</td>
<td>3301</td>
<td>26–Jul–2016</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Pichincha</td>
<td>S00°11.259'</td>
<td>W78°32.432'</td>
<td>10</td>
<td>3897</td>
<td>22–Jun–2016</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Cascha Totoras</td>
<td>S01°43.485'</td>
<td>W78°57.183'</td>
<td>10</td>
<td>3509</td>
<td>13–Jun–2015</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Cajas</td>
<td>S02°47.020'</td>
<td>W79°13.438'</td>
<td>12</td>
<td>3956</td>
<td>20–Jun–2015</td>
</tr>
<tr>
<td>East Andes</td>
<td>5</td>
<td>Cayambe</td>
<td>S00°02.101'</td>
<td>W78°03.608'</td>
<td>12</td>
<td>3743</td>
<td>01–Jul–2016</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Huagrahuasi</td>
<td>S01°05'0.99&quot;</td>
<td>W78°26'36.99&quot;</td>
<td>10</td>
<td>3937</td>
<td>09–Jul–2016</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Releche</td>
<td>S01°38.400'</td>
<td>W78°30.426'</td>
<td>10</td>
<td>3124</td>
<td>08–Jul–2016</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Culebrillas</td>
<td>S02°28.337'</td>
<td>W78°53.719'</td>
<td>12</td>
<td>3799</td>
<td>15–Jun–2015</td>
</tr>
</tbody>
</table>
Table 3. 2. Results from the Analysis of the Molecular Variance (AMOVA) for *P. columbianus*. Data were partitioned to test potential geographical barriers such as western and eastern cordilleras, and Mira, Chimbo, and Chanchan rivers and valleys.

<table>
<thead>
<tr>
<th>No. groups</th>
<th>Partitions</th>
<th>Tests</th>
<th>COI</th>
<th>CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Among groups</td>
<td>Among populations</td>
</tr>
<tr>
<td>2</td>
<td>(1,2,3,4)(5,6,7,8)</td>
<td>E–W</td>
<td>0.52</td>
<td>13.78</td>
</tr>
<tr>
<td>2</td>
<td>(1,2,5,6)(3,4,7,8)</td>
<td>S–N</td>
<td>0.52</td>
<td>13.78</td>
</tr>
<tr>
<td>4</td>
<td>(1)(2,5,6)(3,7)(4,8)</td>
<td>Barriers</td>
<td>–1.58</td>
<td>15.45</td>
</tr>
<tr>
<td>3</td>
<td>(1)(2,5,6)(3,4,7,8)</td>
<td>Barriers II</td>
<td>–3.14</td>
<td>16.38</td>
</tr>
<tr>
<td>2</td>
<td>(1)(2,3,4,5,6,7,8)</td>
<td>Mira Rvr</td>
<td>–6.43</td>
<td>16.44</td>
</tr>
<tr>
<td>2</td>
<td>(7)(1,2,3,4,5,6,8)</td>
<td>7 vs all</td>
<td>25.37</td>
<td>5.35</td>
</tr>
<tr>
<td>2</td>
<td>(6)(1,2,3,4,5,7,8)</td>
<td>6 vs all</td>
<td>–4.01</td>
<td>15.49</td>
</tr>
<tr>
<td>2</td>
<td>(5)(1,2,3,4,5,6,7,8)</td>
<td>5 vs all</td>
<td>17.91</td>
<td>7.31</td>
</tr>
<tr>
<td>3</td>
<td>(5)(7)(1,2,3,4,6,8)</td>
<td>NE vs all</td>
<td>28.30</td>
<td>–1.48</td>
</tr>
<tr>
<td>2</td>
<td>(5,7)(1,2,3,4,6,8)</td>
<td>NE vs all</td>
<td>13.01</td>
<td>7.36</td>
</tr>
<tr>
<td>3</td>
<td>(5)(7)(6)(1,2,3,4,8)</td>
<td>–</td>
<td>24.71</td>
<td>–3.07</td>
</tr>
<tr>
<td>5</td>
<td>(1)(5)(6)(7)(2,3,4,8)</td>
<td>–</td>
<td>22.79</td>
<td>–4.60</td>
</tr>
<tr>
<td>6</td>
<td>(1)(5)(6)(7)(2,3)(4,8)</td>
<td>–</td>
<td>19.71</td>
<td>–4.42</td>
</tr>
</tbody>
</table>
Table 3.3. Overview of the populations genetic indexes for *P. columbianus* populations.

N refers to the number of individuals sampled; S number of segregating sites; Ps number of polymorphic sites informative sites; \( \pi \) is a measure of nucleotide diversity; \( \theta \) is a measure of genetic diversity; Nm number of migrants per generation; D represents the Tajima’s D a neutrality test statistics.

<table>
<thead>
<tr>
<th>Gene</th>
<th>N</th>
<th>S</th>
<th>Ps</th>
<th>( \Theta )</th>
<th>( \pi )</th>
<th>Nm</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>COI</td>
<td>84</td>
<td>50</td>
<td>49</td>
<td>0.01567</td>
<td>0.00418</td>
<td>1.02</td>
<td>–2.10276 (p=0.05)</td>
</tr>
<tr>
<td>CAD</td>
<td>76</td>
<td>37</td>
<td>37</td>
<td>0.01082</td>
<td>0.00429</td>
<td>1.50</td>
<td>–1.82500 (p=0.05)</td>
</tr>
</tbody>
</table>

Table 3.4. Comparison of \( F_{ST} \) values between localities. For COI (lower diagonal) and CAD (upper diagonal).

<table>
<thead>
<tr>
<th>Populations</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. EA</td>
<td>–</td>
<td>0.00</td>
<td>0.00</td>
<td>–0.09</td>
<td>–0.10</td>
<td>0.31</td>
<td>0.01</td>
<td>–0.05</td>
</tr>
<tr>
<td>2. PI</td>
<td>0.03</td>
<td>–</td>
<td>0.00</td>
<td>0.11</td>
<td>0.00</td>
<td>0.50</td>
<td>0.08</td>
<td>0.13</td>
</tr>
<tr>
<td>3. CT</td>
<td>0.06</td>
<td>0.02</td>
<td>–</td>
<td>0.08</td>
<td>–0.10</td>
<td>0.40</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>4. CA</td>
<td>0.10</td>
<td>0.20</td>
<td>0.10</td>
<td>–</td>
<td>0.02</td>
<td>0.28</td>
<td>–0.01</td>
<td>–0.09</td>
</tr>
<tr>
<td>5. CY</td>
<td>0.40</td>
<td>0.24</td>
<td>0.41</td>
<td>0.60</td>
<td>–</td>
<td>0.52</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>6. HU</td>
<td>0.11</td>
<td>0.12</td>
<td>0.11</td>
<td>0.25</td>
<td>0.50</td>
<td>–</td>
<td>0.10</td>
<td>0.35</td>
</tr>
<tr>
<td>7. RE</td>
<td>0.08</td>
<td>0.03</td>
<td>0.04</td>
<td>0.41</td>
<td>0.14</td>
<td>0.04</td>
<td>–</td>
<td>0.05</td>
</tr>
<tr>
<td>8. CU</td>
<td>0.12</td>
<td>0.08</td>
<td>–0.07</td>
<td>0.21</td>
<td>0.40</td>
<td>0.22</td>
<td>0.09</td>
<td>–</td>
</tr>
</tbody>
</table>
Figure 3.1. Map showing localities in the Ecuadorian Andes where *Pelmatellus columbianus* was sampled. Major features examined in AMOVA are highlighted, western and eastern cordilleras, and Mira, Chimbo, and Chanchan Valleys.
Figure 3.2. Bayesian 50% rule consensus tree for A) COI and B) CAD. Posterior probabilities are shown above the branches and bootstrap support values for the ML tree are shown below branches.
Figure 3.3. TCS haplotype network for the COI and CAD genes in *Pelmatellus columbianus*. Geographical distribution of haplotypes is color coded. Each segment represents a single mutation, where a black dot represents intermediate haplotypes not sampled. Size of the circles is proportional to haplotype frequency.
Figure 3.4. Timing of the *Pelmatellus columbianus* clade lineage based on a relaxed molecular clock for both gene data sets.
Figure 3.5. Maximum entropy models for A) past (21000 yrs) and B) present distribution of *Pelmatellus columbianus*, using five bioclimatic variables
CHAPTER FOUR
THE ROLE OF DISPERsal FOR SHAPING PHYLOGEOGRAPHIC STRUCTURE OF FLIGHTLESS GROUND BEETLES IN THE ANDES

INTRODUCTION

The distribution of a species is determined by environmental conditions, ecological interactions of species and dispersal dynamics. In particular, the dispersal ability of species plays an important role in determining the species range and biogeographical structure (Lester et al. 2007). The evolution of wings in insects is considered a major factor of their success and diversity (Stone and French 2003, Nicholson et al. 2014) because it has allowed them to avoid predators, capture prey and disperse (Stone and French 2003). However, the secondary loss and reduction of wings is recorded across many insect lineages (Wagner and Liebherr 1992), and wing length polymorphisms in insects, from reduction to complete loss of wings and wing muscles, suggest that flightlessness strongly influenced dispersal and biogeographical patterns in these lineages (Gutierrez and Menendez 1997, Mcculloch et al. 2017).

Flightlessness has been recorded in insect faunas with stable habitats such as oceanic islands and caves (Darwin, 1859; Wagner & Liebherr, 1992), as well as in species that live in harsh environments, such as polar and alpine regions (Somme and Block 2012). Selection against wings in beetles was hypothesized by Darwin (1859; pp. 135–136), based on the observation of beetles from an oceanic island. The absence of wings was attributed to natural selection and disuse of the wings, given that wings could cause
displacement due to high wind in open areas, and maintaining functional wings is energetically expensive (Darlington 1943, den Boer et al. 1979). Accordingly, flightlessness can have an effect on the dispersion capabilities and geographical range of species (Gutierrez and Menendez 1997, Ikeda et al. 2012). Studies across beetle species show that most winged species have a wider geographical distribution than species with reduction of wings (Gutierrez and Menendez 1997, Ikeda et al. 2012). Therefore, the loss of flight capabilities might promote allopatric speciation due to limited dispersal power (Ikeda et al. 2012). Still, beetle communities in the mountain systems are often composed of winged species (macropterous), species with reduction of wings (brachypterous), species with small wings (micropterous), as well as species that exhibit a range in the length of wings also known as wing polymorphic (Nilsson et al. 1993, Moret 2005).

Flightlessness in carabid beetles has been the focus of several studies, which show some evidence that wing loss might have promoted genetic isolation across beetle populations (Sota and Nagata 2008, Homburg et al. 2013). However, lack of genetic structure across populations has also been reported for flightless ground beetles, where populations appear to maintain low levels of gene flow (Chatzimanolis and Caterino 2007). Comparatively, the genetic diversity of macropterous and flightless species of ground beetles from eastern United States has revealed that both winged and wingless carabids have comparable levels of genetic heterogeneity (Liebherr 1988). This broad range of observations suggests that population differentiation for ground beetle species might be the result of a combination of factors, such as dispersal capability, discontinuity of the habitat and resource availability (Liebherr 1988, Vogler 2012).
Phylogeographic studies of high elevation species from the Andes are limited and mostly centered around plants (Madriñán et al. 2013, Hughes and Atchison 2015) and some vertebrate species (Páez–Moscoso & Guayasamin, 2012; Rodríguez Saltos & Bonaccorso, 2016), with few examples for insects species (Hines 2008, Elias et al. 2009). Nevertheless, these studies show that alpine faunas are species rich, with a high proportion of endemic species. In general, the evolution of Andean species is attributed to allopatric speciation due to the uplift of the Andes, as well as the effect of the contraction and expansion of glaciers during the Pleistocene (Gregory–Wodzicki, 2000; Hughes & Eastwood, 2006; Madriñán et al., 2013). Indeed, many highland species diverged in recent times (2–5 Mya; Hughes & Eastwood, 2006; Garzione et al., 2008; Madriñán et al., 2013).

The present study focuses on two flightless ground beetle lineages from páramo, a tropical alpine ecosystem in northern Andes (Luteyn 1999, Sklenář et al. 2011). Most of the ground beetles present in páramo are micropterous species (76%), with only a few macropterous and brachypterous species reported (Moret 2005). The aim of this study is to test if the secondary loss of wings in these two flightless beetle lineages has affected their phylogenetic structure and population connectivity, since these beetles’ populations are restricted to a small elevational range in the Andes. I address the following questions: 1) has the loss and/or reduction of wings in these flightless ground beetles lowered gene flow between populations, 2) Do potential geographical barriers show higher effects in flightless beetles when compared to a winged ground beetle, *Pelmatellus columbianus* (Reiche, 1843), which is also present in páramo (Munoz–Tobar, unpub), 3) Do winged
and wingless ground beetle lineages from páramo share similar patterns of genetic diversity; and lastly, 4) Is the origin of these flightless ground beetle lineages contemporary with plant species from páramo (2–5 Mya; Madriñán et al., 2013)?. I predict that flightless ground beetles present a higher population structure because of less genetic flow between sites than recorded for *P. columbianus*, given that flightless ground beetle lineages inhabit a smaller elevation range than *P. columbianus*. The genetic diversity of these flightless ground beetle lineages was assessed with the use of two molecular markers, one mitochondrial and one nuclear protein–coding gene, and using phylogenetic, population genetic analyses and niche modeling for species in the genus *Dercylus*.

**MATERIALS AND METHODS**

**Study species**

Two flightless ground beetle lineages were selected for these comparative analyses, both recorded across the east and west cordilleras of the Ecuadorian Andes: the ground beetle *Dyscolus alpinus* Chaudoir, 1878 (Coleoptera, Carabidae) and three species in the genus *Dercylus*: *D. orbiculatus* Moret & Bousquet, 1995, *D. praepapilatus* Moret & Bousquet, 1995, and *D. cordicollis* (Chaudoir, 1883).

*Dyscolus alpinus* is member of the Platyninae subfamily of ground beetles. *Dyscolus* Dejean, 1831, is a new world genus present mainly in the Neotropical Region, from Mexico to Argentina, with some representatives in the southern United States.
A total of 320 species have been described in the genus *Dyscolus*, with 89 species from Ecuadorian páramo (2750–4200 m). All species from the Ecuadorian páramo are described as micropterous, with no flight ability (Moret 2009). Samples were collected in six localities (Figure 4.1, Table 4.1), between 3501 m to 3897 m, over two field seasons during summers 2015 and 2016.

The second beetle lineage analyzed in this study is *Dercylus* Castelnau de Laporte, 1832, which is a New World genus of ground beetles from the Neotropics. This genus belongs to the subfamily Harpalinae. A total of 35 species have been described within this genus (Bousquet 2012), including five species from the Ecuadorian Andes (Moret and Bousquet 1995, Moret 2005). All Ecuadorian species belong to the subgenus *Linodercylus* Kuntzen, 1912. Members of this subgenus are micropterous – with no ability to fly. In this study, I focused on three species: *Dercylus cordicollis*, *Dercylus praepilatus*; *Dercylus. orbiculatus*, which do not show overlapping distributions. *Dercylus cordicollis* is present in the northwestern cordillera, whereas *Dercylus praepilatus* is known only from few localities in the province of Bolivar and Chimborazo; and *Dercylus orbiculatus* is present in the southern portion of the Ecuadorian Andes on both side of the mountain range. Multiple species from the group *Dercylus* (*Linodercylus*) were chosen for analyses, to compare diversification at a population and clade level, since these species appear to represent a monophyletic group. Samples were collected in five sites in the Ecuadorian Andes, between 3501 – 4010 m (Figure 4.1, Table 4.1). An additional sample was provided and identified by Pierre
Moret as *D. cordicollis* (SIMT265) from Cotacachi. This sample was included for the analysis of *D. cordicollis* populations.

Both ground beetle lineages, *Dercylus* (*Linodercylus*) spp. and *Dyscolus alpinus*, are described as micropterous. Yet, during the identification of the samples I observed variation in the length of the vestigial wing. Considering this, three specimens per populations for each beetle lineage were dissected to determine the proportion of wing reduction and to determine if the wing muscle was still present.

**Study area**

The study area encompassed the Ecuadorian Andes, where all the sampling took place above 3100 meters in the páramo ecosystem. The selection of the sites was based on previous records of occurrence and potential geographical barriers. Some of these potential barriers include: dry valleys, rivers, faults and the split of the Andes mountain range (Krabbe 2008, Guayasamin et al. 2010, Quintana et al. 2017). Species in the Ecuadorian Andes appear to me limited by the present of the rivers Pastaza, Chanchan and Mira, and their corresponding dry valleys which appear to limit the distribution of some birds, amphibians and plants species (Krabbe 2008, Guayasamin et al. 2010, Quintana et al. 2017). Similarly, habitat continuity is affected by the presence of the Pallatanga fault, a prominent NE–SW strike–slip fault crossing the western cordillera (Baize et al. 2015). Another geographic barrier considered in this study was the split of the Ecuadorian Andes into east and west cordillera, which limits the distribution of vertebrates and plants (Chaves et al. 2007, Guayasamin et al. 2010, Sklenář et al. 2011).
Collection permits for samples were previously obtained (MAE–DNG–ARGG–CM–2014–004). A total of eight sites were assessed during two field seasons (Summer 2015–2016, Table 4.1, Figure 4.1). Beetles were collected using three methods: manual collection (underneath rocks and on vegetation), pitfall trapping placed in two 100 m transects (1 trap every 10 m), where traps ran for 2–3 days; and through the collection of leaf litter samples from the páramo floor. Three samples of leaf litter were collected per site and were processed in the lab using Berlese funnels. Adult beetles were collected in 100% EtOH for DNA preservation. The specimens were identified using Moret’s (2005) taxonomic keys.

**DNA extraction and sequencing**

Total DNA was extracted from 110 specimens (with an average of 10 individuals per site per species) using GeneJet Genomic DNA Purification Kit (Thermo Fisher Scientific, Vilnius, Lithuania) following the manufacturer’s instructions. Voucher specimens will be deposited in the Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ) after the study is completed. For the phylogeographic inference and populations genetic analyses, I used two molecular markers, one mitochondrial gene (COI) using the primers TL2–N–3014 (5’–
TCCAATGCACAAATCTGCCATATA–3’ and C1–J–2183 (5’–
CAACATTTATTHTGTATTITGTG–3’; Simon et al., 1994) and the amplification profile described in Caterino & Tishechkin (2014); and one nuclear coding gene (CAD), using the primers CD439F (5’–TTTCACTGTACTYAYCAYCAYC–3’) and
CD688R (5’– TGTATACCTAGGGATCDACRTTYTCCATRTTRCA–3’).

Heminested PCRs were done for difficult samples, using CD439F (5’–
TTCAGTGACARTTYCAYCCHGARCAYAC–3’) and CD1098R (5’–
TTNGGNAGYTGNCCNCCCAT– 3) following Wild & Maddison (2008). All PCRs
were performed in 25 μl reactions, containing 17.5 μl water, 2.5 μl 10x buffer, 0.5 μl
dNTPs, 0.75 μl MgCl₂, 0.1 μl DreamTaq DNA Polymerases (Thermo Fisher Scientific)
and 1 μl of each primer (5 nm). PCR products were cleaned using ExoSAP–IT
(USB/Affymetric, CA, U.S.A). Sequencing was done commercially by Macrogen USA,
Inc. (Rockville, MD, U.S.A).

**DNA sequence analysis**

DNA sequences were edited using Geneious R8 (Biomatters Ltd., Auckland NZ) and
aligned in MAFFT v.7 (Katoh and Standley 2013). Models of sequence evolution were
tested using JModeltest 2.0 (Darriba et al., 2012) for each data set, where GTR+I+G
model was in the 100% confidence interval for both COI and CAD data. Phylogenetic
analyses were performed to determine relationships among individuals for each beetle
lineage. Mitochondrial and nuclear data sets were analyzed separately, and combined
data were used to generate phylogenetic hypotheses for each beetle lineage using Mr.
Bayes 3.2 (Huelsenbeck and Ronquist 2001) and RAxML version 8.2.8 (Stamatakis
2014) launched from Mesquite’s Zephyr package (Maddison and Maddison 2015) with
1000 bootstrap replicates. In addition to the phylogenetic inferences, haplotype diversity
was identified through TCS (Clement et al., 2000) and DnaSP (Rozas et al., 2003).
To understand the timing and rate of diversification for each ground beetle lineage, divergence times were estimated using an uncorrelated relaxed clock in BEAST 2.0 (Bouckaert et al. 2014) for the COI and CAD data matrixes. For *Dyscolus alpinus*, two points of calibration were used to designate minimum node ages, both representing taxa within the same subfamily as *Dyscolus*, Platyninae: a fossil of *Platynus* from the middle Eocene (44.1 Mya) of Baltic origin (Larsson 1978), and a fossil of *Agonum* from the early Eocene (49 – 52 Mya) from Green River Colorado (Scudder 1980). The divergence time for the *Dercylus* lineage was estimated using one point of calibration: a fossil of *Amara* (also a Harpalinae) from the Middle Eocene (44.1 Mya) of Baltic origin (Larsson 1978). Log files from Beast were examined in the software Tracer 1.5 5 (http://tree.bio.ed.ac.uk/software/tracer/) to ensure that the effective sample sizes (ESS). Trees were recovered in TreeAnnotator 2.0.02 (http://beast.bio.ed.ac.uk), using maximum clade credibility (MCC) after a 10% burn–in.

For population genetic analyses, population structure was assessed for *Dyscolus alpinus*, *Dercylus orbiculatus* and *Dercylus* species together (*D. orbiculatus*, *D. cordicollis*, *D. praepilatus* and *Dercylus* spp. from Atillo and Cotacachi) to contrast inter– and intrapopulation variability. Estimated haplotype diversity (h), nucleotide diversity (π), number of polymorphic sites (S), and Tajima’s D neutrality test statistics (D) were calculated using DnaSP (Rozas et al., 2003). Φst was calculated to assess the population structure, while the analysis of the molecular variance (AMOVA) was performed to test the influence of geographical barriers, such the presence of rivers, dry valleys and east and west mountain range. A Mantel test of correlation between genetic...
and geographic distances was performed using 1000 randomizations. Analyses were performed in Arlequin 3.5.2.2 (Excoffier & Lischer, 2010). Lastly, Structure 2.3.4. (Pritchard et al. 2000) was used to determine the number of populations by calculating the K.

**Maximum entropy models of species distribution for species in the genus Dercylus**

The ecological niche of each species of *Dercylus* was modeled to determine if species distribution overlapped, using present bioclimatic variables at 2.5’ spatial resolution (WorldClim v.1.4; Fick & Hijmans, 2017) with the use of MAXENT 3.3.3 (Phillips et al. 2006). *Dercylus* from Cotacachi and Atillo were excluded from these analyses given the few records available. For other species of *Dercylus*, sites mentioned in Moret (2005) were georeferenced using GeoLocate (Rios and Bart 2010) and combined with the distribution data generated in this study. A total of 41 occurrence records were gathered for analyses. The model of distribution was based on five bioclimatic variables: BIO1 = Annual Mean Temperature, BIO2 = Mean Diurnal Range (Mean of monthly (max temp – min temp), BIO5 = Max Temperature of Warmest Month, BIO6 = Min Temperature of Coldest Month, BIO12 = Annual Precipitation, BIO13 = Precipitation of Wettest Month and BIO14 = Precipitation of Driest Month. The analyses ran for 500 iterations and ten replicates using a 10% training presence, using the default settings for the convergence threshold ($10^{-5}$). For each species, the output from the average run was reclassified using ArcMap 10.4.1 (ESRI, 2016) with a 10% threshold. Model performance was evaluated using the area under the curve (AUC)
calculated by MAXENT, where values between 0.7 and 0.9 indicated a good discrimination (Swets 1988).

RESULTS

Data analyses for *D. alpinus*

The portion of the COI gene analyzed for the *D. alpinus* samples was 767 bp long, with 41 parsimony informative sites. A total of 6 haplotypes were recovered from the COI data set (Figure 4.2A; GenBank accessions X to X). Haplotype 1 is the most widespread across populations, found in four out of the six sites (Figure 4.2A). Unique haplotypes were recorded for the localities of Atillo (H3), Mojanda (H5) and Cayambe (H6, Figure 4.2A). Haplotype diversity (h) is shown in Table 4.2 for each population, where Mojanda records the highest haplotypic diversity among sampled site (h= 0.71). The overall nucleotide diversity of COI was low (π= 0.02). Results from the Structure analyses based on mt–DNA tended to overestimate the number of populations, results from multiple simulations show that the number of populations using this data set is one population (K=1). The overall value of neutrality test (D= −0.80; p=0.37) suggests that this species is going through a population expansion after a recent bottleneck, but the test was not supported by its p–value. On a population basis, positive and negative values of Tajima D are revealed (Table 4.2), though without statistically significant values except for Atillo (D= −1.79, p=0.05*) and Cayambe (D=−2.09, p=0.00), which might be going through expansion. However, a high proportion of rare alleles, indicative of population expansion, was not found at these two sites (two haplotypes per site; Figure 4.2A).
Additionally, a Mantel test showed no correlation (p=0.13, $R^2=0.08$) between genetic ($\phi_{ST}$) and geographical distances.

The nuclear CAD data set for *D. alpinus* spanned 744 bp, with 64 parsimony informative sites. A total of five haplotypes were recorded (GenBank accessions X to X), with no heterozygote individuals observed for the CAD locus, which was inferred with DnaSP (Rozas et al. 2003). Haplotype 1 is recorded in four out of the six populations assessed. Unique haplotypes were recorded from the Atillo (H4) and Cayambe (H5) populations. Haplotype networks for both genes show that most haplotypes are present at multiple sites (Figure 4.2B). The overall haplotypic (h) and nucleotide diversity was low for CAD data set (Table 4.2). Yet, among all the sites analyzed, Mojanda showed the highest haplotypic diversity (h=0.51). The structure analyses from the nuclear data set showed three populations (K=3). Similar to the results from the mitochondrial data, CAD suggests that *D. alpinus* may be going through an expansion process (overall value $D=−0.86$; $p=0.36$), without statistical support. Positive and negative values of Tajima’s $D$ are recorded across populations of *D. alpinus* without significant p–values. Two exceptions are the sites Cayambe ($D=−1.78$; $p=0.00$) and Pichincha ($D=−1.40$; $p=0.04$). Neutrality test values suggests that these populations are going through an expansion process, but only few haplotypes are recoded for each site, which does not support a population expansion scenario. Results from the Mantel test show there was no correlation between genetic and geographic distances (p=0.81, $R^2=0.01$). Lastly, through the dissection of voucher specimens confirmed that all specimens had reduced wings. The vestigial wing varies in length across populations, and through the dissection I found some
underdeveloped wing muscle. The original description mentions this species is micropterous, but dissections show that there is variation in the length of wings across individuals across populations (1–2 mm).

The AMOVA of nuclear and mitochondrial data of *Dyscolus alpinus* shows that most of the variation is present within populations (Table 4.3). These results seem to contrast with the overall $\phi_{ST}$ values (COI $\phi_{ST} = 0.54$; CAD $\phi_{ST} = 0.26$), which suggest there are some moderate levels of population subdivision among populations of *D. alpinus*. When $\phi_{ST}$ values were compared a population–by–population basis, some genetic differentiation was found among populations (Table 4.4). COI data exhibited an overall higher genetic differentiation between sites, from moderate to high level population subdivision ($\phi_{ST}$=0.32–0.90; Table 4.4). Some exceptions are noticed between sites that are closer in distance (less than 36 km). For example, low $\phi_{ST}$ values were recorded between Cayambe and Mojanda $\phi_{ST}=0.10$ (separated by 30 km), and La Virgen and Pichincha $\phi_{ST}=-0.10$ (separated by 36 km). The CAD data set shows lower genetic differentiation among all populations of *D. alpinus* (Table 4.4). However, higher $\phi_{ST}$ values are observed when Cashca Totoras is compared among the other sites ($\phi_{ST} =0.5–0.65$), except when compared to Cayambe ($\phi_{ST} =–0.08$). The overall number of migrants between *Dyscolus* populations ($nm= 0.31$) that there is population subdivision across sites. Higher levels of genetic connectivity were observed between Mojanda and Cayambe ($nm=1.79$), results that are also supported by the low $\phi_{ST}$ values between these two sites (Table 4.4).
Data analyses for *Dercylus*

In the case of *Dercylus* species, the portion of the COI gene analyzed was 776 bp, with 76 parsimony informative sites (GenBank accessions X to X). A total of 16 haplotypes were identified for all *Dercylus* species, with no shared haplotypes among sites (Figure 4.3A). Eight haplotypes were recorded for populations of *D. orbiculatus*, whereas two haplotypes were recovered from specimens identified as *D. cordicollis*. The specimen provided by Moret from Cotacachi appears to be a distinct clade of *Dercylus* closely related to *D. cordicollis*, based on the TCS analyses and phylogenetic inference, where only one haplotype was found. For specimens of *D. praepilatus* from Salinas, one haplotype was recorded, while four haplotypes were recorded from specimens collected from the locality of Atillo. Based on the morphological similarity with *D. orbiculatus*, specimens from Atillo were thought to be another population of *D. orbiculatus*; however, based on haplotype designation and other phylogenetic analyses, this population appears to be a distinct clade of *Dercylus*. Haplotype diversity indexes are shown in Table 4.2 for each species and population, where populations of *D. orbiculatus* from Culebrillas show the highest haplotypic diversity (h=0.87) among *Dercylus* species. However, the overall nucleotide diversity of species in the genus *Dercylus* is low (π=0.04; Table 4.2). Structure analyses were done for *D. orbiculatus* populations, where K=1 populations, even though these two sites do not share haplotypes. These analyses were also performed for the entire clade, and one population was found (K=1). The overall value of the neutrality test for the *Dercylus* lineage suggests that it is going through a population contraction (D=0.26, p=0.77), but this index was not supported by its p–value and number of haplotypes.
Similarly, positive and negative values of Tajima’s D are recorded without statistical support for each species and site. Lastly, the Mantel test performed with $\phi_{ST}$ and geographic distances show no significant correlation ($R^2=0.05$, $p=0.3$) between genetic and geographical distances.

The portion of the CAD gene amplified for *Dercylus* species was 669 bp with 24 parsimony informative sites (GenBank accessions X to X). A total of 13 haplotypes were identified across the *Dercylus* lineage. Four haplotypes were recovered from *D. orbiculatus* populations, and three haplotypes from *D. cordicollis*, with one haplotype shared with *Dercylus* from Cotacachi (H5, Figure 4.3B). Four haplotypes were identified in the population of *Dercylus* from Atillo, and three haplotypes from *D. praepilatus*. Two haplotypes were shared between *D. praepilatus* and *Dercylus* from Atillo (H8 and H11; Figure 4.3B). The highest haplotypic diversity is recorded for the *Dercylus* from Atillo, followed by *D. orbiculatus* from El Cajas and *D. cordicollis* (Table 4.2). The overall nucleotide diversity was also considered low across this ground beetle lineage ($\pi=0.02$). The structure analyses done only for *D. orbiculatus* populations showed only one population (K=1), whereas results from the analyses of the whole clade show five distinct populations (K=5), corresponding to each collecting site. The overall value of Tajima’s D for the *Dercylus* lineages suggests the lineage is going through population contraction ($D=0.09$; $p=0.81$), with no support from the $p$–value. This was also the case for each species and site (Table 4.2), which showed no statistical support for these values. Results from the Mantel test show no significant correlation ($p=0.8$, $R^2=0.02$) between genetic ($\phi_{ST}$) and geographical distances.
The AMOVA showed that most of the genetic variation was found among groups when testing the effect of geographical barriers (Table 4.5). The high variation among groups is consistent with working with multiple species, since species in this genus do not have widespread distributions, with the exception of _D. orbiculatus_, present in the southern Ecuadorian Andes (Figure 4.1; Moret, 2005). The highest percentage of variation in the AMOVA when parsing the data by species was seen around the Chimbo and Chanchan dry valleys and rivers, which overlap with another potential geographical barrier, the Pallatanga depression. These results were also supported by the overall $\phi_{ST}$ values, which show high levels of differentiation among species and sites (COI $\phi_{ST}$= 0.89; CAD $\phi_{ST}$= 0.95). A high level of genetic differentiation was also observed when $\phi_{ST}$ values were compared between populations and species in COI data set (Table 4.6). In this data set, the lowest $\phi_{ST}$ values were observed between populations of _D. orbiculatus_ Cajas and Culebrillas ($\phi_{ST}$=0.50).

In the case of the nuclear coding gene CAD, the overall value of $\phi_{ST}$ ($\phi_{ST}$= 0.93) shows high levels of population differentiation. When each population was compared against each other, we found moderate to complete population subdivision between some sites ($\phi_{ST}$=0.77–1.00, Table 4.6). Particularly when comparing against populations of _D. orbiculatus_ from Cajas and Culebrillas. Lower $\phi_{ST}$ values were found between the two populations of _D. orbiculatus_ Cajas and Culebrillas ($\phi_{ST}$= 0.10), as well as for _Dercylus_ of Pichincha and Cotacachi ($\phi_{ST}$= 0.00), Pichincha and Salinas ($\phi_{ST}$= 0.00), Salinas and Atillo ($\phi_{ST}$=0.03), and Salinas and Cotacachi ($\phi_{ST}$= 0.00). The number of migrants per generation was calculated for two populations of _D. orbiculatus_, which revealed low gene...
flow (nm=0.39). Lastly, I corroborated that species in the genus *Dercylus* are micropterous through dissections of voucher specimens.

**Phylogenetic relationships and divergence time estimation**

Phylogenetic analyses were conducted separately for each gene data set using Bayesian and Maximum Likelihood (ML) methods. The phylogenetic inference for *D. alpinus* using the mitochondrial gene shows trees with similar support (Figure 4.4A). Most of the individuals analyzed were found in the *Dyscolus alpinus* clade, except for haplotype 6, which corresponds to individual collected in Cayambe (SIM332). This individual is other species in the genus *Dyscolus*, sister to *D. alpinus* (Figure 4.4A). Results of phylogenetic inference are consistent with TCS haplotype network, which shows most samples are contained within one network, with the exception of haplotype 5 (Figure 4.2A). The analyses of COI haplotypes demonstrate that most haplotypes are present in multiple sites (Figure 4.4A), with only two unique haplotypes. Unique haplotypes were among individuals of Atillo (H3) and Mojanda (H5). Five out of the six sampled sites record individuals with haplotype 1 (Mojanda, Cayambe, Pichincha, La Virgen, Cashca Totoras), followed by haplotype 2 (Atillo, Mojanda) and haplotype 4 (Cayambe, Mojanda; Figure 4.2A). The highest haplotypic diversity was found in specimens from Mojanda (H1, H2, H4, H5) with a total of 4 haplotypes, while the lowest haplotypic diversity was in samples at the sites Cashca Totoras (H1) and Pichincha (H1) with only one haplotype recorded for each site. A similar pattern was recovered for the CAD data set of *D. alpinus*. Most samples belonged to the *D. alpinus* clade, with the
exception of an individual collected in Cayambe (SIM332), that is other species in the
genus *Dyscolus* sister to *D. alpinus* (Figure 4.4B). The CAD data set has lower
haplotypic diversity, with only five haplotypes recorded (Figure 4.4B). Most sampled
sites yielded haplotype 1 (Mojanda, Cayambe, Pichincha, La Virgen, Cashca Totoras,
Figure 4.2B), with only one unique haplotype (H4) observed from an individual from
Atillo. The Mojanda populations showed the highest haplotypic diversity (H1, H2, H3),
whereas Pichincha (H1) and Cashca Totoras (H1) only revealed one haplotype.

Phylogenetic inference for a combined data set of *D. alpinus* does not show a
clear pattern of vicariance across sites as expected. For both genes, dominant haplotypes
are present across the range of distribution, with some distinct haplotypes only present in
the northwestern population (Mojanda) or in southeastern populations (Atillo). A relaxed
molecular clock was used on the combined matrix for *D. alpinus* to calculate divergence
time estimates for the clade. These analyses suggest that the *D. alpinus* clade originated
during the Miocene 6.32 Mya (5.1–8.9 Mya). The timing of splitting–events for the *D.
alpinus* clade (Figure 4.5) is slightly older than most páramo plant species (2–5 Mya;
Madriñán et al., 2013) but contemporary with the evolution of highland species in the
northern Andes mountain chain, which arose during the Miocene (Weir 2006, Hines
2008).

The phylogenetic analyses for flightless genus *Dercylus* reveal different patterns
depending on the gene examined. The monophyly of *D. orbiculatus* was not recovered in
the COI gene tree, where individuals of *D. orbiculatus* appear at multiple points in this
tree topology (Figure 4.6A). For the other *Dercylus* species, each clade represents a
distinct clade supported by bootstrap and posterior probability values (Figure 4.6A). COI data show that *Dercylus* individuals from Atillo are more closely related to *D. cordicollis*, whereas *Dercylus* individuals from Cotacachi, previously identified as *D. cordicollis*, is a distinct lineage of *Dercylus*. These results were also supported by the TCS analyses, in which individual from Cotacachi and specimens collected in Atillo appear in a separate haplotype networks (Figure 4.3A). In contrast, the CAD gene tree of the *Dercylus* shows less resolution among species (Figure 4.6B). The nuclear gene tree supported the monophyly of *D. orbiculatus*. But then again, the relationships between species and populations of *Dercylus* in this tree are poorly resolved (Figure 4.6B). Correspondingly, TCS analyses grouped together all haplotypes into one haplotype network for all species, with shared haplotypes between *D. praepilatus* and the *Dercylus* from Atillo (H8, H11), and *D. cordicollis* and *Dercylus* from Cotacachi.

The analyses of the combined matrix (COI and CAD) exhibit a higher resolution among species and populations of *Dercylus*. Distinct clades for *D. orbiculatus*, *D. praepilatus*, *D. cordicollis*, and the distinct *Dercylus* spp. from Atillo and Cotacachi were recovered with the combined data analyses, with support from bootstrap and posterior probability values. Additionally, a relaxed molecular clock was also used to calculate divergence time estimates for members of the *Dercylus* clade using a combined data set (Figure 4.8). Estimates show that the *Dercylus* clade originated during the Oligocene (30 Mya). Thus, most of the sampled species in this study originated prior to the evolution of páramo, with *D. orbiculatus* (20.08 Mya), *D. cordicollis* (5.3 Mya), *Dercylus* from Cotacachi (22.9 Mya) and *Dercylus* spp. 2 (7.9 Mya) originating during the Miocene (22–
7.9 Mya), whereas *D. praepilatus* (3.29 Mya) originated during the Pliocene. Only this last diversification is contemporary with the diversification of most páramo plants (2–5 Mya, Madriñán et al., 2013).

**Niche modeling for species in the genus *Dercylus***

The AUC values for the three species of *Dercylus* showed a high predictive ability of the ecological niche model under a random model, with AUC values between 0.998–0.999. Populations of this ground beetle lineage are mainly present in the Andean region of Ecuador, with potentially overlapping distributions (Figure 4.9). Compared to other species analyzed, the maximum entropy model for *D. orbiculatus* shows that this species is present at high elevation, but its distribution also extends to lower elevations, especially towards the southern Ecuadorian Andes (Figure 4.9). In contrast, *D. praepilatus* has the smallest potential distributional range according to this model. Lastly, niche modeling also revealed that suitable habitats for species of this genus also extend into the Andean region of Colombia and Peru. To test if the distribution of the species extends into these areas, further sampling is needed to support this model.

**DISCUSSION**

Flightless beetles have been the subject of several studies in mountain systems around the world (Bruhl 1997, Gutierrez and Menendez 1997, Ikeda et al. 2012). The effect of wing reduction varies across flightless beetles lineage, from high phylogeographic
structure attributed to limited dispersal capabilities (Gutierrez and Menendez 1997, Ikeda et al. 2012), to flightless beetles that maintain low levels of gene flow and show limited structure in their populations (Chatzimanolios and Caterino 2007, Huang and Lin 2010). In tropical mountains, wingless and wing dimorphic beetles dominate beetles communities (Erwin 1985). This study focused on beetle lineages from the Andean region, an area that is characterized for its high diversity (Myers et al. 2000, Veblen et al. 2007), but few studies have explored the genetic diversity of Andean insect lineages (Hines, 2008; Maddison, 2014; De–Silva et al., 2016). The role of wing reduction and dispersal capabilities of flightless beetles from the Ecuadorian Andes is still less explored, although this mountain system has a high number of beetle species with reduction of wings (Ahn and Ashe 1996, Moret 2005).

Results from the analyses of two flightless ground beetles from páramo show different extents of population subdivision, from low levels of gene flow to complete population isolation, depending on the gene data set used and beetle lineage assessed. For Dyscolus alpinus, a ground beetle that has wing polymorphism (from micropterous to brachypterous individuals), there are fewer haplotypes recorded through sampled populations. One dominant haplotype is found in four out of the six populations analyzed for both molecular markers. Among all sites analyzed, Mojanda showed the highest haplotypic diversity (h=0.5–0.7, Table 4.2) when compared to other localities. The diversity seen at this site could be associated with multiple colonization events. Changes in the landscape of the Mojanda area, such as volcanic activity dated prior to the Last Glacier Maximum (Mt. Mojanda and Mt. Fuya Fuya, Robin et al., 2009), and periodic
burns during the Holocene (Frederick et al. 2018), could have affected the high genetic diversity in the population of *D. alpinus* of Mojanda.

The AMOVA across populations of *D. alpinus* revealed that most of the genetic variation is present within populations (Table 4.3). However, each data set shows different levels of genetic differentiation. The overall $\phi_{ST}$ value generated with the nuclear gene presents less genetic differentiation among populations of *D. alpinus* ($\phi_{ST}$=0.3). The highest $\phi_{ST}$ values in this data set were seen when Cashca Totoras was compared to other sites (Table 4.3). In a closer look at the $\phi_{ST}$ values generated with the mitochondrial data, a higher population structure was detected (overall $\phi_{ST}$=0.51). Populations at opposite sides of the cordillera present high population structure (e.g. Pichincha – Attillo; $\phi_{ST}$=0.86, Table 4.4). High $\phi_{ST}$ values were also found among sites on the same side of the cordillera, but separated by distance (Table 4.4). For example, in the eastern mountain range the population of Attillo appears to be a genetically distinct when compared to Cayambe and La Virgen ($\phi_{ST}$=0.50–0.85). The genetic differentiation between these sites might be driven by distance (<186 km), but could be also influenced by the presence of the Pastaza river. This geographical barrier separates Attillo from the other sites and, in previous studies, this river has been proposed to be a factor that limits the distribution of some species that live in the Ecuadorian Andes (Krabbe 2008, Guayasamin et al. 2010). High $\phi_{ST}$ values were also found among sites in the western cordillera. For example, between Pichincha – Mojanda ($\phi_{ST}$=0.50) and Cashca Totoras – Pichincha ($\phi_{ST}$=0.90). In the case of Cashca Totoras and Pichincha ($\phi_{ST}$=0.90) these populations are separated by a considerable distance (<177 km). The Pichincha and
Mojanda populations are located relatively close in distance (~47 km), where habitat discontinuity could be playing an important role. These localities are separated by a lower elevation dry valley (Guayllabamba valley; Quintana, 2010). The change in elevation and abiotic conditions could be affecting genetic flow between sites. In contrast, the highest levels of genetic connectivity were recorded between Cayambe and Mojanda ($\phi_{ST}=0.10$), and La Virgen and Pichincha ($\phi_{ST}=-0.10$). These sampling sites are situated at opposite sides of the cordillera, but separated by a relatively small distance (~30–47 km). Gene flow was detected between Cayambe and Mojanda ($nm=1.73$). However, this index could not be calculated for La Virgen and Pichincha because of the lack of polymorphic sites between sequences.

The dissimilarity in the results from nuclear and mitochondrial data sets in the population genetic analyses is associated with differences in the rate of nucleotide change between molecular markers (Lin and Danforth 2004). The low levels of genetic variation in the CAD data set might be influenced by the recent origin of the *Dyscolus alpinus* (6.32 Mya, Miocene), whereas the higher population structure in the COI data set might be the product of the faster rate of change that is generally observed in mitochondrial genomes (Lin and Danforth 2004). The phylogenetic inference for both genes shows a well–supported clade for *D. alpinus* (Figure 4.4). This clade contains most of the sampled data, with the exception of haplotype 6, representing a different species of *Dyscolus*. Clear phylogeographic splits are not observed in these topologies since multiple haplotypes are shared between sites. Yet, some haplotypes are only present in northern populations (Figure 4.4). Paleoclimaltical events might have had a greater effect in beetle
populations with smaller elevation ranges and reduction of wings, like *D. alpinus*, that were present during Quaternary glaciations, when high elevation species were moved to lower elevation during the expansion of the glaciers (Villota and Behling 2014, Frederick et al. 2018). The elevational shift of páramo towards the inter–Andean valleys possibly promoted gene flow between ground beetle populations that were previously restricted to higher elevation. These events, in combination with current low levels of gene flow might explain the current genetic diversity of *D. alpinus* populations.

For the species in the genus *Dercylus*, high levels of differentiation are reported at the species and population level for both genes. The mitochondrial marker showed no shared haplotypes among sites and species (Figure 4.3A), whereas CAD showed three shared haplotypes, among specimens collected in Salinas and Atillo, and Pichincha and Cotacachi (Figure 4.3B). Overall, species in the genus *Dercylus* show smaller distributions as documented by Moret (2005). However, results from niche modelling for three species of *Dercylus* using present conditions show that this species could have potentially overlapping distributions. Further fieldwork is needed to test if this maximum entropy model is representative of the actual distribution of the analyzed species.

The limited dispersal ability of members of this beetle lineage appears to be playing an important role in shaping their genetic diversities. A high genetic structure was supported by the high $\phi_{ST}$ values among members of the *Dercylus* clade, a result which suggests that populations of *Dercylus* are genetically distinct. Similarly, high genetic differentiation was observed in the population genetic analyses of the two populations of
*D. orbiculatus* (Cajas and Culebrillas) using the mitochondrial data set. Yet, this high level of genetic differentiation was not seen using CAD, where the populations of Cajas and Culebrillas share no haplotypes in both data sets, probably because they are at opposite sides of the Andean mountain range. The high levels of genetic differentiation were sustained by the restricted gene flow between these two sites, and the analyses in Structure only recovered one population (K=1) with COI and CAD sequences. Similarly, as reported as is seen in populations of *D. alpinus*, Structure analyses done with the mt-DNA data set tended to overestimate the number of populations, in comparison with the analyses using the nuclear coding gene which shows a clear delimitation of populations.

Phylogenetic analyses and the genetic diversity of populations of *Dercylus* show some level of correspondence, since high genetic differentiation is observed at population and species levels. My primary data were composed of three species of *Dercylus* (*D. cordicollis, D. praepilatus* and *D. orbiculatus*) from 5 sites, plus an additional sample from Cotacachi identified by Moret as *D. cordicollis*. In addition to the known species, phylogenetic inference and the TCS revealed two distinct lineages from the samples from Cotacachi and Atillo (Figure 4.6). Different relationships among members of the clade *Dercylus* are supported by each gene tree. The COI gene tree suggests that *Dercylus orbiculatus* is not monophyletic, appearing at multiple points at the base of this tree topology. Only the CAD gene tree recovered the monophyly of *D. orbiculatus* (Figure 4.6B). For the other species, COI shows that most species belong to a distinct clade (Figure 4.6A). This was not the case for CAD, which showed poor resolution among species (Figure 4.6B). The combined analyses of the data revealed a clearer pattern of
speciation between members of *Dercylus* from páramo, where phylogenetic splits correspond more clearly with species (Figure 4.7). Divergence time estimates show that the species in the *Dercylus* clade originated during the Miocene and Pliocene (20–3 Mya; Figure 4.8). Divergence time estimates suggest most species of *Dercylus* from high elevation are older than the ecosystem they live in, since páramo plants are estimated to be between 2–5 Mya (Madriñán et al. 2013), having originated in the Pliocene and Pleistocene. The only exception among species in the *Dercylus* lineages, is *D. praepilatus* (3.29 Mya), that originated in the Pliocene (Madriñán et al. 2013).

The AMOVA for both genes shows higher genetic differentiation among groups in the *Dercylus* lineage when testing for geographical barriers (barriers 1 & 4; Table 4.5). Phylogeographical splits are observed when species are divided around the dry valleys and rivers (Krabbe 2008, Guayasamin et al. 2010, Quintana et al. 2017); in particular, high genetic variation is detected when data is divided around the Chimbo and Chanchan river and valley (Barriers 1 & 4), which overlap with the Pallatanga fault (Baize et al. 2015). The presence of these three geographical barriers in this area of the Andes might explain the phylogeographical split between *D. orbiculatus* and *D. praepilatus*. The division between the population of *D. orbiculatus* from Culebrillas and *Dercylus* from Atillo, both on the same side of the mountain range (47 km apart), could be explained by the presence Mt. Ayapungo (4,730 m) and Mt. Coyay (4,630 m) that separate these two sites. Both mountains maintained small glaciers during Pleistocene and Holocene (Jordan & Hastenrath, 1998), and the change in topography in combination with the effect of Quaternary glaciations might have isolated these beetle lineages. Lastly, the divergence
between *D. cordicollis* and *Dercylus* from Cotacachi was supported by the high levels of genetic variation recorded when these two beetle lineages were considered distinct groups in the AMOVA (Barriers 4). The split between *D. cordicollis* and *Dercylus* from Cotacachi could be explained by the distance (<79 km) between sites, but also by the presence of lower elevation dry valleys around Mt. Pichincha (Quintana 2010).

When results from these two flightless lineages were compared to the patterns observed in *P. columbianus*, a macropterous ground beetle also present in páramo (Munoz–Tobar, unpub), a generalized pattern of the distribution of the genetic diversity for ground beetles that live in páramo is not evident. Flightless beetles appear to have higher levels of population structure when compared to *P. columbianus* (Munoz–Tobar, unpub). Even though both beetle lineages are flightless, *D. alpinus* appears to be a better disperser than species in the genus *Dercylus*. Evidence for this was found in the levels of genetic connectivity between northern populations of *D. alpinus* that show some levels of gene flow (e.g. Mojanda–Caymabe), probably enabled during Quaternary glaciations when páramo moved to lower elevation areas such as the inter–Andean valleys (Villota and Behling 2014). The low genetic differentiation for some populations of *D. alpinus* could also be explained by the recent origin of the lineage (6.32 Mya).

Overall, this study demonstrates the importance of range size and dispersal capability for each beetle lineage from páramo. While macropterous species such as *P. columbianus* presents broad distributional ranges (from the inter–Andean valleys to páramo, 2000–4200 m) and sustain higher levels of gene flow among populations (Munoz–Tobar,
unpub), flightless ground beetles display smaller distributions (2750–4200 m) and different proportions of genetic connectivity among populations. Even though some of the flightless beetles analyzed in this study show potentially overlapping distributional ranges, less genetic cohesiveness was found among populations, possibly as a result of fragmentation of the ecosystem they live in. The dispersal capability of each individual beetle lineage influences the patterns of divergence, where flightless ground beetles are restricted to an elevation range and geographical area. Species with smaller elevation ranges are probably more susceptible to climate change, elevational shifts towards higher elevations have been already been reported among ground beetle species from páramo (Moret et al. 2016). For example, populations of *Dercylus orbiculatus* and *Dyscolus alpinus* from Mt. Pichincha are experiencing expansion of their elevation range into higher elevations (100–400 m upslope; from grass páramo to super páramo), into the range of other species of ground beetles in the area (Moret et al. 2016). The patterns of diversification found from the study of ground beetles from páramo have important implications for the conservation of species from páramo, where conservation efforts should take into consideration local and regional patterns of diversity, recognizing the micro- and macroevolutionary processes that gave rise to species in páramo.
Table 4. 1. Summary of the collecting sites from the Ecuadorian Andes, from which ground beetle species were collected for genetic analysis. Data set compiled from 2015 to 2016.

<table>
<thead>
<tr>
<th>No.</th>
<th>Site Name</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation</th>
<th>Col. Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mojanda</td>
<td>N00°08.710'</td>
<td>W78°16.753'</td>
<td>3715 m</td>
<td>12–Jul–2016</td>
</tr>
<tr>
<td>2</td>
<td>Pichincha</td>
<td>S00°11.259'</td>
<td>W78°32.432'</td>
<td>3897 m</td>
<td>22–Jun–2016</td>
</tr>
<tr>
<td>3</td>
<td>Salinas</td>
<td>S1°24'11.03&quot;</td>
<td>W78°14.051’</td>
<td>3604 m</td>
<td>11–Jun–2015</td>
</tr>
<tr>
<td>4</td>
<td>Cashca Totoras</td>
<td>S01°43.485'</td>
<td>W78°57.183'</td>
<td>3509 m</td>
<td>13–Jun–2015</td>
</tr>
<tr>
<td>5</td>
<td>Cajas</td>
<td>S02°47.020'</td>
<td>W79°13.438'</td>
<td>3956 m</td>
<td>20–Jun–2015</td>
</tr>
<tr>
<td>6</td>
<td>Cayambe</td>
<td>S00°02.101'</td>
<td>W78°03.608'</td>
<td>3743 m</td>
<td>01–Jun–2016</td>
</tr>
<tr>
<td>7</td>
<td>La Virgen</td>
<td>S00°18.477'</td>
<td>W78°13.953'</td>
<td>3694 m</td>
<td>28–Jun–2016</td>
</tr>
<tr>
<td>8</td>
<td>Atillo</td>
<td>S02°11.265'</td>
<td>W78°31.2601'</td>
<td>3501 m</td>
<td>07–Jul–2016</td>
</tr>
<tr>
<td>9</td>
<td>Culebrillas</td>
<td>S02°28.337'</td>
<td>W78°53.719'</td>
<td>3799 m</td>
<td>15–Jun–2015</td>
</tr>
<tr>
<td>10</td>
<td>Cotacachi</td>
<td>N00°19.79952'</td>
<td>W78°20.80830'</td>
<td>3757 m</td>
<td>13–Jul–2016</td>
</tr>
</tbody>
</table>
Table 4.2. Overview of the genetic diversity indexes for *Dyscolus alpinus* and *Dercylus* species and populations. N refers to the number of individuals sampled; S, number of segregating sites; $\pi$, is a measure of nucleotide diversity; and D represents the Tajima’s D, a neutrality test statistic with its corresponding p–value.

<table>
<thead>
<tr>
<th>Species</th>
<th>Site</th>
<th>COI</th>
<th>CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>h</td>
</tr>
<tr>
<td><em>Dyscolus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>alpinus</td>
<td>Mojanda</td>
<td>11</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Pichincha</td>
<td>9</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>C. Totoras</td>
<td>10</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Cayambe</td>
<td>10</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>La Virgen</td>
<td>11</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Atillo</td>
<td>10</td>
<td>0.21</td>
</tr>
<tr>
<td><em>Dercylus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cajas</td>
<td>10</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Culebrillas</td>
<td>10</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Pichincha</td>
<td>10</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Salinas</td>
<td>8</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Atillo</td>
<td>10</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Cotacachi</td>
<td>1</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 4.3. Analysis of the molecular variance for populations of *Dyscolus alpinus*.

<table>
<thead>
<tr>
<th>No. groups</th>
<th>Partitions</th>
<th>Tests</th>
<th>COI</th>
<th>CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Among groups</td>
<td>Among populations</td>
</tr>
<tr>
<td>2</td>
<td>(1,2,4)(6,7,8)</td>
<td>E – W</td>
<td>-1.51</td>
<td>3.96</td>
</tr>
<tr>
<td>2</td>
<td>(1,2,6,7)(8,7)</td>
<td>N – S</td>
<td>-0.41</td>
<td>19.64</td>
</tr>
<tr>
<td>2</td>
<td>(8)(1,2,4,6,7)</td>
<td>7 vs all</td>
<td>11.5</td>
<td>14.32</td>
</tr>
<tr>
<td>2</td>
<td>(4)(1,2,6,7,8)</td>
<td>4 vs all</td>
<td>-8.14</td>
<td>23.15</td>
</tr>
<tr>
<td>4</td>
<td>(1,2)(4)(6,7)(8)</td>
<td>Barriers 1</td>
<td>-10.11</td>
<td>28.36</td>
</tr>
<tr>
<td>3</td>
<td>(1,2)(4)(6,7,8)</td>
<td>Barriers 2</td>
<td>-13.96</td>
<td>30.42</td>
</tr>
<tr>
<td>4</td>
<td>(1,2,4)(6)(7)(8)</td>
<td>Barriers 3</td>
<td>10.91</td>
<td>10.36</td>
</tr>
</tbody>
</table>

Table 4.4. $\Phi_{ST}$ values of *Dyscolus alpinus* for COI (lower diagonal) and CAD (upper diagonal) genes.

<table>
<thead>
<tr>
<th>Populations</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. MO</td>
<td>–</td>
<td>-0.02</td>
<td>0.50</td>
<td>0.22</td>
<td>0.22</td>
<td>0.15</td>
</tr>
<tr>
<td>2. PI</td>
<td>0.50</td>
<td>–</td>
<td>0.53</td>
<td>0.31</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>4. CT</td>
<td>0.32</td>
<td>0.90</td>
<td>–</td>
<td>-0.08</td>
<td>0.65</td>
<td>0.62</td>
</tr>
<tr>
<td>6. CY</td>
<td>0.10</td>
<td>0.56</td>
<td>0.40</td>
<td>–</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>7. LV</td>
<td>0.50</td>
<td>-0.10</td>
<td>0.88</td>
<td>0.55</td>
<td>–</td>
<td>0.01</td>
</tr>
<tr>
<td>8. AT</td>
<td>0.40</td>
<td>0.86</td>
<td>0.81</td>
<td>0.50</td>
<td>0.85</td>
<td>–</td>
</tr>
</tbody>
</table>
Table 4.5. Analysis of the molecular variance for populations and species of *Dercylus*.

<table>
<thead>
<tr>
<th>No. groups</th>
<th>Partitions</th>
<th>Tests</th>
<th>COI</th>
<th>CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Among groups</td>
<td>Among populations</td>
</tr>
<tr>
<td>2</td>
<td>(2,3,5,10) (8,9)</td>
<td>E – W</td>
<td>−11.28</td>
<td>102.46</td>
</tr>
<tr>
<td>2</td>
<td>(2,3,10) (5,8,9)</td>
<td>N– S</td>
<td>11.89</td>
<td>80.09</td>
</tr>
<tr>
<td>2</td>
<td>(8)(2,3,5,9,10)</td>
<td>8 vs all</td>
<td>35.57</td>
<td>55.23</td>
</tr>
<tr>
<td>4</td>
<td>(2,10)(3)(5,9)(8)</td>
<td>Barriers 1</td>
<td>66.33</td>
<td>25.88</td>
</tr>
<tr>
<td>3</td>
<td>(2,10,3)(5)(9)(8)</td>
<td>Barriers 2</td>
<td>10.86</td>
<td>80.79</td>
</tr>
<tr>
<td>5</td>
<td>(2,10)(3)(5)(9)(8)</td>
<td>Barrier 3</td>
<td>22.59</td>
<td>68.99</td>
</tr>
<tr>
<td>4</td>
<td>(2)(10)(3)(5,9)(8)</td>
<td>Barriers 4</td>
<td>73.18</td>
<td>19.05</td>
</tr>
<tr>
<td>2</td>
<td>(5,9)(3,5,8,10)</td>
<td>8,9 vs all</td>
<td>26.22</td>
<td>10.43</td>
</tr>
</tbody>
</table>

Table 4.6. $\phi_{ST}$ values for species of *Dercylus* COI (lower diagonal) and CAD (upper diagonal) genes.

<table>
<thead>
<tr>
<th>Populations</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. <em>D. cordicollis</em> – Pichincha</td>
<td>−</td>
<td>0.00</td>
<td>1.00</td>
<td>0.06</td>
<td>0.95</td>
<td>0.00</td>
</tr>
<tr>
<td>3. <em>D. praepilatus</em> – Salinas</td>
<td>0.99</td>
<td>−</td>
<td>1.00</td>
<td>0.03</td>
<td>0.95</td>
<td>0.00</td>
</tr>
<tr>
<td>5. <em>D. orbiculatus</em> – Cajas</td>
<td>0.98</td>
<td>0.60</td>
<td>−</td>
<td>0.93</td>
<td>0.10</td>
<td>1.00</td>
</tr>
<tr>
<td>8. <em>Dercylus</em> spp. Atillo</td>
<td>0.96</td>
<td>0.97</td>
<td>0.96</td>
<td>−</td>
<td>0.90</td>
<td>−0.77</td>
</tr>
<tr>
<td>9. <em>D. orbiculatus</em> – Culebrillas</td>
<td>0.80</td>
<td>0.84</td>
<td>0.60</td>
<td>0.85</td>
<td>−</td>
<td>0.92</td>
</tr>
<tr>
<td>10. <em>Dercylus</em> spp. Cotacachi</td>
<td>0.98</td>
<td>1.00</td>
<td>0.98</td>
<td>0.95</td>
<td>0.71</td>
<td>−</td>
</tr>
</tbody>
</table>
Figure 4.1. Map showing sites were *Dyscolus alpinus* and species in the genus *Dercylus* where collected. Major geographical barriers examined in the AMOVA are highlighted (east and west cordilleras, rivers and dry valleys).
Figure 4.2. TCS haplotype network for the COI and CAD genes in *Dyscolus alpinus*.
Figure 4.3. TCS haplotype network for the COI and CAD genes in *Dercylus* lineage.
Figure 4. Bayesian 50% rule consensus tree for *Dyscolus alpinus* A) COI and B) CAD. Posterior probabilities are shown above the branches and bootstrap support values for the ML tree are shown below branches.
Figure 4.5. Timing of the *Dyscolus alpinus* clade lineage based on a relaxed molecular clock for all gene data sets.
Figure 4.6. Bayesian 50% rule consensus tree for species of *Dercylus* A) COI and B) CAD. Posterior probabilities are shown above the branches and bootstrap support values for the ML tree are shown below branches.
Figure 4.7. Bayesian 50% rule consensus tree for species of *Dercylus*, using a combined data set (COI + CAD). Posterior probabilities are shown above the branches and bootstrap support values for the ML tree are shown below branches.
Figure 4.8. Timing of the *Dercylus* clade lineage based on a relaxed molecular clock for all gene data sets.
Figure 4.9. Maximum entropy models for present distribution for *Dercylus orbiculatus*, *Dercylus cordicollis* and *Dercylus praepilatus* across the Ecuadorian Andes, using five bioclimatic variables.
CHAPTER FIVE

ORIGIN AND DIVERSIFICATION OF THE ANT–LOVING BEETLES *PANABACHIA* PARK 1942 (COLEOPTERA, STAPHYLINIDAE) FROM THE HIGH ELEVATION IN THE ANDES

INTRODUCTION

The Andes mountain chain situated along the South America continent has a dynamic geological and climatological history. A wide range of geological processes, such as plate subduction, volcanism, crustal shortening and terrain accretion, has shaped the topography and the distribution of the species in the tropical Andes (Gregory–Wodzicki, 2000; Veblen et al., 2007; Hoorn et al., 2010; Antonelli, 2015). The orogenic formation of the Andes started during the Mesozoic and peaked with a massive uplift over the past 30 Ma (Orme 2007). The increase in elevation affected the climatic patterns of the region, which were evident during the Quaternary with the formation of glaciers in the mountain summits (Schubert and Clapperton 1990, Clement et al. 2000, Ehlers et al. 2011). Theses geological and paleoclimatical events have influenced the distribution and genetic diversity of multiple evolutionary lineages that inhabit the tropical Andes, which are characterized by their high number of endemic species (Myers et al. 2000, Hoorn et al. 2010, Madriñán et al. 2013).

These events, in particular the contraction and expansion of glaciers during the Quaternary glaciations, probably had a greater effect on the evolution of tropical alpine ecosystems (Heine 2004, Villota and Behling 2014, Villota et al. 2017, Frederick et al. 2018). In this case, suitable habitats moved from the mountain slopes into the inter–Andean valleys depending on the extent of the glaciers (Villota and Behling 2014). This, in some cases, allowed certain species to exchange genetic material between populations that were usually separated by
elevation (Mac Vean and Schuster 1981); in other instances, it drove the fragmentation of species distribution (Muñoz–Mendoza et al., 2017).

In the northern Andes above 2800 m, isolated patches of páramo, a tropical alpine ecosystem exists as islands (Neil 1999, Anthelme et al. 2014). Multiple factors, including isolation due to elevation and climatic oscillations, have played into shaping the current diversity in the páramo (Neil, 1999; Madriñan et al., 2013). Most species from páramo are thought to be relatively young (0.11–5 Mya; Pliocene and Pleistocene) and possess adaptations to live at high elevation (Somme & Block, 2012; Madriñan et al., 2013). These include morphological, physiological and behavioral adaptations as result of experiencing harsh abiotic conditions, such as extreme temperatures, higher solar radiation, desiccation and reduced oxygen pressure (Hodkinson 2005, Dillon 2006, Somme and Block 2012).

The phylogeographic structure of Andean species has been partially assessed, where most studies that include páramo species are focused on the evolution of vascular plants and vertebrate species in a larger phylogeographical context (Helgen et al., 2009; Guayasamin et al., 2010; Madriñán et al., 2013; Muñoz–Mendoza et al., 2017). These studies have revealed that most páramo lineages are quite young, and that the orogeny of the Andes has played an important role shaping their phylogeographical patterns (Helgen et al., 2009; Guayasamin et al., 2010; Muñoz–Mendoza et al., 2017). Conversely, the few studies done in insect lineages from high elevation have also shown that allopatric speciation is a contributing factor to their diversity patterns (Hines, 2008; Elias et al., 2009; Maddison, 2014, Muñoz–Tobar, unpub). However, these patterns may vary depending on the dispersal capability of each insect lineage (Muñoz–Tobar, unpub). Previous studies focused on widely distributed ground beetles from páramo showed contrasting patterns of genetic distribution. Indeed, while some beetle lineages sustain high
levels of gene flow, others show higher genetic structure across páramo patches (Muñoz–Tobar, unpub). Basic evolutionary processes are not entirely well understood for most insect alpine lineages, and the discrepancies among ground beetle’s lineages from high elevation show the necessity to analyze other beetle lineages in páramo.

The rove beetles (Coleoptera: Staphylinidae) represent one of the most diverse families of beetles (61,300 spp.; Newton, 2015). Its diversity has been attributed to the variety of habitats they inhabit, their feeding behaviors and ecological interactions (Thayer 2005). Many representatives of this family are found in the Neotropical Region, a region that is thought to contain one of the most diverse faunas of rove beetles, though it is still understudied (Newton 2015). In Ecuador, a total of 544 species of rove beetles have been reported (Donoso et al. unpub), mainly from lowland areas. Still, the general diversity of rove beetles in this region it is thought to be much greater than previously documented (Newton 2015), including in mid– and high–elevation areas that present unique Andean microhabitats (Sánchez–Baracaldo & Thomas, 2014; Hubb et al., 2017).

In this study, I focused on diversification patterns in páramo populations of the genus Panabachia Park 1942, a Neotropical genus of ant–loving beetles (Coleoptera, Pselaphinae) that can be identified by the presence of a large trilobed excavation in the pronotum (Park 1942). So far, only two species have been described within this genus: P. vulnerata (Sharp, 1887) from Panama, and P. impressicollis (Sharp 1887) from Guatemala (Newton & Chandler, 1989; Navarrete–Heredia et al., 2002). However, this genus is thought to be present across the Neotropical Region, from Mexico to Bolivia, on leaf litter and on top of bromeliads (Navarrete–Heredia et al., 2002), and during the summer of 2016 Panabachia was collected from leaf litter samples of Ecuadorian páramo. The diversity of this genus is unexplored, and in this study, I
aim to investigate the evolutionary history and genetic diversity in the genus *Panabachia* from páramo. For that, I addressed four specific questions: (1) How many reproductively isolated clades of *Panabachia* are present in the sampled material from páramo? (2) Are genetically isolated clades restricted to specific sites? (3) Is the distribution of the genetic diversity limited by major geographic features such as rivers, dry valleys, and other subdivisions within the Ecuadorian Andes, as observed for some ground beetle lineages from páramo? and (4) Is the timing of diversification of *Panabachia* across páramo contemporary (Miocene–Pliocene), or did it precede the current distribution of this ecosystem like some ground beetle lineages (e.g. *Pelmatellus columbianus*, 11.9 Mya and *Dyscolus alpinus*, 6.32 Mya; Muñoz–Tobar, unpub). These questions will be addressed through the combination methods including species delimitation, phylogenetics and divergence time estimation.

**MATERIAL AND METHODS**

**Field collection**

*Panabachia* individuals were recovered from leaf litter samples from 7 sites across the highlands of Ecuador (Figure 5.1, Table 5.1). Three leaf litter samples were extracted per site, from a variety of litter types (*Polylepis* forest, moss, shrubs and grass). The selection of the sites was based on conservation status, since most of the collecting took place within the network of Ecuadorian national parks and protected areas. Collecting permits for this study were previously obtained (MAE–DNG–ARGG–CM–2014–004). The sifted material was transported to the lab, and processed using Berlese funnels into 100% ethanol. Collected beetles were separated into morphospecies, based on characters examined and genus identification was done with the help of
Dr. Joseph Parker and Dr. Don Chandler. Voucher specimens of this study will be deposited in the Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ) after the study is concluded.

**DNA extraction, amplification and sequencing**

The entire body of each beetle was used to extract genomic DNA using GeneJet Genomic DNA Purification Kit (Thermo Fisher Scientific, Vilnius, Lithuania). Polymerase chain reaction was used to amplify two molecular markers COI and *wingless*. The mitochondrial gene COI was amplified using the primers C1–J–2183 (5’–CAACATTTATTTTGTATTTTTG–3’) and TL2–N–3014 (5’–TCCAATGCACTAATCTGCCATATT–3’, Simon et al., 1994) following the amplification profile described by Caterino & Tishechkin (2014). For the nuclear gene *wingless*, I used the primers wg550f (5’–ATGCCTAGGARTGYAARTGYCAYGGYATGTC–3’) and wgAbRZ (5’–CACTTNACYTCRCARCACCARTG–3’; Wild & Maddison, 2008) following the amplification profile described by Parker & Grimaldi (2014). PCR reactions of 25 µl generally contained 2–3 µl genomic DNA, 17.5 µl water, 2.5 µl 10x buffer, 0.5 µl dNTPs, 0.75 µl MgCl₂, 0.1 µl AmpliTaq® DNA polymerase (Thermo Fisher Scientific) and 1 µl of each primer (10nm). Amplification cycles were performed in a Mastercycler® nexus (Eppendorf). PCR products were purified using ExoSAP–IT (USB/Affymetric, CA, U.S.A), and sequencing was done commercially by Macrogen USA, Inc. (Rockville, MD, U.S.A). Sequences were manually cleaned using Geneious R8 (Biomatters Ltd., Auckland NZ), and aligned using MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/).
Phylogenetic analyses

Models of molecular evolution were assessed using JModeltest 2.0 (Darriba et al. 2012) for each molecular marker, where GTR+I+G model appeared to be in the 100% confidence interval for COI and wingless data. TCSv1.21 (Clement et al., 2000) was used to construct haplotype networks for each data set. To reconstruct phylogenetic relationships among haplotypes RAxML version 8.2.8 (Stamatakis, 2014) was launched from Mesquite’s Zephyr package (Maddison & Maddison, 2015) with 1000 bootstrap replicates. For Bayesian inference I used Mr. Bayes 3.2 (Huelsenbeck & Ronquist, 2001), through 10 million generations.

Species delimitation analyses

The number of species within the genus Panabachia is unknown. Morphology–based species classification is a useful tool to determine species, yet the number of species can be masked by lack of characters in females of Panabachia, since sexual dimorphism is recorded in this genus (Chandler, 2001; Navarrete–Heredia et al., 2002) and a high proportion of females were found among the samples collected. Therefore, I used two sequence based species delimitation methods to determine the number of reproductively isolated clades in samples of Panabachia from páramo. Specifically, I used the Bayesian implementation of the PTP model, in the bPTP server (http://species.h–its.org/ptp/; Zhang et al., 2013); and a single threshold GMYC was implemented in the GYMC server (http://species.h–its.org/gmyc/; Fujisawa & Barraclough, 2013). Species delimitation analyses using these two models were performed using single locus and a multilocus data set.

For analyses in bPTP, trees generated in Mr. Bayes were used as input. Analyses were run through 100000 MCMC generations, with a thinning of 100 and 0.1 of burning. For GYMC,
ultrametric trees were produced in BEAST 2.0 (Bouckaert et al., 2014), using an uncorrelated relaxed clock, a constant coalescent speciation process prior, through 10 000 000 generations and 10% burn–in. Effective Sample Size (ESS) was evaluated in Tracer v1.5 (Rambaut and Drummond 2003), considering runs with ESS values above 200. Output trees were generated in TreeAnnotator 2.0.02 (http://beast.bio.ed.ac.uk), using maximum clade credibility (MCC) after a 10% burn–in and median heights for node heights. Resulting trees were used as input in the GYMC server, using a single threshold. Additionally to these delimitation methods, pairwise distances using Jukes Cantor were calculated for each gene in PAUP v.4.0 (Swofford 2003) to infer intra– and interspecific differences among sister clades, and were an aid to hypothesize species–level clades.

Divergence time estimates

Divergence time estimates were generated in BEAST 2.0 (Bouckaert et al., 2014) using an uncorrelated relaxed clock, with a combined data matrix for COI and wingless. Two points of calibration were used, all designating minimum node age within the Pselaphinae. The first was an undescribed Bythinini from Burmese amber (99 Mya; Parker & Grimaldi, 2014); and a second point of calibration is based on estimates generated in the Parker & Grimaldi (2014) paper, where they estimate higher Pselaphinae to be 150 Mya. Output trees were recovered using TreeAnnotator 2.0.02 (http://beast.bio.ed.ac.uk), with maximum clade credibility (MCC) after a 10% burn–in.
Population structure

Once distinct genetic clusters of *Panabachia* were identified through species delimitation methods, clades represented by individuals from more than one site were considered for population structure. The population structure was assessed through $\phi_{ST}$ values calculated in Arlequin 3.5.2.2 (Excoffier & Lischer, 2010). Standard indices of genetic diversity, such as haplotypic diversity, nucleotide diversity and neutrality tests were calculated in DnaSP (Rozas et al. 2003). These were complemented with the analyses of molecular variance (AMOVA) calculated in Arlequin 3.5.2.2 (Excoffier & Lischer, 2010) to determine the degree of variance between populations found across potential geographical barriers such as east and west mountain ranges, as well the rivers Pastaza and Mira, and their corresponding dry valleys which are thought to have influenced the distribution of plants and animals in the area (Krabbe 2008, Guayasamin et al. 2010). To determine if there was a correlation between genetic and geographical distances, a Mantel test was performed in Arlequin 3.5.2.2 (Excoffier & Lischer, 2010), using $\phi_{ST}$ values, and straight–line geographic distance, as measured by Google Earth. The number of populations per species was calculated in Structure 2.3.4. (Pritchard et al. 2000) by calculating the $K$ in the population.

RESULTS

Sampling and sequence data

Sampling from litter resulted in the collection of 68 adult *Panabachia* from 7 localities (Fig. 5.1). For phylogenetic analyses I used an average 10 individuals per site where possible (Table 5.1), with exception of Releche where the sifted material only yielded 7 individuals. The COI
gene was amplified from 67 samples (GenBank accessions X to X), and the alignment of this gene had a total of 765 base pairs. Of the 765 base pairs, 240 were variable, 66 were parsimony informative (Table 5.2), and 30 distinct haplotypes were identified using TCS (Figure 5.2). The *wingless* gene was amplified from 62 individuals. The alignment for this gene had 445 base pairs with 108 segregating sites from which 105 were parsimony informative (Table 5.2). For the *wingless* data set, 55 haplotypes were identified (Figure 5.3). Amongst the two data sets, only a few haplotypes were shared among sites. Individuals from La Virgen shared COI haplotypes with individuals of Pichincha (H9), Attillo (H11) and Releche (H10) (see Fig 5.2). For the *wingless* data set, only one haplotype (H1) was shared, among the northern populations of Cayambe, Pichincha, and La Virgen (Figure 5.3). The overall nucleotide diversity for each data set was low (\( \pi = 0.047 \); Table 5.2).

**Species delimitation analyses**

Results from single locus and multilocus analyses using two models of species delimitation are summarized in Figure 5.2. These analyses identified 17–20 species, with a high level of congruence among results from bPTP and GYMC, using a combined data set. The single locus analyses showed a high variation among outputs. *Wingless* showed a wide range of results depending on the method used. For example, bPTP presented the highest number of subdivisions, with 51 species identified, and the analyses performed in GMYC revealed only three genetic clusters (Figure 5.4). For the mitochondrial gene, results from bPTP showed a high level of correspondence with results from the multilocus analyses, with 20 species identified. This level of correspondence was not seen in the GMYC, and only 4 species were observed (Figures 5.4).
Overall, the congruence between the analyses of multilocus data sets and COI using bPTP shows that between 17–20 species. These results were partially supported by the pairwise distance matrix of the nuclear gene, which divided the individuals into 11 clades supported by bootstrap and posterior probability values (Figure 5.2, Table 5.3). Clades defined using wingless show within clade variation of 0–2%, whereas genetic variation between clades was above 3% (Table 5.3). These values were significantly higher for the COI data set, where pairwise matrix showed a mean variation within clades of 7% and above 12% among clades.

For further interpretation of phylogenetic relationships and geographical distribution of genetic clusters, a conservative number of species (17) will be considered the most reasonable hypothesis. This approach was based on the statistical support for each species lineage in the phylogenetic inferences and the distribution of haplotypes in the TCS, since splits observed in pBTP multilocus analyses probably represent intraspecific variation in Mojanda. The number of species is also moderately supported by the preliminary morphological analyses: characters in the pronotum of the male, such as the size and shape of the pronotum, show correspondence with the proposed species in this group. Yet, further sampling is needed since there is a higher proportion of female in the sampled material, and not all species lineages identified are represented by male specimens.

**Phylogenetic analyses and divergence time estimates**

Phylogenetic inferences generated through Maximum Likelihood and Bayesian methods show that *Panabachia* is a monophyletic group (Figure 5.5–5.6). Two separate radiations are apparent in these phylogenic trees, where most species lineages identified through species delimitation analyses appear to be well supported by bootstrap and posterior probability values.
Exceptions are found for the species lineages 1, 2 and 17, which do not have strong branch support (Figure 5.2). Gene trees did not recover all the genetic clusters observed in the species delimitation analyses (Figure 5.5–5.6). The COI gene tree shows support for most species lineages (Figure 5.5), with strong bootstrap and posterior probability values. Yet, the species lineages 2, 13 and 17 were not supported by these statistical values (Figure 5.5). In the case of the wingless gene tree, bootstrap and posterior probability values only found support for 5 species out of the 17 proposed (Figure 5.6). When I compared both gene trees, the wingless gene tree shows a lack of phylogenetic resolution (Figure 5.5–5.6).

In reference to the distribution of species lineages, clear geographical splits among sites were not detected in the phylogenetic inferences, since multiple species were identified for the majority of sites (e.g. four species in Mojanda). However, in a closer look to these separate radiations events, divergence among species at opposite sides of the mountain range, east and west cordillera, starts to become apparent. This was the case for species 8 (Pichincha, W) and 7 (Atillo, E), separated by distance and side of the mountain range. Most species with well supported branches show patterns of divergence that might be explained by the distance between sites. This pattern was detected between the species 9 (Mojanda, W) and 10 (el Angel, W), separated by <70 km, a distance that might be reinforced by the presence of the dry valley and Mira river. Similarly, divergence between species 14 (Releche, E) and 15 (Atillo, E) was detected; these species are separated by <60 km, where no major geographical barriers were apparent. Other species of páramo represent unique geographical areas, but the relationships among species is not well supported by posterior probability and bootstrap in the genetic trees or the tree generated with a combined data set.
Divergence time estimates show that the *Panabachia* lineage originated in the Miocene (9.2 Mya), and that most of the species from páramo diverged during the Pliocene and Pleistocene (5.3 – 0.11 Mya), with exception of *Panabachia* species lineage 16, that originate in the Miocene (7.86 Mya). Two parallel radiations are observed through phylogenetic analyses, these radiations started in the Miocene (8–7.85 Mya) and continued throughout the Pleistocene. The first radiation event (7.86–0.47 Mya) gave rise to species 7–17, and a second radiation gave rise to species 1–6 (4.65 –0.24 Mya). This second radiation is composed mostly of northern species (species 1–4, and 6), with the exception of species 5, represented by specimens collected in Atillo (SE). Another exception was found among individuals of the species lineage 2 that contained an individual from the southern site of Releche (SIMT284). Species 2 is a particular lineage in comparison to the other species, because it is composed of individuals from multiple sites (La Virgen, Pichincha, El Angel and Releche) and might represent a widespread species.

**Population structure**

The population structure of the species 2 and 3 was preliminarily explored. These species were chosen for analyses due to the variation in the species delimitation analyses, which either defined the data as one or two species, and the lack of branch support for species 2 in the tree topologies. Some consideration were taken in account prior to the analyses, such as the exclusion of individual SIMT248 (from el Angel) due to missing data in the mitochondrial data set, and the input data were treated as a single species.

The population genetic analyses show moderate levels of population subdivision for both molecular makers (Overall $\phi_{ST}=$0.4–0.7). When comparing $\phi_{ST}$ values on a population by population basis, the COI data set shows high levels of genetic differentiation when the other
population were compared to Cayambe (\( \phi_{ST} = 0.46–0.76 \), Table 5.4). The gene *wingless* shows high differentiation when populations were compared to Releche (\( \phi_{ST} = 0.63–0.76 \), Table 5.4). An AMOVA was used to test potential geographical barriers. For COI, most of the variation was found within populations (Table 5.5). In contrast, the geographical partitioning of the *wingless* data set showed that most of the variation was found among groups when Releche was considered as a separate group, which suggest distance is a factor contributing to population subdivision. There was no correlation between genetic and geographical distances in the Mantel test (\( R^2 = 0.23 \); \( P > 0.22 \)), even though Releche is separated from the other sites by a considerable distance (<193 km). Lastly, the analyses in Structure detected one populations (K=1) using the mtDNA, and the nuclear protein–coding gene.

**DISCUSSION**

High elevation species are particularly interesting given the climatic diversity, high levels of isolation and complex geological history of mountain systems (Madriñán et al. 2013, Antonelli 2015, Hoorn et al. 2018). Alpine beetle faunas from the Andes have only been superficially explored. Previous work in the Ecuadorian páramo showed distinct patterns of genetic distribution in ground beetles, from higher population structure in flightless ground beetles to high levels of genetic connectivity between populations of a macropterous species (Muñoz–Tobar, unpub). Still, the genetic diversity of other alpine beetle lineages from the Andes has not been assessed, which questions whether other alpine beetles are following similar patterns as the ground beetles.

Species delimitation analyses allow the identification of distinct evolutionary lineages within a sample (Fujita et al. 2012, Fujisawa and Barraclough 2013, Zhang et al. 2013), and the use of
multilocus genetic data has proven to be a powerful tool for delimiting species (Fujita et al. 2012). Yet, methods to delimit species vary greatly in parameters and outcomes, and the search for congruence across results from species delimitation models will provide us with a more accurate hypothesis for species boundaries. Results from the species delimitation analyses show that *Panabachia* from páramo is a diverse beetle lineage; 17–20 species were identified with bPTP and the GMYC using a multilocus data set. Both models of species delimitation showed similar outcomes, but bPTP tended to subdivide further the data showing 1–3 more species than GMYC. Evidence found in the phylogenetic inferences, haplotype networks, and percentage of variation between sequences shows that some delimited species using bPTP model might actually represent intrapopulation variation. This seems to be case of species lineage 11 from Mojanda, which is divided by bPTP into two species lineages. For other data subdivisions, for instance species lineage 5 from Atillo, bPTP also divides this beetle lineage into two species. Still, this speciation event is recorded in the phylogenetic inferences, as well as in the TCS analyses which shows the existence of two separate haplotype network. However, sequences for this species lineage show low percentage variation among individuals.

When results from the species delimitation analyses were compared, multilocus versus single locus, the single locus analyses showed a wide variation of results depending on the gene and model of delimitation used. The GMYC tends to detect for both genes a smaller number of species (3–4), which was unexpected since this method has been criticized for over dividing the data (Miralles and Vences 2013, Zhang et al. 2013). In contrast, outputs from the bPTP analyses show a higher number of species. For the *wingless* gene, 51 species were identified, possibly due to the high number of haplotypes found within this data set (55 haplotypes). With the mitochondrial data set, bPTP found 20 species. This last result has a higher correspondence with
the number of species defined using these two models of species delimitation that identified 17 to 20 species. Most of these species lineages detected through species delimitation are supported by bootstrap and posterior probability values (Figure 5.2), with exception of the species lineage 1, 2 and 17 that do not have branch support. Pairwise distances generated using the wingless data set partially supported these data subdivisions, by identifying 11 well supported clades, which in most cases correspond to species delimitations results (Figure 5.2).

In reference to the phylogenetic relationship among species of the lineage Panabachia, two parallel radiations were identified in the phylogenetic trees, with support from bootstrap and posterior probability values in each gene tree (Figure 5.5–5.6). This was not the case for the phylogeny generated using a combined data set, where only one radiation event has strong branch support. Divergence time estimates show that these radiations started during the Miocene (5.59–7.81 Mya), but 14 out of 17 species of Panabachia from páramo originated during the Pleistocene (0.11–4.6 Mya, Figure 5.7). These estimates are contemporary with the environment they live in, since the Andes reached its current elevation during the Pleistocene (Gregory–Wodzicki, 2000). The increase in elevation created suitable conditions for the development of high elevation species (Sklenář et al. 2011), which are thought to have evolved from close relative lineages from the lowland tropical areas, as well as from lineages from temperate regions (Luteyn 1999, Madriñán et al. 2013, Merckx et al. 2015). Studies of plant lineages from páramo show accelerated rates of diversification during this period of time (Madriñán et al. 2013), which is consistent with the patterns observed among species of Panabachia from páramo.

The main factors that affect cladogenetic events are associated with geographical isolation or ecological shifts (Barraclough and Nee 2001). Although most of the genetic clusters of Panabachia are well supported by bootstrap and posterior probability values, and represent...
distinct geographical areas, phylogenetic relationships between species within each radiation are not well supported. Few speciation events associated with the presence of geographical barriers are well supported across tree topologies (Figure 5.4–5.6). A higher level of correspondence with geography was found in the COI gene tree (Figure 5.5). Lineage splits in this tree topology are found between species at opposite sides of the mountain range (species 8–7; Atillo and Pichincha). However, most of the speciation events with well supported branches show divergence between species on the same side of the mountain range but separated by distance were more commonly found (species 8–9 and 14–15). In some instances, for example, between species 8 and 9, the distance might be reinforced by the presence of a major river and dry valley (Mira river and valley). Although phylogenetic relationships among species are not well supported, each lineage typically represents an individual site, which suggests geographic isolation is playing an important role in the evolution of this group, since only a single widely distributed species was found among the sampled data.

While allopatric speciation might explain some of the patterns in the *Panabachia* from páramo, it does not explain the high number of species found in Angel, Mojanda and Atillo, which seem to be sympatric. Understanding the timing of local geological events might give us insight into the factors that influence speciation events of these sites, for example, volcanic activity dates prior to 10,000 years in Mt. Mojanda and Mt. Fuya in Mojanda, and Mt. Chiles in el Angel (Robin et al. 2009, Monsalve and Laverde 2016). For Atillo, evidence of the presence of small glaciers during Pleistocene and Holocene were found in Mt. Ayapungo (4,730 m) and Mt. Coyay (4,630 m; Jordan & Hastenrath, 1998), mountains that are adjacent to Atillo. The high diversity of species from páramo is influenced by geological and paleoclimatrical events (Madriñán et al. 2013). The increased number of species of these particular sites could be the
result of multiple re–colonization events from adjacent areas, as seen in other mountain systems (Edwards and Sugg 1993, Elizalde 2014).

Other factors that might have contributed to the diversity in the sample of *Panabachia* is the method of sampling. Given that páramo has a great diversity of plants (Luteyn 1999, Sklenář et al. 2014), I was able to collect different types of leaf litter, from decomposing grass leaves and roots, *Polylepis* and Compositae leaf litter, and moss over rocks and rotten wood. Several species of *Panabachia* from páramo are present in the same geographical area, and could be associated with a specific type of leaf litter. Yet, further sampling and more information about the natural history of the group is needed to determine if there is an association between leaf litter or food source, since most rove beetles are predatory species.

In comparison with patterns found among ground beetles from páramo, *Panabachia* from páramo diverged in more recent times (mostly in the Pleistocene). Most ground beetle species from Ecuadorian páramo evolved during the Miocene, prior to the evolution of this ecosystem (~6–20 Miocene Mya; Muñoz–Tobar, unpub). Phylogeographic breaks in *Panabachia* are not as clear as in the *Dercylus* lineage (Carabidae, Harpalinae) where the presence of geographical barriers (e.g. rivers, dry valleys and mountain range) had a great effect in the pattern of speciation of this group (Muñoz–Tobar; unpub.). Yet, most species within the *Panabachia* lineage represent a restricted geographical area. When the diversity of *Panabachia* was compared to the widely distributed *Pelmatellus columbianus* (11.19 Mya, Carabidae, Harpalinae), the only species lineage with similar characteristics was the *Panabachia* species lineage 2, which is present in multiple sites. The population structure of species 2 and 3 was analyzed due to the variation in species delimitation analyses that divided these samples into one or two species, depending on the model used. Results show some level of subdivision among
some populations, where individuals from Cayambe and Releche represent distinct genetic clusters. This was partially supported by AMOVA results using wingless, which reveal that most of the variation is found among groups when Releche is considered a separate group (Table 5.5). This widely distributed lineage of Panabachia has a higher genetic diversity when compared to populations of P. columbianus, but these results are still preliminary due to the small number of samples. Hence, a more comprehensive analysis will require additional samples from each population. Nevertheless, widely distributed species of ground beetles have been reported at the same sites as this widespread species of Panabachia. Such is the case in P. columbianus (Cayambe, La Virgen, Pichincha, Releche) and Dyscolus alpinus (Cayambe, Pichincha, La Virgen; Muñoz–Tobar, unpub), which suggests similar factors are affecting the evolution of these northern beetle lineages. Specially, the effect of Quaternary glaciation might have enabled gene flow between sites now isolated by elevation (Mac Vean and Schuster 1981).

The study of multiple beetle lineages from páramo provides a better understanding about the evolution of high elevation beetle faunas. An increasing number of studies have concentrated on ground beetle species from the Ecuadorian Andes (Moret 2005, 2009, Anthelme et al. 2014). Other beetle lineages from páramo have been less studied, but results from these studies show that high elevation faunas have an elevated number of endemic species (Mac Vean and Schuster 1981, Moret 2005, 2009, Martinez et al. 2007, Anthelme et al. 2014). Numerous species have yet to be described, as seen in Panabachia, which reveals a large number of species across sampled sites. The high diversity in the ant–loving beetles confirms the importance of conserving high elevation ecosystems. Although most of the sampled sites are already protected areas (Cuesta, Peralvo, et al. 2017), many high elevation areas across the Ecuadorian Andes are not part of this network of national parks.
Overall, *Panabachia* represents a promising model for the study of diversification of beetles from high elevation areas in the Andes. Most of the species identified are restricted to a specific area, and with the few examples studied so far, the age of each species is contemporary with the ecosystem they live in (Figure 5.2). The distribution of genetic diversity of *Panabachia* is complex, and a generalized pattern for alpine beetles of the Ecuadorian Andes has yet to emerge. Multiple factors appear to be shaping the genetic diversity of alpine beetle species, including mountain isolation and habitat discontinuity, as well as dispersal capability, and the distribution of genetic clusters in the *Panabachia* lineage shows indications of all these factors. The pattern of divergence observed across the tree topologies in this study certainly does not capture the entire genetic diversity of the *Panabachia* lineage, since sampling was only focused on isolated páramo patches. A more comprehensive picture of the distribution of phylogenetic diversity of *Panabachia* would be revealed if specimens from lower elevation (e.g. cloud and montane forest) were included in the analyses, since the genus is reported to be present in lower elevation areas (Navarrete–Heredia et al., 2002).
Table 5.1. Population and site information for each sample of Panabachia from páramo sites.

<table>
<thead>
<tr>
<th>Mountain range</th>
<th>No.</th>
<th>Site</th>
<th>N</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation</th>
<th>Collecting Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>West</td>
<td>1</td>
<td>El Angel</td>
<td>10</td>
<td>N00°42.3521'</td>
<td>W77°57.985'</td>
<td>3301 m</td>
<td>26–Jul–16</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Mojanda</td>
<td>10</td>
<td>N00°08.710'</td>
<td>W78°16.753'</td>
<td>3715 m</td>
<td>12–Jul–16</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Pichincha</td>
<td>11</td>
<td>S00°11.259'</td>
<td>W78°32.432'</td>
<td>3897 m</td>
<td>22–Jun–16</td>
</tr>
<tr>
<td>East</td>
<td>4</td>
<td>Cayambe</td>
<td>10</td>
<td>S00°02.101'</td>
<td>W78°03.608'</td>
<td>3743 m</td>
<td>1–Jun–16</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>La Virgen</td>
<td>10</td>
<td>S00°18.477'</td>
<td>W78°13.953'</td>
<td>3694 m</td>
<td>28–Jun–16</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Releche</td>
<td>7</td>
<td>S01°38.400'</td>
<td>W78°30.426'</td>
<td>3124 m</td>
<td>8–Jul–16</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Atillo</td>
<td>10</td>
<td>S02°11.265'</td>
<td>W78°31.2601'</td>
<td>3501 m</td>
<td>7–Jul–16</td>
</tr>
</tbody>
</table>

Table 5.2. Overall genetic indexes for the genus Panabachia. N refers to the number of individuals sampled; S, number of segregating sites; Ps, number of parsimony informative sites; \( \pi \), is a measure of nucleotide diversity; \( \theta \) is a measure of genetic diversity; D represents Tajima’s D, a neutrality test statistics.

<table>
<thead>
<tr>
<th>Gene</th>
<th>N</th>
<th>S</th>
<th>Ps</th>
<th>( \theta )</th>
<th>( \pi )</th>
<th>( D )</th>
</tr>
</thead>
<tbody>
<tr>
<td>COI</td>
<td>67</td>
<td>240</td>
<td>66</td>
<td>0.054</td>
<td>0.113</td>
<td>1.73 (p&gt;1.78)</td>
</tr>
<tr>
<td>wingless</td>
<td>62</td>
<td>118</td>
<td>105</td>
<td>0.056</td>
<td>0.047</td>
<td>-0.51 (p&gt;0.10)</td>
</tr>
</tbody>
</table>
Table 5.3. Mean uncorrected pairwise distances for COI and wingless data sets for within and among clades of Panabachia.

<table>
<thead>
<tr>
<th></th>
<th>among clades</th>
<th>within clades</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>range</td>
</tr>
<tr>
<td>COI</td>
<td>0.12</td>
<td>0.07–0.18</td>
</tr>
<tr>
<td>Wingless</td>
<td>0.03</td>
<td>0.03–0.13</td>
</tr>
</tbody>
</table>

Table 5.4. $\phi_{ST}$ values for populations for species 2 and 3 of Panabachia lineage. In the upper diagonal $\phi_{ST}$ values from the mitochondrial marker (COI), and in the lower diagonal $\phi_{ST}$ values from the nuclear gene data set (wingless).

<table>
<thead>
<tr>
<th>Populations</th>
<th>Pichincha</th>
<th>Cayambe</th>
<th>La Virgen</th>
<th>Releche</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pichincha</td>
<td>–</td>
<td>0.46</td>
<td>0.12</td>
<td>–0.76</td>
</tr>
<tr>
<td>Cayambe</td>
<td>–0.09</td>
<td>–</td>
<td>0.75</td>
<td>0.76</td>
</tr>
<tr>
<td>La Virgen</td>
<td>–0.02</td>
<td>0.06</td>
<td>–</td>
<td>–0.33</td>
</tr>
<tr>
<td>Releche</td>
<td>0.63</td>
<td>0.75</td>
<td>0.76</td>
<td>–</td>
</tr>
</tbody>
</table>
Table 5.5. AMOVA for populations for species 2 and 3. Numbers in the partitions represent site number.

<table>
<thead>
<tr>
<th>Partitions</th>
<th>Tests</th>
<th>COI</th>
<th>wingless</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Among groups</td>
<td>Among populations</td>
<td>Within populations</td>
<td>Among groups</td>
</tr>
<tr>
<td>(3,4,5) (6)</td>
<td>N–S</td>
<td>-48.36</td>
<td>67.03</td>
<td>81.34</td>
<td>74.53</td>
</tr>
<tr>
<td>(3)(4,5,6)</td>
<td>E–W</td>
<td>18.96</td>
<td>28.97</td>
<td>52.07</td>
<td>-56.02</td>
</tr>
<tr>
<td>(4) (3,5,6)</td>
<td>Cayambe vs all</td>
<td>20.04</td>
<td>24.12</td>
<td>55.84</td>
<td>-47.20</td>
</tr>
<tr>
<td>(5)(3,4,6)</td>
<td>La Virgen vs all</td>
<td>-15.49</td>
<td>53.51</td>
<td>61.98</td>
<td>-50.70</td>
</tr>
<tr>
<td>(6)(3,4,5)</td>
<td>Releche vs all</td>
<td>-48.36</td>
<td>67.03</td>
<td>81.34</td>
<td>74.53</td>
</tr>
<tr>
<td>(3)(3,4,6)</td>
<td>Pichincha vs all</td>
<td>18.96</td>
<td>28.97</td>
<td>52.07</td>
<td>-56.02</td>
</tr>
<tr>
<td>(3)(4,5)(6)</td>
<td>Barriers 1</td>
<td>2.28</td>
<td>40.18</td>
<td>57.54</td>
<td>40.12</td>
</tr>
</tbody>
</table>
Figure 5.1. Map of the collecting sites for *Panabachia* in the Ecuadorian Andes. Potential geographical barriers are highlighted in this map.
Figure 5.2. TCS haplotype networks for COI gene of Panabachia.
Figure 5.3. TCS haplotype networks for the nuclear gene, *wingless* of *Panabachia*.
Figure 5.4. Bayesian inference based on a combined data set for *Panabachia* species. Colored bars represent site information, and species delimitation analysis using bPTP and GYMC are represented by bars on the right side of the phylogeny, color on these bars signify that belong to the same group. Posterior probabilities are shown above the branches and RAxML bootstrap values are shown below branches. Branch lengths are not proportional with the number of changes.
**Figure 5.** Posterior probability tree for the mitochondrial gene COI of *Panabachia*. Branch lengths are in proportion to the number of substitutions per sites, in reference with the scale bar. Posterior probabilities are shown above the branches and bootstrap support values for the ML tree are shown below branches.
Figure 5.6. Posterior probability tree for the nuclear coding gene *wingless* of *Panabachia*.

Branch lengths are in proportion to the number of substitutions per sites, in reference with the scale bar. Posterior probabilities are shown above the branches and bootstrap support values for the ML tree are shown below branches.
**Figure 5.7.** Divergence time estimation for *Panabachia* based on a relaxed molecular clock, using a combined data set.
CHAPTER 6
CONCLUSIONS AND FUTURE DIRECTIONS

Understanding the pattern of distribution of the genetic diversity and the processes that
gave origin to alpine lineages from the Andes is a fascinating task that relies on disentangling
macro and microevolutionary processes. Commonly, the distribution of a species is presumed to
be determined by abiotic factors, ecological interactions of species, and dispersal dynamics
(Lester et al. 2007). For species in mountain systems, we also have to take into consideration the
series of orogenic events that gave rise to the mountain range and the paleoclimatical events
associated with the increase in elevation. In the northern Andes, the biological diversity is
attributed to multiple vicariant events caused by the uplift of the Andes, and by the ecological
opportunity originated from the new niches (Kattan et al. 2004). This raises the question about
patterns of genetic diversify for highland species, and the effect that each factor (e.g. geology,
dispersal) had throughout the Andean radiations, given that the northern Andes are a highly
diverse area (Smith et al. 2014). Discerning these patterns will help conservation efforts for high
elevation species of the Andes, by identifying areas of high diversity of species, as well as by
clarifying which areas are important for maintaining large scale connectivity among regions (e.g.
use of biological corridors).

My comparative study of four beetle lineages from the Ecuadorian Andes, including
ground beetles (Coleoptera, Carabidae) and ant–loving beetles (Coleoptera, Staphylinidae,
Pselaphinae), reveals that the processes underlying the evolution of beetles from páramo are
complex. In contrast with my vicariance hypothesis, which suggested beetle species from páramo
had similar patterns of genetic structure, analysis of these beetle lineages indicates no general
pattern of diversification in beetles from páramo. My work combined with other phylogenetic
studies shows that the effect of mountain isolation varies across beetle taxa (Wagner and Liebherr 1992, Chatzimanolis and Caterino 2007, Ikeda et al. 2012, Vogler 2012). The distribution of the genetic diversity for each beetle lineage may be influenced by several factors such as divergence time, range size, dispersal capability (in some cases is affected by the presence or absence of functional wings), as well as geological and paleoclimatic events that occurred in the Miocene–Pleistocene (Madriñán et al. 2013).

Each factor has influenced, to different degrees, the pattern of genetic structuring of each beetle lineage analyzed in this study. For example, dispersal ability and range size may have a greater effect on ground beetle species. The widely distributed species *Pelmatellus columbianus* appears able to overcome geographical barriers and therefore sustain high connectivity between populations. The presence of wings in this species probably enables the dispersal of the species across elevational gradients (inter–Andean valley to páramo). Winged beetles generally have a wider geographical distribution when compared to flightless species (Gutierrez & Menendez, 1997; Ikeda et al., 2012). Nevertheless, populations of this ground beetle are not entirely homogenous; population differentiation was found when populations were separated by greater distances (>97 km). The flightless ground beetle species *Dyscolus alpinus* and species in the genus *Dercylus* tend to exhibit smaller geographic ranges and could be affected by the presence of geographical barriers; especially for species in the genus *Dercylus*, where genetic clusters represent distinct geographical areas. East and west vicariance can be traced in the phylogeny of *Dercylus* (e.g. between *Dercylus cordicollis* and *Dercylus* spp. from Atillo), but does not explain the other vicariance events that could be associated with the presence of dry valleys, mountains and rivers (e.g. Chimbo, Chan Chan river and valley). For populations of *Dyscolus alpinus*, high levels of gene flow were found among adjacent populations, even though they have lost their
ability to fly. However, with increased distance, moderate to high levels of population subdivision are noticeable. Similarly, population structuring seems to coincide with the presence of dry valleys (Guayllabamba, San Antonio, Chimbo and Chan Chan), major rivers (Pastaza, Chimbo and Chan Chan) and side of the mountain range in some cases.

The analyses of macropterous and flightless ground beetles reveal that flightless ground beetle species have a higher phylogeographical structure, as hypothesized. Yet, the level of mountain isolation is dependent on the amount of gene flow among beetle populations, since gene flow tends to promote homogeneity across populations (Slatkin 1987). The reduction of wings in flightless ground beetles has not completely eliminated the gene flow between adjacent populations (>37 km), and morphological traits such as increased length of legs (e.g. *Dyscolus alpinus*) might be enabling their land dispersal. The high levels of genetic exchange within some species (e.g. *Pelmatellus columbianus* and *D. alpinus*) in the northern Andes was probably enabled by the expansion of glaciers during Pleistocene glaciations, which caused the movement of alpine faunas to lower elevation areas (Villota and Behling 2014), promoting gene flow among populations previously separated by elevation.

Comparing the ages of diversification events has put into perspective how geological and paleoclimatirical events have influenced the evolution of beetles from high elevation areas. Among the analyzed populations, most ground beetle lineages originated prior to the evolution of the páramo ecosystem, which occurred 2–5 Mya during the Pliocene and Pleistocene (Figure 6.1; Madriñán et al., 2013). Six out of the seven species of ground beetles analyzed evolved during the Miocene: *Pelmatellus columbianus* (11.19 Mya), *Dyscolus alpinus* (6.32 Mya), *Dercylus orbiculatus* (20.08 Mya), *Dercylus cordicollis* (5.39 Mya), *Dercylus* from Atillo (7.97 Mya) and *Dercylus* from Cotacachi (22.99 Mya, Table 6.1). During the Miocene, the northern portion of
the Andes was going through crustal accretion and had not reached its current elevation (Gregory–Wodzicki, 2000). These ages contradict the ages predicted from the hypotheses that beetle species arose contemporary with vascular plants from páramo. The only ground beetle species here hypothesized to have evolved in more recent time was *Dercylus praepilatus*, which originated during the Pliocene (3.9 Mya), a time period when páramo emerged above the tree line (Sklenář et al. 2011). By contrast, and contemporaneous with the evolution of plant species from páramo, most species in the genus *Panabachia* evolved during the Pleistocene (2.11–0.11 Mya, Table 6.1). Molecular clock divergence estimates in ground beetles and *Panabachia* beetles are consistent with previous studies that have documented Andean radiations, occurring in the Miocene and continuing throughout the Pleistocene (Hughes and Eastwood 2006, Hines 2008, Elias et al. 2009, Hoorn et al. 2010). For example, highland species of *Bombus* (Hymenoptera, Apidae) and *Ithomia* (Lepidoptera, Nymphalidae, Table 6.1), as well as several other lineages (Madriñán et al. 2013, Merckx et al. 2015), diverged in the Miocene 6–8 Mya (Hines 2008, Elias et al. 2009).

Signatures of microevolutionary process as predictors of patterns for other species is still highly contested (Barraclough and Nee 2001, Reznick and Ricklefs 2009). Possibly one of the most relevant results from the analyses of flightless ground beetles here pertains to the high levels of genetic differentiation at both species and population levels for members of the genus *Dercylus*. The analysis of the population genetic diversity gives insight into the mechanisms that cause differences within and between populations. From the analyses of two populations of *D. orbiculatus* from Cajas and Culebrillas, population genetic indexes show these populations are highly subdivided, as a consequence of being on opposite sides of the Andes (east vs west cordillera). This result of high subdivision is supported by the estimates of low number of
migrants per generation between these two sites, which suggests that this flightless ground beetle has poor dispersal ability. In comparison, macroevolutionary methods, such as use phylogenetic analyses, is helpful for discerning diversification events over larger time scales. For species in the genus *Dercylus*, phylogenetic analyses using COI sequences revealed that interspecific clades are representative of their collecting site, with no shared haplotypes among species. This phylogenetic inference also revealed two distinct clades from specimens collected in Atillo, and those from Cotacachi, which appear to represent two new undescribed species of *Dercylus*. However, this high level of genetic differentiation among species associated to their collecting sites was not completely recovered with the nuclear gene since *D. praepilatus* and *Dercylus* from Atillo, as well as *D. cordicollis* and *Dercylus* from Cotacachi, shared haplotypes. However, some level of genetic differentiation was recorded among some species in this genus using $\phi_{ST}$ values of CAD sequences.

Niche modeling analyses of the three species within the genus *Dercylus* revealed that all species have overlapping potential distributions, but these species seem to be restricted to a smaller elevation range and geographical areas in accordance with previous studies (Moret 2005). Which suggests that the dispersal capabilities of this beetle lineage are poor. Therefore, the presence of geographical barriers possibly had a greater effect on the divergence pattern of members of *Dercylus*. When geographical barriers were tested through an AMOVA analyzing all species within the genus *Dercylus*, the highest variability was found when data were divided around dry valleys, rivers and sides of the mountain range, which, for the most part, could explain the distribution of these genetic clusters. Yet, no clear explanation for the split between *Dercylus* from Atillo and *D. orbiculatus* (Culebrillas) was found. Both species live in the southern portion of the eastern mountain range, separated by a short distance where no apparent
geographical barrier is present. In a closer look at the geological history of the area, two mountain summits, Mt. Ayapungo (4,730 m) and Mt. Coyay (4,630 m) show evidence of the formation of glaciers on their peaks (Jordan and Hastenrath 1998). The presence of the mountain and glaciers during the Quaternary could have influenced the divergence of these two beetle lineages. Overall, genetic diversity of populations and species in the genus *Dercylus* shows that mountain isolation and poor dispersal ability is shaping the current distribution of genetic diversity.

The analyses of the rove beetle lineage *Panabachia* show this beetle lineage is much more species rich than previously reported. Between 17 and 20 species were identified using two models of species delimitation to infer putative species boundaries, the Poisson tree processes (PTP) and the generalized mixed Yule coalescent model (GMYC) using multi–locus data and single locus data, where congruence between results was used to determine species clusters. The number of species found through species delimitation analyses was higher than expected. From the preliminary analyses of morphological characters, I estimated 10–11 species, mainly based on male characters (beetles in this group are often characterized by male secondary sexual characters), with females lacking strong characters for species recognition (Park, 1942; Navarrete–Heredia et al., 2002). Potentially informative characters were also found in the median fovea of the pronotum (variation of size and shape), different patterns of distribution of setae, and in details of the male genitalia. However, a high proportion of females were collected, and some species are only represented by female individuals, which has limited the morphological portion of the study. The increase in the number of samples and a more comprehensive study of the morphological characters for each species is necessary to analyze in detail this beetle lineage. The diversity of the genus *Panabachia* is still understudied, since only
two species have been described from Central America (Park, 1942; Navarrete–Heredia et al., 2002). Phylogenetic inferences of *Panabachia* show good branch support for each species proposed through species delimitation. Yet, support for the relationships among species within each radiation was weak. But it is evident that the high diversity in the lineage *Panabachia* appears to be driven in part by montane isolation; most species appear to be present in small geographical areas, as in some ground beetles (e.g. *Dercylus*), with exception of one species that seems to be a species of wide distribution. However, the number of sympatric species in El Angel, Mojanda and Atillo cannot be explained by allopatric speciation. The high diversity of these sites might be explained by local adaptation (e.g. food or microhabitat). Still, additional sampling and more information about the natural history are needed to understand in more detail the evolution of this rove beetle lineage.

Both north/south and east/west vicariance patterns were found among the beetle lineages analyzed in this study. Although east/west vicariance is commonly reported in northern Andean species such as humming birds (i.e. species in the genus *Adelomya*; Chaves et al., 2007), this vicariance pattern was found in few instances among beetles from páramo, probably because of more complex evolutionary processes. For example, in the genus *Dercylus*, *Dercylus cordicollis* (east) and *Dercylus* from Atillo (west) are present on opposite sides of the Andean mountain. Similarly, east/west vicariance was reported between species lineages 8 and 7 of *Panabachia* from Atillo (east) and Pichincha (west). In contrast, north/south genetic differentiation was found between some species pairs in the lineage *Panabachia* in the COI gene tree (e.g. species 9–10 and 14–15). Regarding the general patterns of genetic distribution of populations of *Dyscolus alpinus* and *Pelmatellus columbianus*, populations separated by significant distances north/south appear to show higher levels of genetic differentiation. For example, high genetic differentiation
is found in the populations of *Pelmatellus columbianus* from Cayambe (north) and el Cajas (south), as well as for multiple populations of *Dyscolus alpinus* (e.g. Mojanda–Cashca Totoras).

The analyses of ground beetles and rove beetles show some level of correspondence in the distribution of widespread species of ground beetles and the widespread species of the *Panabachia* lineage. Populations of *Pelmatellus columbianus* (El Angel, Cayambe, La Virgen, Pichincha, Releche) and *Dyscolus alpinus* (Cayambe, Pichincha, La Virgen) are present in the same sites as the widespread lineage of *Panabachia* (El Angel, Cayambe, La Virgen, Pichincha, Releche). This pattern of distribution suggests factors that are structuring these beetle lineages of wide distribution might be shared. The effect of Quaternary glaciation probably had an important role in maintaining genetic connectivity among isolated populations of páramo. Palynological studies in the southern Ecuadorian Andes revealed that páramo extended its distribution towards lower elevations during this period (Villota and Behling 2014). The expansion of glaciers from surrounding mountains (Mt. Cayambe, Mt. Antisana, Mt. Altar (Jordan and Hastenrath 1998) possibly pushed high elevation species into inter–Andean Valleys, allowing gene flow between populations that were previously restricted to high elevations. A similar scenario was proposed for bess beetles (Passalidae) from páramo in Costa Rica, where dispersal among species of higher elevation occurred during the glacial periods, when cold temperature caused species to move to lower elevations (Mac Vean and Schuster 1981).

A big limitation of this study was the few widespread species for analysis, due to the high level of endemic species in páramo (Moret and Bousquet 1995, Luteyn 1999, Sklenář et al. 2011). This factor, in addition to the challenge of gathering enough specimens for population genetics analyses (10 individuals), excluded several taxa from further studies. During the initial phases of this project, I aimed to include different beetle families (e.g. weevils). Given the
challenges that come with working with tropical faunas, such as the high number of undescribed species and the limited number of papers related to the diversity of the area, this research was mainly focused on ground beetles, a family that is well understood in Ecuadorian páramo. Nevertheless, the comparative study of four beetle lineages, including an ant–loving beetle, points out how different factors shape the genetic diversity of these high elevation beetle lineages, and reveals some interesting patterns for each beetle lineage analyzed.

Results found in this study have some important implications for the conservation of species from páramo. Given the variation of patterns among beetle lineages, it is important that conservation efforts take into consideration local and regional patterns of diversity. Currently, elevational shifts due to climate change have already been reported for several ground species in the Ecuadorian Andes (Moret et al. 2016), which particularly would affect species with smaller elevational ranges like *Dyscolus alpinus* or species in the genus *Dercylus*. While some páramo areas are already included in the system of national parks and reserves (Cuesta, Muriel, et al. 2017), not all high elevations sites in the Ecuadorian Andes are protected. These areas are particularly threatened by the expansion of agriculture and cattle ranching towards higher elevations (De Brievre and Calle 2011). On the other hand, some of the species in páramo extend their distributional ranges to lower elevations, where the implementation of biological corridors to connect páramo patches would help maintain the genetic connectivity across species populations with wider elevational ranges.

Future efforts will focus on elucidating the connection between high elevation species of beetles to lower elevation species. Through this project, I found two promising beetle lineages to continue studying the evolution of high elevation beetle species at different scales. The lineage *Panabachia* appears to be a good candidate to understand the evolution of endemism in tropical
mountains, given that the genus is reported to be also present in lower elevation areas such as montane and cloud forest. Comparing low to high elevation species will give us insight into the processes that gave rise to these alpine faunas. The study of populations of Pelmatellus columbianus at a wider geographical scale will provide some insight into the dispersal patterns of this species of ground beetle from the inter–Andean valleys to high elevation. Similarly, examining the biological diversity of beetle lineages across elevational gradients in the Andes will help us to better understand the origin of beetle faunas at high elevation. In particular, the analyses of elevation gradients across the eastern and western cordillera will reveal how coastal and Amazonian beetle lineages have contributed towards the current diversity of Andean species of beetles.

Increasing the numbers of studies focused on high elevation lineages will lead to a better understanding of the factors influencing the evolution in Andean species including the role that dynamic interactions, ecological and evolutionary processes have in shaping the community structure (Johnson and Stinchcombe 2007). In particular for páramo species, studying how the high rates of diversification found in vascular plants in páramo (Sklenář et al. 2011, Madriñán et al. 2013) has influenced the evolution of herbivorous species (e.g. weevils) will probably show some distinct patterns.
**Table 6.1.** Summary of divergence time estimates for Andean species, with emphasis on ground beetles and rove beetles from páramo.

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Figure 6.1. Summary of divergence time estimates ground beetles and rove beetles from páramo of Ecuador.
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