Biogeografía histórica reciente de los vertebrados del "hotspot" de Chile Mediterráneo:

Dinámicas de distribución y nicho climático de linajes intraespecíficos, desde el Ultimo Máximo Glacial hasta el presente.

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Dedicado a mi familia.

"En el gran mundo como en una jaula

afino un instrumento peligroso…"

Enrique Lihn

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Lista de abreviaturas

AUC: Area under the ROC curve

DNA: Desoxyrribonucleic acid

ESUs: Evolutionary significant units

GCC: Global climate change

LGM: Last glacial maximum

mtDNA: Mitochondrial DNA

PCR: Polymerase chain reaction

ROC: Receiver operating characteristic curve

SDM: Species distribution model

Introducción general

Se ha señalado recientemente la necesidad de considerar los efectos del cambio climático global (CCG) en los niveles de biodiversidad por debajo de la categoría de especie. En este contexto y a pesar del consenso general respecto de la importancia de delimitar las unidades evolutivamente significativas (UESs) en las estrategias de conservación, existe un serio déficit de estudios de efecto de CCG al nivel de la diversidad genética intraespecífica en comparación a la gran cantidad de publicaciones en los niveles de organización comunitaria y ecosistémica (Fraser & Bernatchez 2001). En esta línea de argumentos, Bálint et al. (2011) han demostrado que las especies con estructura genético-poblacional experimentarán masivas pérdidas de diversidad al nivel intraespecífico en un escenario de cambio global. Es decir, las estimaciones del impacto del CCG en la diversidad basadas en criterios de morfo-especies cometerían severas subestimaciones de la pérdida de diversidad críptica, y por tanto las estrategias de conservación de la diversidad biológica basadas en los niveles de organización comunitaria, ecosistémica y específica podrían ser una aproximación sobre-simplificada toda vez que no pueden cuantificar la pérdida de linajes intraespecíficos. Por estas razones, será crítico para las futuras estrategias de conservación no solo cuantificar la cantidad de diversidad críptica en riesgo (ej. variantes genéticas locales con potencial adaptativo, variabilidad relacionada al fitness o linajes intraespecíficos con una distribución geográfica característica), sino además comprender cuáles serán las respuestas ecológicas y evolutivas de las UESs bajo el nivel de especie al CCG.

Algunos trabajos han enfatizado de distintas formas la independencia en las respuestas distribucionales de los linajes de una especie, pero la integración explícita de estructura genealógica en modelos de distribución de especies (MDEs) es aun un área escasamente explorada: Integrando MDEs y medidas de la diversidad genética (Schorr et al. 2012) han concluido que esta aproximación integrada a nivel intraespecífico puede cambiar la historia distribucional inferida a partir de la información filogeográfica por sí sola. Estudiando la relación entre el proceso de formación de linajes y la variación en el nicho ecológico en el grupo de especies de *Peromyscus maniculatus (Kalkvik et al. 2011)* han demostrado que la mayoría de los linajes genéticos al interior de una especie en efecto ocupan diferentes

nichos ambientales. En otra aproximación integrada de filogeografía y MDEs, (Fontanella et al. 2012) concluyeron que los haploclados principales encontrados en la lagartija *Liolaemus petrophilus* presentan distintas respuestas distribucionales al CCG en el Pleistoceno. Estos hallazgos en conjunto sugieren que los cambios de rango de distribución gatillados por cambio climático podrían ser complejos si las especies poseen estructura filogenética y algún grado de de divergencia de nicho intraespecífica asociada a estos linajes filogenéticos crípticos. Por este motivo, predicciones realistas de los cambios en rango de distribución para futuros escenarios de CCG deben necesariamente considerar información filogeográfica para implementar modelos de distribución de linajes, pues las especies no necesariamente responderán como unidades ecológicas simples a futuras alteraciones del régimen climático.

En esta tesis, se eligió como modelo de estudio de las respuestas distribucionales de linajes crípticos ante un forzamiento climático, cinco especies de vertebrados endémicos de un área con alto grado de endemismo y que ha sido sometida a forzamientos climáticos drásticos en el pasado geológico reciente: la subregión biogeográfica de Chile central (Morrone, 2006). Esta extensión de territorio (28°S – 36°S) corresponde aproximadamente con la definición original del hotspot de Chile mediterráneo (Myers 2000) y alberga al 50% de los vertebrados endémicos de Chile en un área que no supera el 16% de la superficie continental del país (Simonetti 1999). Si bien esta subregión no estuvo cubierta por masas de hielo durante la última glaciación, se sabe que los glaciares descendieron en la Cordillera de los Andes hasta los 1100 m, con el consecuente descenso en temperatura e incremento de las precipitaciones en Chile central (Heusser, 1983; Heusser, 1990; Lamy *et al.*, 1999). Se ha demostrado a través de evidencia palinológica que este forzamiento climático durante el último máximo glacial (LGM) desencadenó el desplazamiento de los cinturones vegetacionales de la Cordillera de los Andes hacia menor altitud, alcanzando el valle y la Cordillera de la Costa; una vez que los hielos retroceden, la vegetación Andina habría recuperado su distribución previa a la glaciación, dejando ínsulas de vegetación andina remanente en las cumbres de la Cordillera de la Costa (Darwin, 1859; Simpson, 1983; Heusser, 1990; Villagrán & Armesto, 1991; Villagrán & Hinojosa, 2005). El motivo por el cual estos forzamientos climáticos son relevantes en el contexto de la distribución de la diversidad críptica de vertebrados, es que dinámicas de distribución del tipo de las

descritas para la vegetación andina, son una buena analogía para predecir el comportamiento de este sistema en futuros escenarios de cambio climático y variaciones drásticas en la cantidad de hábitat disponible. En este contexto, la hipótesis más general de esta tesis, de la cual derivan otras más específicas, es simple: *La distribución de vertebrados terrestres de Chile central debió sufrir variaciones asociadas al desplazamiento de los cinturones vegetacionales andinos, ya sea porque este fenómeno implica el desplazamiento del hábitat de los vertebrados, o bien porque están directamente determinados por la configuración climática.*

Respecto de la relación entre la distribución de vertebrados terrestres de Chile central y las glaciaciones del Pleistoceno, la única hipótesis que relaciona la diversificación de este grupo y su distribución presente con las glaciaciones, es de larga data y fue postulada en un trabajo titulado *"Lizards & Rodents: an explanation for their relative species diversity in Chile"* (Fuentes & Jaksic 1979). En general, esta hipótesis postula que la diversidad relativa de especies de lagartijas y roedores de Chile central es explicada por diferentes modos de especiación en ambos grupos, como resultado de la interacción entre la geografía de los dos cordones montañosos de la región, glaciaciones del Pleistoceno y los rasgos ecológicos de ambos grupos. Específicamente, se propone que roedores y lagartijas obedecen a dos modos de especiación asincrónicos: i) la llamada "especiación de montaña" ocurre durante períodos interglaciales y su mecanismo de acción es el alto recambio altitudinal de especies sumado al aislamiento entre montañas; ii) la "especiación de valle" ocurriría durante los períodos glaciales, en donde las especies debiesen ocupar rangos de menor altitud. Dados los atributos ecológicos de ambos grupos, se espera que las lagartijas presenten ambos modos de especiación, pues dada su reducida vagilidad este grupo experimentaría severa disminución de conectividad en el valle durante períodos glaciales, y también en las montañas en el interglacial; esto debido a su ámbito de hogar, alta especificidad de hábitat y alto recambio de especies entre montañas. Esta hipótesis también predice que los roedores solo experimentarían el modo de especiación de valle, pues este grupo sufriría una disminución de conectividad en el valle durante el período glacial, pero no aislamiento en las montañas durante el interglacial, debido a su mayor vagilidad y menor recambio de especies entre montañas en comparación con las lagartijas. Estas diferencias en modos de especiación serían finalmente explicadas por diferencias en

movilidad entre ambos grupos, como consecuencia de sus diferentes modos de termorregulación y requerimientos energéticos. Si bien el objeto de esta tesis no es la explicación de la diversidad relativa de los distintos grupos de vertebrados, existe una relación con el mecanismo hipotético de diversificación para roedores y lagartijas explicado más arriba, a través de la modelación explícita de la distribución de linajes de vertebrados en el presente y durante el último máximo glacial, pues la hipótesis general del presente trabajo también descansa en la interacción entre los forzamientos climáticos de los ciclos glaciales, rangos de distribución y sistemas montañosos de Chile central; se espera que la evidencia presentada sea al menos de modo indirecto un contraste empírico de la hipótesis de diversificación de Fuentes & Jaksic, que es hasta el momento la única propuesta de un mecanismo general de diversificación de vertebrados en el área de estudio.

Desde una perspectiva teórica, este trabajo explora el supuesto implícito en muchas predicciones de cambios de rango de distribución: homogeneidad en la respuesta distribucional de una especie frente a una transición climática. Esta tesis introduce la idea de que la estructura filogenética intraespecífica a menudo determina dinámicas de distribución mixtas al interior de una misma especie; predicciones realistas de los efectos del cambio climático futuro debiesen considerar la posibilidad de que linajes crípticos presenten respuestas independientes del resto de la especie.

Estructura de la tesis

La presente tesis está organizada en tres capítulos, cada uno de ellos es el manuscrito de una publicación científica pronta a ser sometida a evaluación.

El primer capítulo versa sobre una particular segregación altitudinal de linajes al interior de una especie de roedor endémico, cuya actual distribución disyunta es potencialmente explicada como una recolonización postglacial diferencial a través de valles y cordones montañosos.

El segundo capítulo extiende las conclusiones del capítulo 1, esta vez con un diseño de muestreo específicamente ideado para dilucidar la historia de las poblaciones de cumbres de montaña para dos especies de roedores; a su vez se explora la relación entre la genealogía y distribución de dichas poblaciones con los conocidos desplazamientos de

vegetación altoandina hacia la Cordillera de la Costa, durante y después del LGM. Se incluye una hipótesis para el origen de la diversificación intraespecífica asociada a las transiciones glacial/interglacial del Pleistoceno.

El tercer capítulo pretende generalizar las conclusiones de los dos capítulos precedentes; para este fin se implementó una aproximación filogeográfica comparada para 4 especies de vertebrados endémicos, que sintetiza información genealógica y modelos de distribución en hipótesis biogeográficas explícitas contrastadas empíricamente.

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Running head: Molecular Phylogeography of *Phyllotis darwini*.

Integrating phylogeography and species distribution models: cryptic distributional responses to past climate change in an endemic rodent from the central Chile hotspot.

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ABSTRACT

Biodiversity losses under the species level (i.e. cryptic diversity) may have been severely undersestimated in future global climate change scenarios. Therefore, it's important to characterize diversity units at this level, and also understand its ecological responses to climatic forcings. We have chosen an endemic rodent from a highly endangered area as a model to look for cryptic distributional responses below the species level: *Phyllotis darwini* in the central Chile biodiversity hotspot. This area harbors a high amount of endemic species, and it's known to have experienced vegetational displacements between two mountain systems during and after the Last Glacial Maximum. We've implemented an approach which integrates phylogeographic information into species distribution models. Our major findings are that the species is compossed of two major phylogroups: one of

them has a broad distribution mainly accros valley but also in mountain ranges, meanwhile the other displays a disjunct distribution across both mountain ranges and always above a 1500 m altitude limit. Lineage distribution model under LGM climatic conditions sugget that both lineages were codistributed in the southern portion of *P. darwini's* current geographic range, and mainly at the valley and coast. We've concluded that present distribution of lineages in *P. darwini* is the consequence of this cryptic distributional response to climate change after LGM, with a postglacial colonization with strict altitudinal segregation of both phylogroups.

INTRODUCTION

It is well known that climate regime may affect species distribution and therefore future climate change could potentially induce geographical range dynamics such as contraction, expansion or geographical range shifts (Walther *et al.*, 2002; Pearson & Dawson, 2003; Summers *et al.*, 2012). It is also agreed that the climatic impact on species distribution can be extrapolated in community and ecosystem shifts (Walther *et al.*, 2002; Hamann & Wang, 2006). For these reasons, the species level might be critical for conservation issues in a global climate change scenario (GCC).

At the species level, climate change is expected to affect the geographical range mainly through physiological restrictions as temperature and precipitation tolerances in conjunction with species dispersal abilities (Walther *et al.*, 2002). Altogether, those factors may determine species ability to keep up with climate change. From an ecological perspective, expected species responses to climate change are i) to tolerate or spread in the new climatic set up (either by physiological tolerance or phenotypic plasticity) ii) to change

its distribution in order to catch up the new climate regime iii) to get extinct (Pettorelli, 2012). From an evolutionary perspective, range shifts may change the distribution of genetic diversity and range contractions will most likely reduce the genetic diversity (Alsos *et al.*, 2012; Pauls *et al.*, 2012). Nevertheless, the emphasis on the role of evolution in species responses to climate change has been usually focused on evolutionary adaptations and the relationship between species' adaptation speed and climatic change rate (Hoffmann & Sgrò, 2011).

It has been recently pointed out the need to consider GCC effects on biodiversity below the species level, other than the usually advocated adaption potential and phenotypical plasticity issues. There exists a severe lack of studies on GCC effects on biodiversity, at the level of intraspecific genetic diversity. This is highly evident if we compare it with the vast amount of publications at the ecosystem, community and species levels (Fraser & Bernatchez, 2001), despite the general agreement on the importance of Evolutionary Significant Units (ESUs) delimitation for conservation planning. On this line of arguments, (Bálint *et al.*, 2011) demonstrated that species with strong population genetic structure will face massive losses of diversity at the intraspecific level in a GCC scenario. That is to say that morpho-species based estimation on GCC impacts on biodiversity will severely underestimate cryptic diversity loss; therefore conservation strategies based solely on species, community, or ecosystem organization levels could be an oversimplified approach.

For the above reasons, it might be critical for future conservation planning not just to quantify the amount of cryptic diversity at risk (i.e. local genetic variants with adaptative potential, fitness related variability or intraspecific lineages with a characteristic distribution), but to also understand what could be the ecological and evolutionary

responses of ESUs below the species level to GCC. There exist a substantial number of publications trying to understand the relationship between evolutionary and ecological species's responses to climate change, mainly through phylogeographical information and species distribution models (SDMs) (Carstens & Richards, 2007; Waltari *et al.*, 2007; Kozak *et al.*, 2008; Provan & Bennett, 2008; Cordellier & Pfenninger, 2009; Marske *et al.*, 2009; Waltari & Guralnick, 2009; Buckley *et al.*, 2010; Lim *et al.*, 2010; Allal *et al.*, 2011; Eckert, 2011; Gugger *et al.*, 2011; Marske *et al.*, 2011; Svenning *et al.*, 2011; Marske *et al.*, 2012; Qi *et al.*, 2012). However, approaches using intraspecific phylogenetic information to build lineage distribution models (lineages bellow species level) are less frequent. Even though this approach has been used with success in systematics for species delimitation and cryptic speciation issues (Raxworthy *et al.*, 2007; Rissler & Apodaca, 2007; Engelbrecht *et al.*, 2011; du Toit *et al.*, 2012; Florio *et al.*, 2012), the need to discern lineage-specific ecological responses to GCC at the intraspecific level (and its potential applicability in conservation strategies, as a measure of distributional shifts experimented by cryptic phylogenetic lineages) is a relatively new issue in the scientific literature. Several efforts have been recently made on this concern: by using SDMs and genetic diversity measures Schorr *et al.* (2012) have concluded that this integrated approach may change the distributional history inferred from phylogeographic information alone at the species level. Studying the relationship between lineage formation and variation in the ecological niche in the *Peromyscus maniculatus* species group (Kalkvik *et al.*, 2011) have demonstrated that the majority of genetic lineages within species does occupy distinct environmental niches. In another integrated approach using SDMs and phylogeography, Fontanella *et al.* (2012) concluded that two main haploclades in the lizard *Liolaemus petrophilus* shared different distributional responses to climate change during the Pleistocene. Those findings suggest

that geographical range shifts triggered by climatic shifts might be complex if the species share phylogenetic structure, and some degree of intraspecific niche divergence related to cryptic phylogenetic lineages. Therefore, realistic predictions of range shifts for future climate change scenarios should consider phylogenetic information in order to perform lineage-specific distribution models, because species might not necessarily respond as an ecological unit to future GCC.

In this work, we expect to hindcast the geographic range responses of an endemic rodent species from a biodiversity hotspot to past climate change. We will specifically evaluate the responses to climate change after Last Glacial Maximum (LGM), by integrating intraspecific diversity information and intraspecific lineage distribution models for present and past climatic conditions. This information will be very valuable to understand what should be the relevant implications of considering cryptic diversity in conservation strategies planning, compared to approaches based only in species or ecosystem levels. This kind of information should be relevant for conservation strategies in general, by setting more realistic assumptions on species responses to GCC.

We have chosen a sigmodontine rodent species, endemic to a biodiversity hotspot area in central Chile as our study model because: i) We want to know how vertebrate species have responded to climate change in a currently endangered area; there is a strong need of that information for future conservation planning; ii) There is some evidence that general genetic structure of species in a hotspot shows features that are characteristic of the region and its particular evolutionary and geo-climatic history. Among the world biodiversity hotspots, the African "Cape Floristic Region" (CFR) is one of the better known with respect to the evolutionary origin of its biota (Verboom *et al.*, 2009a). In fact, a

phylogeographic study in the genus of dwarf chameleons *Bradypodium* (Fitzinger, 1843) suggested that there was a concerted phylogeographic pattern between species of this genus, and between these and two other endemic lizards associated to the emergency of the Cape Flora (Tolley *et al.*, 2006; Tolley *et al.*, 2009). At he same time, the Cape Flora may display a complex diversification history with ancient and recent speciation events, probably caused by a combination of climatically induced fragmentation and adaptative radiation (Verboom *et al.*, 2009b). On the other hand, a comparison of the phylogeographic pattern conducted in six taxonomic groups, endemic to the "California Floristic Province" hotspot, concluded that the genetic structure of these taxa show major splits highly consistent across most taxa (Calsbeek *et al.*, 2003). The latter authors concluded that diversification can be spatially and temporally explained by the climatic and geographic history of the California hotspot. Finally, in the "Brazilian Atlantic Forest" hotspot, Carnaval *et al.* (2009) demonstrated that climatic stability in Pleistocene refugia is a good predictor of the current genetic diversity within this hotspot. Altogether those evidences suggest that biodiversity hotspots may be appropriate systems to study the relationship between climate dynamics and genetic differentiation in currently endangered areas.

Sixty one species of endemic vertebrates have been described in the hotspot of central Chile representing 0.2% of the global vertebrate diversity (Simonetti, 1999).The central Chile area, or Mediterranean ecoregion of Chile, is one of the 25 areas proposed as hotspots based on the levels of endemism and threat of habitats (Myers *et al.*, 2000). Regarding mammals, of the 150 species described for Chile, 56 are distributed in central Chile with nine endemics, with rodents representing the greatest diversity (12 species; Palma, 2007). One of the endemic forms in central Chile is our model species *Phyllotis*

darwini (Waterhouse, 1837), the Darwin's leaf-eared mouse. This is a sigmodontine mouse distributed along a narrow fringe from Atacama southward to Bío Bío regions (27 to 36˚ S), and from coastal areas up to 2000 m (Redford & Eisenberg, 1992). The species is part of the tribe Phyllotini (46 species, 19 in Chile; Spotorno *et al.*, 2001; Iriarte, 2008) whose original diversification area was the southern Altiplano (Reig, 1986; Spotorno *et al.*, 2001). *P. darwini* and *P. magister* are sequential sister groups to *P. xanthopygus* from the Andes and southward to central Chile (Steppan *et al.*, 2007). The systematics of *P. darwini* does not recognize sub-specific forms as was earlier demonstrated by the complete hybrid sterility between true *P. darwini* (formerly known as *P. darwini darwini*) and the current recognized subspecies *P. xanthopygus vaccarum* (formerly known as *P. darwini vaccarum* (Walker *et al.*, 1984). Therefore, *P. darwini* is an endemic taxon from the Mediterranean ecoregion of Chile, which have no previously described intraspecific lineages, and its geographic distribution roughly agrees with the central Chile "hotspot".

We expect that potential genetic splits in *Phyllotis darwini* might be associated to the altitudinal gradient given by the Andes, the valleys, and the Coastal Cordillera. The latter topography constitutes the most important geographic feature in the central Chile hotspot. We also expect that the history of the species' geographic range may display signatures of one of the most important geo-climatic events in central Chile: the Last Glacial Maximum (LGM). This event was chosen to test the hypothesys because in addition to being one of the greatest episodes of recent climate change, it is known that Quaternary glaciations in general have been one of the most important factors in determining the current genetic structure of many populations, species and communities (Hewitt, 2000). Even though central Chile was not extensively covered by ice sheets at

LGM, the glacial advanced northward through the Cordillera de los Andes (Clapperton, 1990; Clapperton, 1994) descending to about 1,100 m with a subsequent drop in the temperature and an increased rainfall in the whole region (Heusser, 1983; Heusser, 1990; Lamy *et al.*, 1999). The palinological evidence has demonstrated that high Andean vegetational belt shifts downwards the valley during the glacial advance, and then moved upwards, reaching its original high altitude distribution after glacial retreat. This vegetational shift may have given rise to the existing biogeographical insulas of Andean vegetation in both, the Andes and the Coastal cordilleras of central Chile (Darwin, 1859; Simpson, 1983; Heusser, 1990; Villagrán & Armesto, 1991; Villagrán & Hinojosa, 2005). In addition, it has been suggested that lizards and rodent's relative species diversity in central Chile is explained by different speciation modes in both groups, as a result of differential interaction between mountain geography, Quaternary glaciations, and ecological features in both groups (Fuentes & Jaksic, 1979).

Having the former scenario, the goals of this paper were i) to evaluate the genetic and phylogenetic structure of *Phyllotis darwini* in order to look for cryptic intraspecific lineages; ii) to build lineage distribution models at present and at LGM, to assess the existence of lineage-specific distributional responses to climate change, in order to investigate if species have behaved as a single distributional unit in response to past climate change. To achieve these goals, we sequenced the Hypervariable domain II of the mitochondrial DNA (mtDNA) control region in *Phyllotis darwini* to recover the morphologically cryptic phylogenetic lineages, and build distribution models for each lineage at present and at LGM. We expect to discern if *Phyllotis darwini* have shifted its geographical range as a single ecological unit since LGM to present, or if there exist cryptic

phylogenetic lineages which shared independent distributional responses to past climate change after glacial retreat.

METHODS

Specimens and localities.- Sixty eight specimens of *Phyllotis darwini* were analyzed representing 18 localities across central Chile (Fig. 1). The southern distribution of *Phyllotis* was poorly represented because we were unable to capture individuals between 34° S and 36° S. The same is true for the northernmost portion of the range since we did not obtain samples between "Parque Nacional Pan de Azúcar" (26° S) and "Parque Nacional Llanos de Challe" (28°S). Voucher specimens were deposited in the Colección de Flora y Fauna Profesor Patricio Sánchez Reyes (SSUC), Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile, and the Museum of Southwestern Biology (MSB), University of New Mexico, Albuquerque, New Mexico. The list of specimens, localities and abbreviations is given in Appendix I. Tissues and data associated to each specimen are cross-referenced and stored in the collection under a field catalog number: NK is the field catalog used by the SSUC and the MSB; ER is the field catalog of Dr. Enrique Rodriguez-Serrano. We followed the ASM guidelines during the collection and care of the animals used in this work (Sikes & Gannon, 2011)

DNA extraction and sequencing.- We used frozen liver for DNA extraction using the Wizard Genomic DNA Purification Kit (PROMEGA, Madison, Wisconsin). The DNA extraction in *Phyllotis magister* (used as outgroup) was performed from ethanol preserved ear tissue. We amplified via the Polymerase Chain Reaction (PCR) the Hypervariable domain II (HV2) of the mitochondrial DNA (mtDNA) control region in 72 individuals. We

used HV2 instead of the traditionally Hipervariable domain I (HV1) because the substitution rate of the former was enough to solve the evolutionary relationships among *Phyllotis darwini´s* haplotypes. Although the HV1 has a significantly higher variability than HV2 (measure as nucleotide diversity), the former was about only 10% more variable than HV2 (data not shown). Primers used for PCR were 282 and 283 (Bacigalupe *et al.*, 2004), and the thermal profile was: initial denaturation at 95°C for 7 min, followed by 30 cycles of 94°C (30 s), 59°C (16 s), and 72 °C (1 min 15 s). A final extension followed at 72 °C for 4 min. PCR products were purified with PCR Preps (QIAGEN). Cycle sequencing (Murray 1989) was performed using primer 283 labeled with the Big Dye terminator kit (Perkin Elmer, Norwalk, Connecticut). Sequencing reactions were analyzed on an Applied Byosystems Prism 310 (Foster City, California) automated sequencer. We sequenced a total of 412 base pairs of the mtDNA control region and those sequences have been deposited in GeneBank (GeneBank accession numbers JN226664 - JN226735). Sequences were aligned using BIOEDIT (Hall, 1999) and by eye. In addition, the complete sequence of *Auliscomys pictus* mtDNA control region (GeneBank accession number AF296272) was used as a reference for alignment. Finally, saturation of the molecular marker was evaluated using the Xia test (Xia *et al.*, 2003a) implemented in DAMBE (Xia & Xie, 2001). The assumption of neutrality was tested calculating Tajima's D index (Tajima, 1989) implemented in the DnaSP 4.1 software (Rozas *et al.*, 2003), as well as the nucleotide and haplotype diversity indexes.

Haplotype Network and *Intraspecific Phylogeny*._Haplotype network and demographic analyses were performed over an haplotype file, built in the DnaSP 4.1 software (Rozas *et al.*, 2003). For phylogenetic analyses four specimens of *Phyllotis*

magister, the sister species of *P. darwini* (Steppan, 1998), was used as outgroup. The Markov Chain Monte Carlo (MCMC) method within a Bayesian framework (hereafter BMCMC) was used to estimate the posterior probability of phylogenetic trees. The MCMC procedure ensures that trees are sampled in proportion to their probabilities of occurrence under the model of gene-sequence evolution. Approximately 10,000,000 phylogenetic trees were generated using the BMCMC procedure, sampling every 1000^{th} trees to ensure that successive samples were independent. The first 50 trees of the sample were removed to avoid including trees sampled before convergence of the Markov Chain. The pattern of molecular evolution from the control region in mammals is very complex. In rodents, a strong rate heterogeneity among sites has been detected, as well as a variable length and number of tandem repeated elements, even between subspecies. Moreover, the HV2 domain may feature heterogeneous patterns of molecular evolution because it posses three conserved blocks, and it is functionally important given the presence of the replication origin of the H (Heavy) strand (Larizza *et al.*, 2002). Because of this, we used a general likelihood-based mixture model (MM; Pagel & Meade, 2004), based on the general time-reversible (GTR) model of gene-sequence evolution to estimate the likelihood of each tree. This model accommodates cases in which different sites in the alignment evolved in qualitatively distinct ways, but does not require prior knowledge of these patterns or partitioning of the data. These analyses were conducted using the software BayesPhylogenies, available at the website http://www.evolution.rdg.ac.uk/SoftwareMain.html. In order to find the best mixture model of gene-sequence evolution, we obtained the likelihood of the trees by first using a GTR matrix plus the gamma distributed rate heterogeneity model (1GTR + G) and then continuing to add up to five GTR + G matrices were determined. For the posterior analyses,

only the combination of matrices with the fewest number of parameters that significantly increased the likelihood was used. Posterior probabilities for topologies were assessed as the proportion of trees sampled after burn-in, in which that particular topology was observed.

To assess whether the hierarchical relationships between haplotypes (inferred from BMCMC) were consistent with its reticulate associations, and to explicitly assess the geographical pattern and frequency associated with each haplotype, a network of haplotypes was calculated using the median joining algorithm (Bandelt *et al.*, 1999) implemented in NETWORK 4.5 (Rohl & Mihn, 1997).

Population genetic analysis._ To evaluate the genetic structure within *P. darwini* we identified the populations within the species using the GENELAND software (Guilliot *et al.*, 2005). This approach is a Bayesian cluster analysis that uses individual geo-referenced genetic data to detect the number and geographic position of populations (Guilliot *et al.*, 2008). The algorithm identifies genetic discontinuities while estimates both the number and locations of populations without any a priori knowledge on the populational units and limits. Once the number and limits of populations are established, the population membership probability is calculated from the posterior probability distribution of the MCMC. First, one independent run was performed by 10,000,000 of generations, sampling every 1000 generations of the markov chain and treating the number of populations as unknown. Then, we choose the better of five independent runs, each of 10,000,000 of generations and sampling every 1,000 but now treating the number of populations as a fix parameter estimated from the first independent run. From the posterior

distribution, we draw a map of probability isoclines of population membership, one for each population or cluster inferred by the model.

Once the geographic location of cluster units and phylogenetic relationships was known, we assigned the haplotypes to each of the two major phylogenetic groups according to its geographic location and performed a Mantel test (Mantel, 1967). Across populations of each clade, we evaluated correlations between the genetic and the geographic distance to test for isolation by distance (Rousset, 1997). The Mantel test was performed in the PopTools software (Hood, 2010); first we computed two matrices for populations of each of the two major clades, one of genetic differentiation index (pairwise Fst), and another of pairwise geographic distances between localities. The frequency distribution of correlation coefficients expected by chance was approximated through randomization of both genetic and geographic distances matrices between the haplotypes, with 10.000 replicates for each matrix. The significance of the correlation between genetic and geographic distances was assessed as the cumulative probability of the correlation coefficients from the random distribution that exceeded the value of the observed correlation coefficient between genetic and geographic distances.

To achieve insights about the demographic history of *Phyllotis darwini* that could explain the genealogical patterns, we evaluated the sudden expansion model in the distribution of pairwise genetic differences (Rogers & Harpending, 1992; Schneider & Excoffier, 1999). This analysis was performed from the haplotypes dataset using an infinite-sites model that took into account multiple substitutions and allow mutation rates to vary through DNA sequence. To compute this Mismatch distribution and test its goodness-of-fit to the sudden expansion model (Schneider & Excoffier, 1999), we used the software

ARLEQUIN 3.1 (Schneider *et al.*, 2000). The least-squares deviation method was used as a test of goodness-of-fit (Schneider & Excoffier, 1999).

Clock calibration.- The age of intraspecific divergence events was estimated in a relaxed molecular clock approach implemented in the software BEAST v.1.7.4 (Drummond *et al.*, 2006). The node ages for the main phylogroups recovered inside *Phyllotis darwini* were co-estimated from a subsample of the intraspecific phylogeny, but rooted with a D-loop sequence from *Auliscomys pictus* (GeneBank accession number AF296272). To make a gross estimation, we used the 3.0 -5.1 Myr basal split in *Phyllotis* suggested by (Steppan *et al.*, 2007) and 10% molecular divergence rate estimated for D-loop in rodents (Brown, 1986). The analysis implemented a GTR + G + I model with rate variation (four gamma categories) and a Yule branching rate prior. Rate variation across branches was assumed to be uncorrelated and lognormally distributed (Drummond *et al.*, 2006). The MCMC chain was run for 10 000 000 generations (burn-in 10 000 generations), with parameters sampled every 1000 steps.

Distribution models .- We modeled the climatic niche of each intraspecific lineage to approximate the whole species' current distribution, and its distribution during the LGM under the assumptions that: (1) climate is an important factor driving the species' distribution; (2) the climatic niche of species remained conserved between the LGM and present time, and (3) Overlapped lineage's distribution ranges will approach the whole species geographic range. The latter assumption was tested by overlapping distribution models of each intraspecific lineage in order to approach the full species distributional range, as the sum of ranges estimated for each lineage. The resultant distributional range

was roughly contrasted with another model built for the whole species without considering phylogenetic structure.

The climatic niches were reconstructed using the methodology of ecological niche modeling, where environmental data are extracted from occurrence records and random points (represented by geographic coordinates). Habitat suitability was evaluated across the landscape using program specific algorithms (Elith *et al.*, 2006). The current models were then projected on the climatic reconstructions of the LGM. For occurrence records, we used our unique sampling localities. In addition to full geographic distribution models for the species, we built climatic models for each major lineage recovered in the intraspecific phylogeny following the same approach. As a test of consistency we overlap the intraspecific lineage distribution models, in order to compare it to the full species distribution models.

The current climate was represented by bioclimatic variables from the WorldClim dataset v. 1.4 (http://www.world clim.org/; Hijmans *et al.*, 2005) that are derived from monthly temperature and precipitation data, and represent biologically meaningful aspects of local climate (Waltari *et al.*, 2007; Jezkova *et al.*, 2009).

For environmental layers representing the climatic conditions of the LGM, we used ocean–atmosphere simulations (Harrison, 2000) available through the Paleoclimatic Modeling Intercomparison Project (Braconnot *et al.*, 2007). These reconstructions of the LGM climate are based on simulated changes in concentration of greenhouse gases, ice sheet coverage, insulation and topography (caused by lowering sea levels). We used two models that have been previously downscaled for the purpose of ecological niche modeling (Waltari *et al.*, 2007): Community Climate System Model v. 3 (CCSM; Otto-Bliesner *et al.*, 2006) and the Model for Interdisciplinary Research on Climate v. 3.2 (MIROC; Hasumi &

Emori, 2004). The original climatic variables used in these models have been downscaled to the spatial resolution of 2.5 min under the assumption that changes in climate are relatively stable over space (high spatial autocorrelation) and were converted to bioclimatic variables (Peterson & Nyári, 2007).

Climatic niche models were built in the software package MAXENT v. 3.2.1 (Phillips *et al.*, 2006), a program that calculates relative probabilities of the species' presence in the defined geographic space, with high probabilities indicating suitable environmental conditions for the species (Phillips *et al.*, 2004). Trapping coordinates of each individual captured for DNA extraction were used as presence points. We used the default parameters in MAXENT (500 maximum iterations, convergence threshold of 0.00001, regularization multiplier of 1, and 10 000 background points) with the application of random seed and logistic probabilities for the output (Phillips & Dudik, 2008). We masked our models to four altitudinal categories resuming both, the abrupt altitudinal clines characteristic of central Chile, and some known altitudinal distribution limits for several vertebrate taxa in this area (Fuentes & Jaksic, 1979). This procedure was conducted because reducing the climatic variation being modeled to that which exists within a geographically realistic area improves model accuracy and reduces problems with extrapolation (Pearson *et al.*, 2002; Thuiller *et al.*, 2004; Randin *et al.*, 2006). We ran 10 replicates for each model, and an average model was presented using logistic probability classes of climatic niche suitability. The presence– absence map was determined using the 'maximum training sensitivity plus specificity logistic threshold' where the omission error of all occurrence records is set to zero (i.e., locations of all occurrence records are predicted as 'suitable'). Nevertheless, we arbitrary defined a second threshold as the 50% highest logistic probability values observed between the maximum training sensitivity plus specificity logistic threshold and the

maximum observed logistic value, in order to depict the areas with highest probability of suitability. We used the receiver operating characteristic for its area under the curve (AUC) value to evaluate the model performance (Fielding & Bell, 1997; Raes & ter Steege, 2007). AUC values range from 0.5 for a random prediction to 1 for perfect prediction (Phillips *et al.*, 2004).

RESULTS

Molecular Marker.- The Tajima's D value of 0.54865 was not significative ($p > 0.10$), thus the neutral mutation hypothesis could not be rejected for the HVII domain. The **I**ndex of **S**ubstitution **S**aturation (**Iss**) obtained with the Xia test (Xia *et al.*, 2003b) was significantly lower than the **C**ritical **I**ndex of **S**ubstitution **S**aturation Value (**Iss.c**), with Iss=0.4211 Iss.c=0.7035 and a p value of 0.0053. Therefore, the molecular marker shows a very small saturation and it meets both, the neutrality and non-saturation assumptions.

Thirty seven haplotypes were recovered representing 68 sequences of *P. darwini*, whereas the four sequences of *P. magister* corresponded to haplotypes Phm3_7 and Phm4_5. Most of the *darwini* haplotypes were private haplotypes (32 haplotypes) and 27 of them were represented by only one individual. Among the five most frequent haplotypes three were shared haplotypes and two had a broad geographic distribution encompassing several localities (see Appendix II): DIV1 (11 individuals from Llanos de Challe and Observatorio la Silla, four individuals from Fray Jorge, two from Pelambres, two from Chillepín and one from Quebrada del Tigre); DIV2 (five individuals from Fray Jorge, Chillepín, Cerro Santa Inés and two from San Carlos de Apoquindo) (Appendix II). In summary, haplotype diversity in *Phyllotis darwini* D-loop HV2 consists mostly of private

haplotypes of low frequency and two frequent haplotypes with exceptionally wide distribution.

Intraspecific Phylogeny and Haplotype Network ._ The intraspecific phylogeny confirms *Phyllotis darwini* as a monophyletic group, and shows a clear division in two major clusters with a posterior probability value of 1.0 (Fig.2). This pattern disagrees with the genetic homogeneity suggested for this species by Steppan (2007). One of the recovered clades included haplotypes from almost all localities sampled in this work (hereafter "Lineage B"; Fig. 2). In fact, lineage B included two widely distributed haplotypes, namely DIV1 and DIV2, whose distributional range encompasses from Llanos de Challe to Quebrada del Tigre (28°-32° S), and from Fray Jorge to San Carlos de Apoquindo (30°-33° S), respectively (Figs. 1 and 2). The phylogenetic topology suggests some structuring within lineage B towards the southernmost distributional range (Agua Tendida and Quirihue, 36˚ S; Fig.1). Despite the low number of localities representing the southernmost range, we recovered the haplotypes from Agua Tendida and Quirihue as a well-supported subclade, reciprocally monophyletic with respect to the northernmost haplotypes belonging to lineage B. Taken together, haplotypes from B are distributed throughout almost the entire latitudinal range of *P. darwini*. The other well supported group (hereafter Lineage A) is restricted to the northernmost locality of Pan de Azúcar National Park (26°S) in the Coastal Desert, and to the highland localities of Observatorio La Silla, Pelambres, and Tranque de Relaves Barahona in the Andean Cordillera, and to Cerro el Roble in the Coastal Cordilllera (Figs. 1 and 2). Lineage A is clearly differentiated from the geographically widespread lineage B. All haplotypes in lineage A were sampled in disjunct

localities, with altitudes above 1500 m with the exception of one haplotype sampled in the coast (Pan de Azúcar National Park).

The Median Joining Network was totally congruent in recovering both major groups (lineages A and B) inferred from MultiBayes (Figs. 2 and 3). It is remarkable that lineage B included the most frequent and widespread haplotypes. It is also interesting the large number of mutational steps (34) that separate lineages A and B despite the geographical proximity between both phylogroups (less than 30 km at the narrower points in the valley). This contrasts with the nine mutational steps that separates Agua Tendida and Quirihue haplotypes (Fig. 1), 400 km away from the rest of haplotypes of lineage B.

Molecular clock.-

The 95% highest posterior density (HDP) interval for node ages are 0.47 – 2.9 MYa for lineage B, and 0.057 – 1.99 Mya for lineage A (Fig 5). Those values are not meant to be a precise estimation of phylogroup's ages, because clock calibration using the estimated age for the entire genus is somehow an indirect way to calibrate intraspecific divergence times. Nevertheless, those values allow us to demonstrate that main intraspecific lineages in *Phyllotis darwini* were established long before LGM (21 KYa).

Population Genetics Analyses._ In the first run of Geneland we treated the number of clusters as an unknown parameter in order to establish the most probable number of populations within the species. After 50,000 generations burn in, the highest posterior probability density occurs in a value of three for the number of populations parameter. Then, we set the number of populations at three for the next independent runs; we choose

the highest average posterior probability density as the better run to estimate both, the posterior probability cluster membership of each individual, and the geographic position of the clusters. The output of the best run was used to draw a map with an estimation of the geographic position of each cluster, as well as the isoclines of population membership (Fig 4). We recovered three populations within the species, one restricted to the southernmost part of the distribution range around 36° S at the coast (fig 4 A, cluster 1). The geographic extension of this population could be broader than the estimated because we failed to capture individuals around Quirihue and Agua Tendida neither northward nor in the Andean cordillera. The second population (Fig 4 B, cluster 2) ranges between 28° S and 34° S, mostly in the central valley and the Andean slopes at altitudes not exceeding 1500 m. This population encompasses the major portion of the species distributional range. Individuals from the third population (Fig 4 C, cluster 3) appeared as belonging to three disjunct high altitude locations and also to the northernmost locality of Pan de Azúcar in the Coastal Desert. Those three populations inferred within the species agreed exactly with the major genealogical clusters inferred from the intraspecific phylogeny and the haplotype network analysis.

Mantel test. The next step was to assess if there was some evidence of isolation by distance. We divided the localities in the two major clades because we hypothesized the broad latitudinal extension of each clade as a potential source of genetic differentiation in a stepping stone pattern. Lineage B included localities from the coast and from the valleys, as well as localities from the slopes of the Andes and Coastal cordillera. Lineage A included high altitude localities from the Andes and Coastal cordillera, being Pan de Azúcar the exception to this group since it is a lowland locality. The results indicated that there exists a

significant correlation, major than the expected by chance, between genetic differentiation and geographic distance into the populations belonging to lineage B with a p-value of 0.049. Meanwhile, in the localities belonging to lineage A the correlation was not significantly distinct from the expected by chance with a p-value of 0.4915. We repeated the test for lineage A now excluding the locality of Pelambres because it is the only one that shares haplotypes assigned to both major phylogroups. However, the correlation was still not significantly distinct from the expected by chance with a p-value of 0.2073.

Distribution of Pairwise Genetic Differences.- Since there is an haplogroup distributed throughout the range of *P. darwini* (lineage B), whereas the other it is mainly restricted to high altitude locations (lineage A, which agreed with the clusters inferred from GENELAND), we considered each lineage as separate demes. We evaluated if each haplogroup had independently experienced an abrupt demographic expansion during its evolutionary history. Finally, to test the goodness of fit from the observed mismatch distribution to the simulated distribution under the assumption of sudden expansion, we implemented the least square deviation method. The Sum of Square Deviations (SSD) value was 0.025 for lineage B, and 0.031 for lineage A; its associated p-value was 0.85 and 0.29 respectively. Accordingly, the hypothesis of sudden expansion cannot be rejected and we concluded that populations of both clades have experienced at least one important and sudden population size expansion across its evolutionary history

Distribution models .-

In order to test the assumption that overlapped lineage distribution models may approximate whole species distribution models, we compared our estimations of *Phyllotis*

darwini's distribution range from i) all trapping localities as a single distributional unit (Fig. 6, table 1), and ii) independent models for each intraspecific lineage as an independent distributional unit (Fig 7, Tables 1 and 2). The result shows that the whole species's range model is an accurate approximation of the observed distribution range for *P. darwini* (26°S to 36°S observed, 25°S to 36.5°S predicted) and also, with high model performance (AUC, Table 1), whereas overlapped lineage distribution models appeared to slightly over-predict northern distribution (22°S to 36.5°S). Both models (whole specie's and overlapped lineage's ranges) are good and consistent approximations of current *Phyllotis darwini's* geographic range.

As expected, distribution model for lineage B encompasses the whole distributional range of *P. darwini* (Fig 7, table 2). Interestingly, when considering an arbitrary high threshold (50% highest logistic probability value, red area in Figs. 6 and 7) the predicted range is mainly restricted to the lowlands in the valley and the coast. On the other hand, the distribution model for lineage A shares lower AUC value and clearly over-predict the observed distribution of this phylogroup. Nevertheless, when considering our arbitrary high threshold, the estimated distribution for lineage A is surprisingly well delimited and restricted almost exclusively to Andean mountain ranges above the 1500 m elevation limit detected for this phylogroup in this work. In summary, overlapped lineage distribution models has slightly worst performance and some over-prediction compared to the whole species's distribution model, but when considering our arbitrary 50% highest logistic probability threshold, lineage distribution models reproduced very accurately the altitudinal pattern reported for both phylogroups at present. This information is missed in the whole species' distribution range model (Fig. 6).

Past lineage distribution estimated by both LGM models is conflicting (CCSM and MIROC, Fig. 7, table 2): the CCSM model predicts a distributional gap during LGM for both phylogroups, but MIROC based distribution models predicts that both phylogroups were restricted to the southern portion of *Phyllotis* present distribution range. Nevertheless, both models consistently predicted the area between 31°S-35°S as suitable for both phylogroups during LGM. This latitudinal distribution dynamics must be considered with caution because downscaled climatic data may not represent local geographic complexity with accuracy. It is important to emphasize that altitudinal particularities reported for both lineages at present were already established during LGM: lineages A and B might have been restricted to approximately the same latitude, but only lineage A displayed suitability areas at Andean mountain range during LGM (Table 2), which also has been sampled mainly at the Andes and at localities above 1500 m. at present.

DISCUSSION

One of the most important features in the genetic structure of *Phyllotis darwini* is the major split found between the widespread haplogroup (lineage B) and the high altitude haplogroup (lineage A). The phylogroup B has the most frequent and widely distributed haplotypes, whereas lineage A shares only private haplotypes separated by 34 mutational steps from lineage B, and it is mostly restricted to high altitude localities. Lineage's ages are at least 47 and 57 KYa for lineages A and B, respectively (lowest 95% HPD value), and according to GENELAND analysis, the inter-lineage gene flow appeared to be restricted at present. Meanwhile, both lineages displayed signal of past population expansions; only

lineage B had a significative isolation by distance pattern. Altogether, those phylogenetic and populational features suggested that main lineages in *P. darwini* had ancient and independent evolutionary trajectories. Since the pioneer work of Fuentes & Jaksic, (1979) it has been hypothesized that there exist two asynchronous speciation modes for lizards and rodents in central Chile: i) Mountain speciation occurred during interglacial periods, because of high species replacement with altitude and between mountains isolation, and ii) valley speciation should occur during glacial periods, when species may not reach high altitude elevations and connectivity in the valleys is reduced. Given the ecological attributes of both groups, lizards are expected to display both speciation modes because this group might be affected by severe decrease in connectivity in the valley during glacial periods, and also are restricted in high altitude localities during interglacial periods because its low vagility, high habitat specificity, and high species turnover between mountains. Rodents would only exhibit the valley speciation mode because they might have been affected by a decrease in connectivity in the valley during glacial periods, but not by high altitude isolation during interglacial, because its high vagility and lower "between-mountains species turnover" compared to lizards. Those differences in speciation modes would be finally explained by differences in mobility between groups, as a consequence of its different thermoregulation modes and energy requirements (Fuentes & Jaksic, 1979). Our results show that *P. darwini* displays differentiation inside the lowland phylogroup (Lineage B) with an isolation by distance pattern and restricted gene flow between subgroups. This could be considered as evidence of the valley speciation mode inside this endemic rodent species. Nevertheless, the fact that postglacial recolonization in mountain ranges has occurred only in the apparently high-altitude adapted lineage A, suggests that mountain speciation mode could be most likely the cause of the origin for this lineage,

which show non-latitudinal structure despite being distributed in several disjunct localities along mountain ranges. In conclusion, and contrary to the "lizards and rodents" hypothesis, the lineages in this species appear to have originated by the same speciation mode suggested for lizards in the central Chile area (both, valley and mountain speciation modes in the whole species). The specific historic event which could have triggered those intraspecific diversification events remains elusive, because lineage ages in *P. darwini* could be older than Quaternary times.

The fact that *P. darwini*'s distribution range at present can be estimated by overlapping lineage distribution models is non-trivial. Even though model performance is slightly worst in lineage distribution models compared to whole species distribution model, it is clear that by using below species level ESUs and more restrictive probability thresholds in SDMs, we are able to recover very important distributional information. In fact, in this approach we have demonstrated that *P. darwini* is composed of two ancient lineages which, despite their latitudinal overlap, it shares a very strict segregation in its altitudinal distribution at present. An important methodological consideration is that intraspecific lineages may have more restricted climatic niches than the whole species, and given the low resolution of downscaled climatic models regards local conditions, it is not surprising that the distribution models at intraspecific level suffered more over-prediction than the whole species distribution models (Merow *et al.*, 1992; Laughlin *et al.*, 2012). Therefore, more restrictive probability thresholds for distribution models below species level must be considered.

Once we have established the phylogenetic and population structure, main lineage´s ages and meaningful lineage distribution models at present, the next step was to project our

lineage distribution model to climatic conditions at LGM. The rational|e behind this procedure is as follows: if we can estimate geographic distribution for intraspecific lineages at present and also at some point in the past, with very different climatic conditions, then we can compare past to present lineage distribution, and interpret the differences between those geographic ranges as distributional responses to climate change. LGM was chosen because is one of the biggest recent climatic events (Heusser, 1990; Clapperton, 1994; Heusser *et al.*, 2006), and it has been hypothesized as a major forcing in vegetation range dynamics in the central Chile biodiversity hotspot (Villagrán & Armesto, 1991; Villagrán & Hinojosa, 2005). In this context, the results shows that at LGM, both lineages were restricted to aproximately the same latitudes (28°S-31°S) but only lineage A displayed suitability areas at the high altitude andean mountain ranges. After the LGM event, temperature may have rise and precipitation may have declined at those latitudes, and besides other minor climatic oscilations, present day temperature is higher and precipitation is lower than during LGM. Therefore, this comparison suggests that after post-LGM warming, both lineages expanded their northern distribution to their present geographic range limits around 26°S. However, only lineage A colonized Andean mountain ranges above 1500 m altitude, being the lineage that retained its Andean distribution during the maximum northward glacial advance through the Andes during LGM (Clapperton, 1990; Clapperton, 1994). In conclusion, after glaciation, both lineages expanded their distribution northward to the same latitudes, but clearly not to the same altitudes. This would explain the present day segregation of lineage B which shows a wide distribution although restricted to the lowlands and the coast, whereas lineage A is mainly distributed through the Andes and the coastal mountain ranges above a threshold of 1500 m aproximately. Specifically, the distributional response to an increase of temperature and a decline of

precipitation was independent for each lineage: lineage B colonized a broad latitudinal range but restricted to low elevations, whereas lineage A was able to colonize mountain ranges.Thus,we hypothesize that both lineages will display independent distributional responses to future GCC scenarios, as they did in the past. This conclusion would be impossible to achieve if we consider that species behaves as simple ecological units to climate change.

It is important to notice that we refer to the distribution range for both lineages as restricted to 30°S- 35°S during LGM, but the distribution model projected in CCSM climatic data disagree with this interpretation and it predicts another relict between 25°S-28°S. We can not rule out this possibility; in fact, it could be a good explanation for the only lowland locality in wich lineage B has been sampled, the current northern *P. darwini* distribution's limit. We have deliberately chosed the MIROC based distribution model at LGM because this area is predicted as suitable by both models. We do not expect to provide precise distribution models because high resolution climatic models wich reproduce the local conditions and the complex geography of the central Chile hotspot are lacking. Nevertheless, the essential altitudinal pattern and independent post-LGM colonization with altitudinal segregation for intraspecific lineages is supported by both, CCSM and MIROC based distribution models.

In conclusion, this work is an example not only that species in endangered areas had cryptic diversity below the species level, but also those lineages appeared to have responded independently to climate change in the past, and therefore species may not behave as ecological units to future GCC scenarios. In order to prevent massive cryptic biodiversity losses in the future (Bálint *et al.*, 2011), the integration of genetic and ecological tools must

be encouraged (May *et al.*, 2011) as a way to understand the complex distributional responses of species and biotas. This could be the only way to make realistic predictions for conservation planning.

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Tables and Figures

Table 1.

Model	AUC	AUC stdv.
P. darwini whole specie's range	0.971	0.022
P darwini lineage A	0.968	0.025
P. darwini lineage B	0.83	0.15

Table 2._

	P darwini lineage A	*P. darwini lineage B*
Present	25°S - 34°S (Highest logistic probability values at the valley and at the coast)	24°S - 36°S (Highest logistic probability values exclusively at the Andean mountain range)
CCSM	Distributional gap between 28°S-31°S. Not distributed in the Andean mountain range.	Distributional gap between 28°S -31°S
MIROC	31°S-35°S (mainly distributed in the valley and the coast)	30°S - 35°S (andes, valley and coast)

FIG. 1

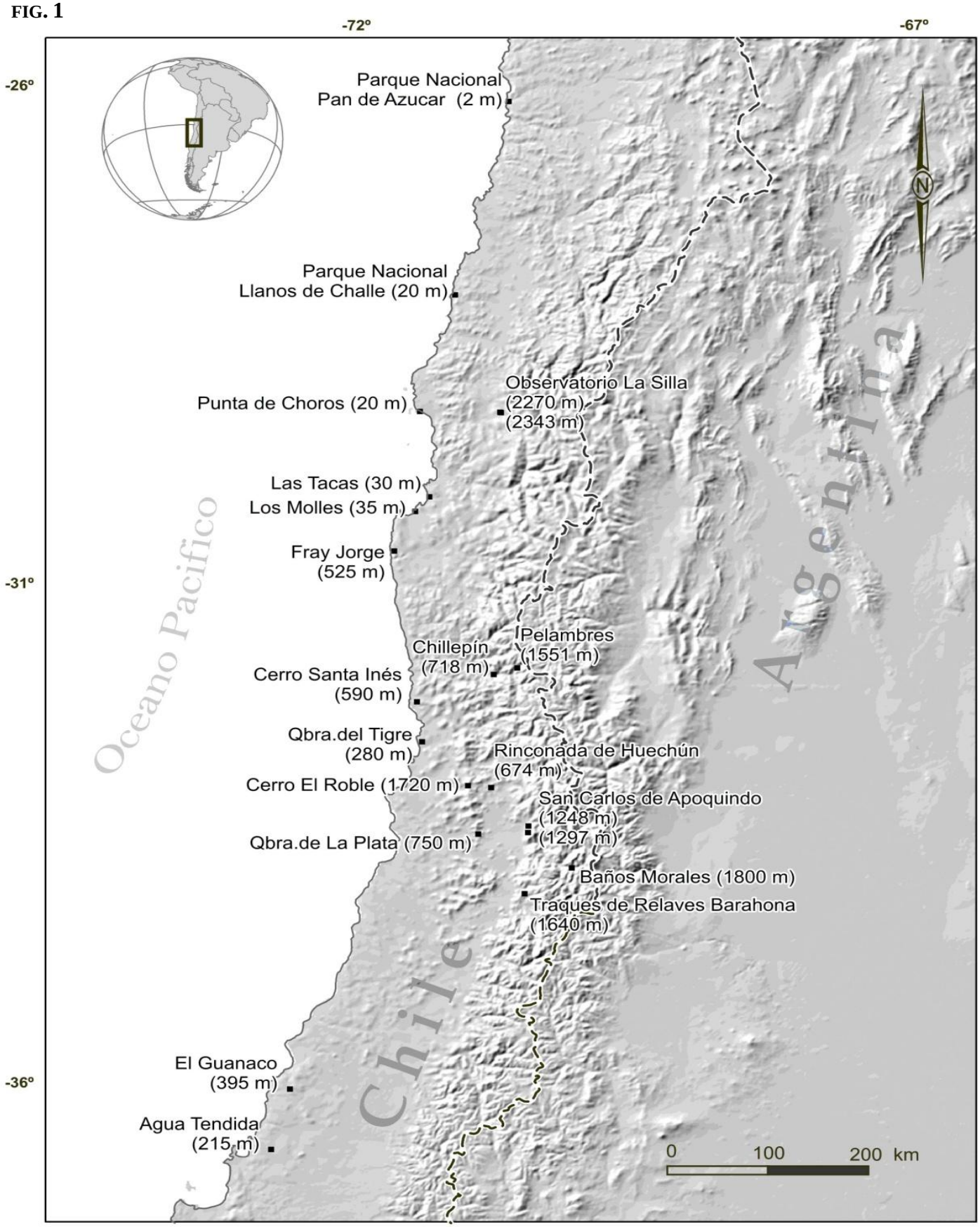

-72°
-67°
-26°
-31°
-36°
Parque Nacional
Pan de Azucar (2 m)
Parque Nacional
Llanos de Challe (20 m)
Observatorio La Silla
(2270 m)
(2343 m)
Punta de Choros (20 m)
Las Tacas (30 m)
Los Molles (35 m)
Fray Jorge
(525 m)
Pelambres
(1551 m)
Chillepín
(718 m)
Cerro Santa Inés
(590 m)
Qbra.del Tigre
(280 m)
Rinconada de Huechún
(674 m)
Cerro El Roble (1720 m)
San Carlos de Apoquindo
(1248 m)
(1297 m)
Qbra.de La Plata (750 m)
Baños Morales (1800 m)
Traques de Relaves Barahona
(1640 m)
El Guanaco
(395 m)
Agua Tendida
(215 m)
Oceano Pacifico
Chile
Argentina
N
0
100
200 km

FIG. 2.-


Ate51
Ate68
Qui51_53_57_58_59_60
Qui52
DIV2
RHu68
SA19
CR69
Pe75
DIV1
Pe91
RHu73
LT27
LT28_29_31QPI38
SA17
LT26
LCh04
LT33
CSI48_49
SA23
SA12
SA49
Paz83_84
OS46
OS55
OS47
OS64
TrBa17
TrBa20
CR37_41_46
Pe95
TrBa22
TrBa25
TrBa16_21_24
TrBa23
Phm3_7
Phm4_5
0.95
1.0
1.0
0.64
1.0
0.98
1.0
B
A
0.01

FIG. 3

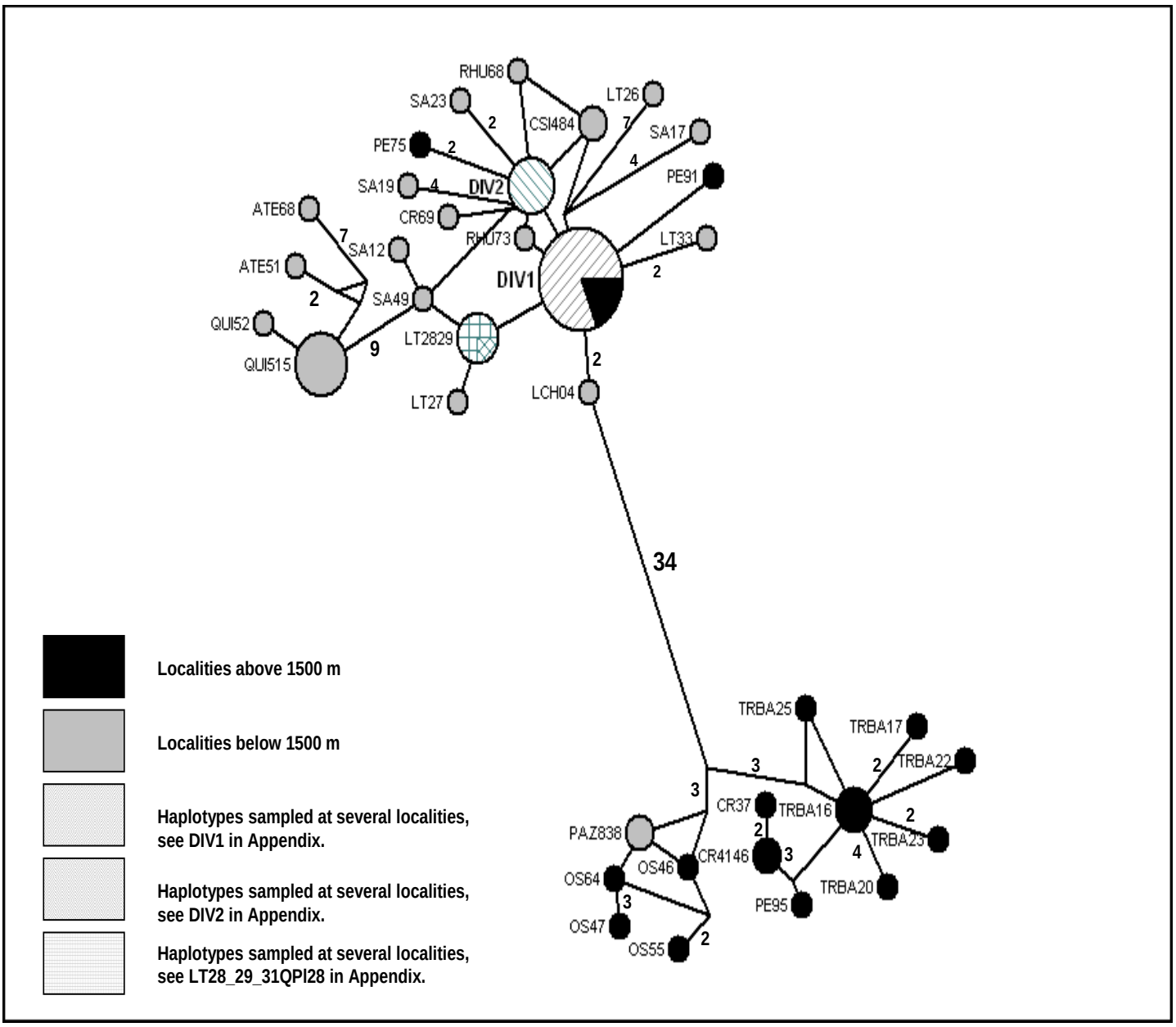

RHU68
SA23
LT26
CSI484
SA17
PE75
PE91
SA19
DIV2
ATE68
CR69
RHU73
LT33
SA12
ATE51
DIV1
SA49
QUI52
LT2829
QUI515
LT27
LCH04
34
TRBA25
TRBA17
TRBA22
CR37
TRBA16
TRBA23
PAZ838
CR4146
OS64
OS46
TRBA20
PE95
OS47
OS55
Localities above 1500 m
Localities below 1500 m
Haplotypes sampled at several localities, see DIV1 in Appendix.
Haplotypes sampled at several localities, see DIV2 in Appendix.
Haplotypes sampled at several localities, see LT28_29_31QPI28 in Appendix.

FIG. 4

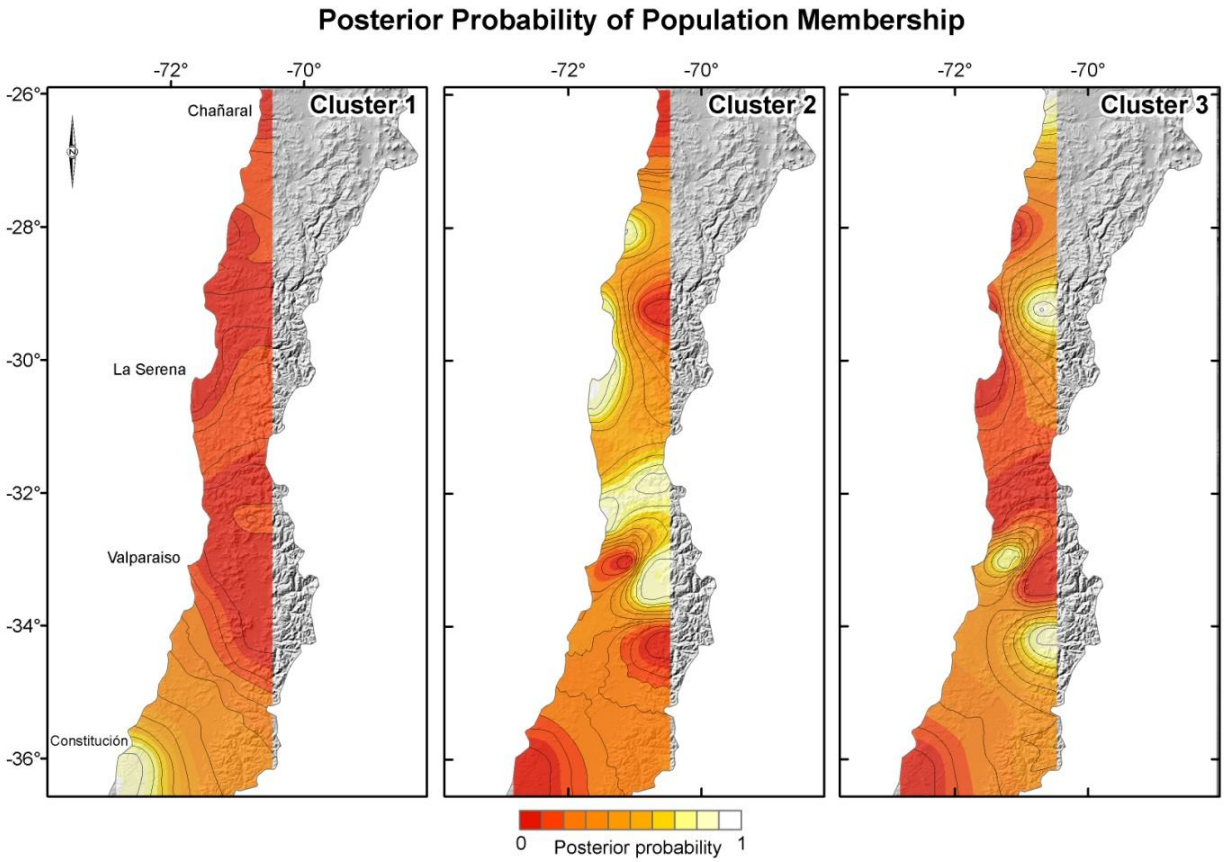
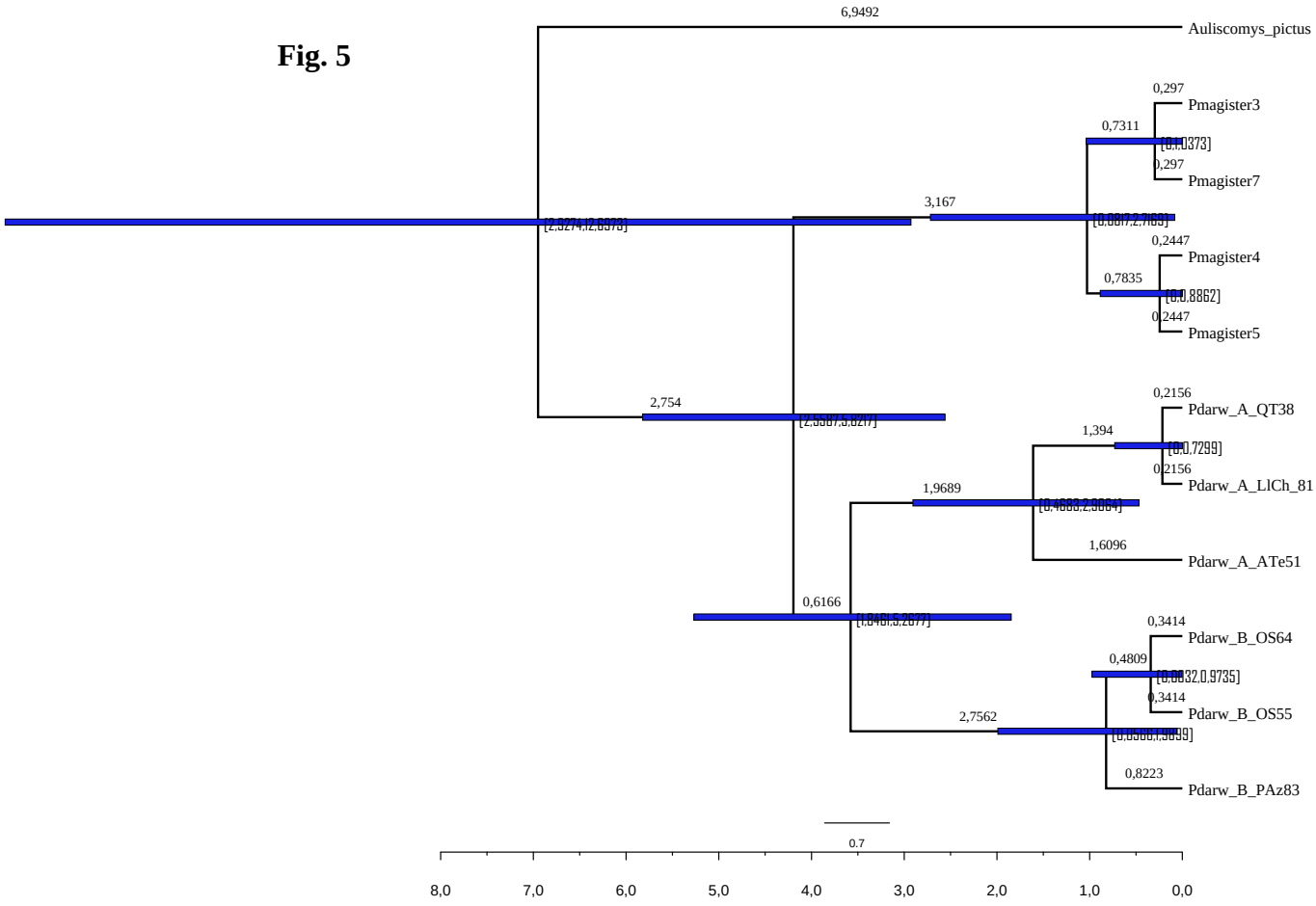

Posterior Probability of Population Membership
-72°
-70°
-26°
-28°
-30°
-32°
-34°
-36°
Chañaral
Cluster 1
Cluster 2
Cluster 3
La Serena
Valparaiso
Constitución
0
Posterior probability
1

Fig. 5


6,9492
Auliscomys_pictus
0,297
Pmagister3
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0,297
Pmagister7
3,167
0,2447
Pmagister4
0,7835
0,2447
Pmagister5
2,754
0,2156
Pdarw_A_QT38
1,394
0,2156
Pdarw_A_LlCh_81
1,9689
1,6096
Pdarw_A_ATe51
0,6166
0,3414
Pdarw_B_OS64
0,4809
0,3414
Pdarw_B_OS55
2,7562
0,8223
Pdarw_B_PAz83
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7,0
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0,0

FIG. 6

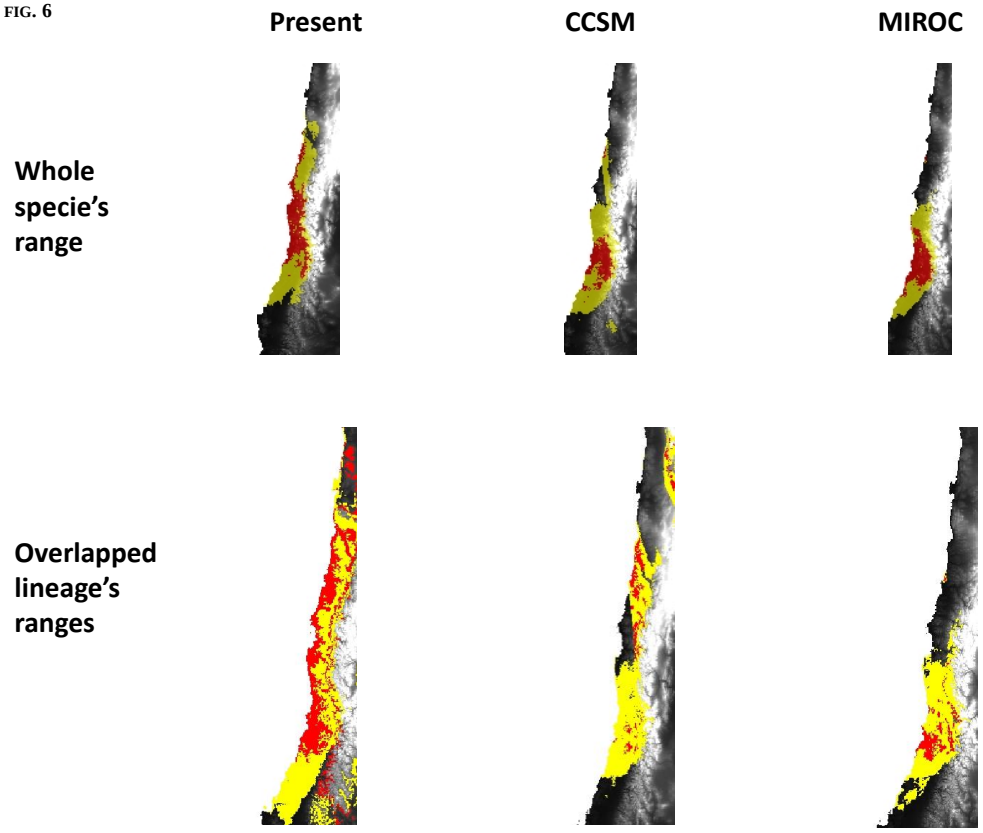

Present
CCSM
MIROC
Whole specie's range
Overlapped lineage's ranges

Fig. 7

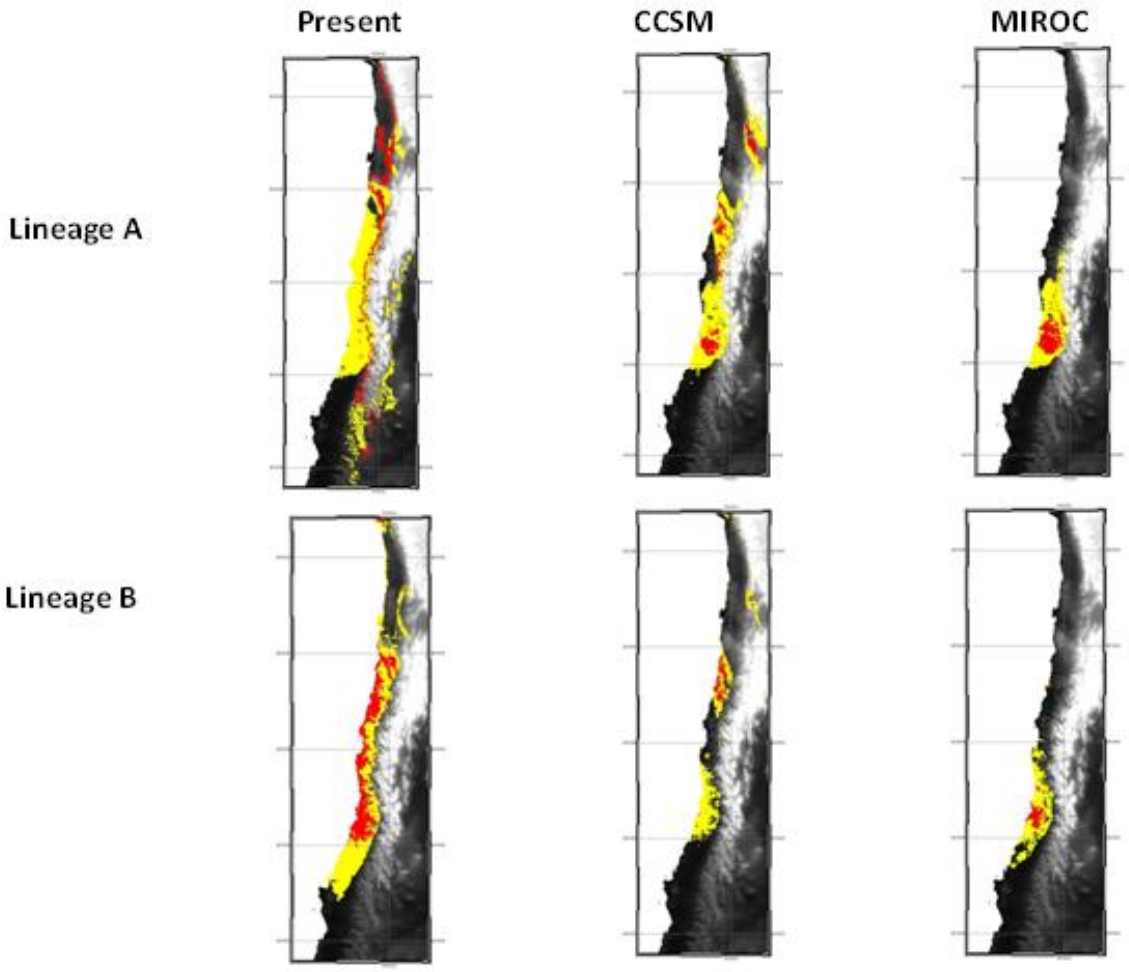
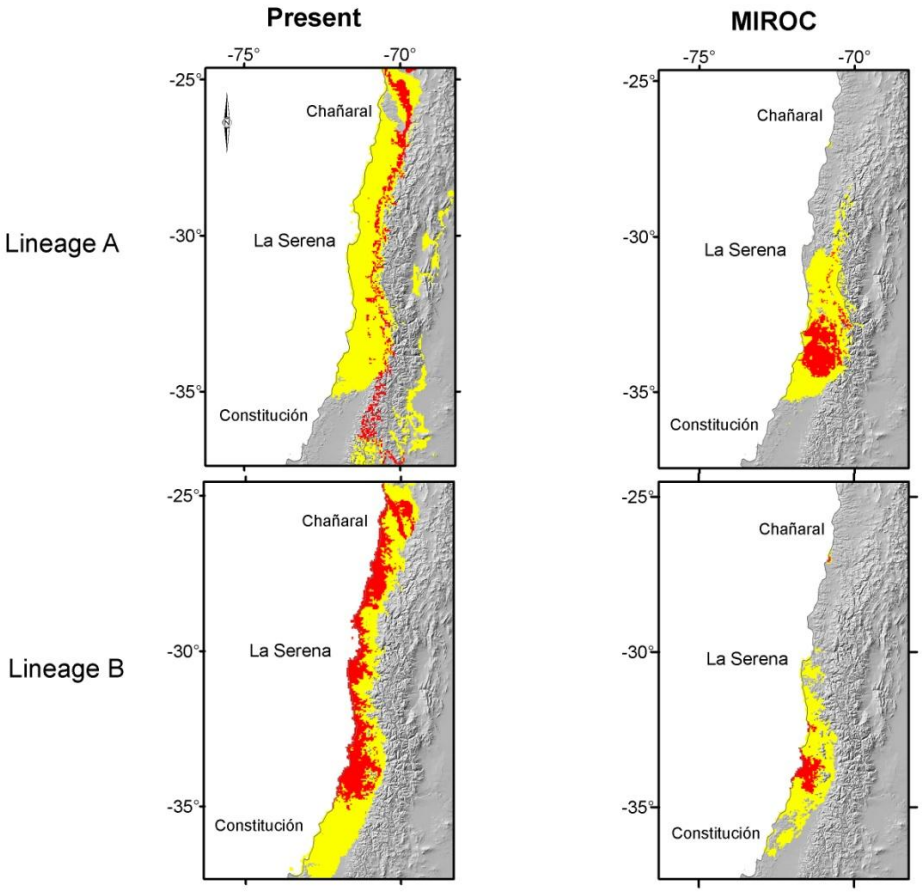


Fig. 7.1


Present
MIROC
Lineage A
Lineage B
-75°
-70°
-25°
-30°
-35°
Chañaral
La Serena
Constitución

Legends

Table 1_ AUC values and standard deviation for whole species's distribution and individual lineage's distribution models.

Table 2_ Lineage´s distribution model summary, at present and at LGM (current conditions, CCSM and MIROC).

Figure 1 _ Geographic distribution of localities sampled in *Phyllotis darwini* distributional range.

Figure 2 _ *Phyllotis darwini* intraspecific phylogeny based on Bayes Markov Chain Monte Carlo method (BMCMC). The phylogeny was obtained for the Hipervariable Domain II (HV2) from the mitochondrial control region sequence data, whereas for BMCMC represents a consensus tree from the n = 9950 trees from the converged Markov chain. Posterior probability values over 0.5 are represented on each node.

Figure 3 _ Median-Joining haplotype network for the *Phyllotis darwini* mitochondrial DNA dataset. The size of the Haplotype tip is proportional to its frequency. Numbers on the branches are mutational steps between haplotype tips; when the branch has no number the tips are separated by just one mutational step. Filled black and grey are private haplotypes sampled at localities above and below 1500 m altitude respectively. Dashed are shared haplotypes. The DIV 1 is a shared haplotype, but the proportion of individuals sampled above 1500 m altitude are designated by filled black.

Figure 4 _ Map of population membership posterior probability. According to Geneland analysis, the species is composed of three genetic units, designated as cluster 1, 2 and 3. Map depicts posterior probability iso-lines of belong to each cluster.

Figure 5 _ D-loop based Phylogeny for intraspecific lineages in *P. darwini* and other related species. Diversification times (expressed in millions years) appears above the branches. Blue bars on the nodes represent 95% highest prior density estimates (95% HPD, range in brackets) for the molecular rate.

Figure 6 _ *P. darwini's* distribution models. The figure shows specie's distribution models for present and two LGM climatic models (columns). Models were built for the whole distribution range as a single unit, and also by overlapping independent lineage distribution models (rows). Yellow represents suitability areas for the specie according to the maximum training sensitivity plus specificity logistic threshold; red areas represents suitability areas according to an arbitrary restrictive threshold, defined as the 50% highest values observed between the maximum training sensitivity plus specificity logistic threshold, and the maximum logistic probability value for each model.

Figure 7.1 _ Lineage distribution models. The figure shows the species distribution models for present and two LGM climatic models (columns). Models were built independently for each *P. darwini*'s intraspecific lineage (rows). Yellow areas represents suitability areas for the lineages according to the maximum training sensitivity plus specificity logistic threshold; red areas represents suitability areas according to an arbitrary restrictive threshold, defined as the 50% highest values observed between the maximum training

sensitivity plus specificity logistic threshold, and the maximum logistic probability value for each model.

7.2 Detailed view of 4 lineages from figure 7.1

Mountaintops phylogeography: a case study using small mammals from the Andes and the Coast of central Chile.

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Running head: Phylogeography of mountaintop small mammals

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ABSTRACT

Aim We evaluated if selected species of small vertebrate taxa (and its intraspecific lineages) at the Andes and Coastal mountaintops of central Chile had experienced distributional shifts due to altitudinal movements of plant biota and climate change during and after the Last Glacial Maximum (LGM) of the Pleistocene. We used two sigmodontine rodent taxa inhabitant of both mountain ranges, *Phyllotis darwini* and *Abrothrix olivaceus*, as study models. The major hypothesis to test was that during LGM populations of both, *P. darwini* and *A. olivaceus*, experienced altitudinal descents due to range shifts of habitats from the Andes to the Coast. The retraction events during postglacial may have leave remnants of these two rodent species populations on the Coastal Cordillera of central Chile, leaving disjunct populations on the mountaintops of the Cordillera de la Costa and Cordillera de los Andes.

Location Coastal Cordillera and Cordillera de los Andes, central Chile

Methods We sampled specimens of both study model taxa from the Andes and the Coast of central Chile. Samples were phylogeographically analyzed based on nucleotide sequences of the mitochondrial control region and the intron 7 of the nuclear b-fibrinogen gene (FGB). Intraspecific phylogenies were reconstructed for each and concatenated molecular markers using maximum likelihood and Bayesian methodology; a haplotype network was also reconstructed for each studied species. Structuring of populations was analyzed using Geneland. Finally, we modeled the climatic niche of the two rodent species' lineages to approximate the species' current distribution and distribution during the Last Glacial Maximum (LGM).

Results We recovered a strong and well supported phylogenetic split within *P. darwini* and *A. olivaceus*, patterns that were also evident with network analyses. Current distribution in *A. olivaceus* displays one lineage with shared haplotypes between both mountain systems, whereas the other haplogroup is restricted to the Andean mountain range, contrary to the shared haplotype pattern between the Andes and the Coast for both *P. darwini*'s intraspecific lineages. Geneland analyses recovered coastal and Andean localities conforming panmictic units for *P. darwini* despite

current disjunct distribution, whereas for *A. olivaceus* there exists an strictly Andean panmictic unit, and another which includes localities from the valley, coast, and Coastal Cordillera. Finally, the niche modelling analyses depicted differential postglacial expansions in several lineages, mainly from the valley and coastal mountain tops towards the Andes and coastal cordilleras, since the LGM to present.

Main conclusions Our results suggested that historical events as the LGM, would have triggered the descending of populations from the Andes to lower elevations and refuge areas in the lowlands and the Coastal Cordillera. Further movements of populations backwards after glacial retreats may have followed, leaving population isolates on the mountaintops of the Coastal Cordillera. The haplotype admixture between phyllogroups sampled at both mountain ranges, along with evidence of postglacial expansion of the climatic niche for both species's lineages, suggest that current distribution of those mammals is the outcome of climate change and habitat reconfiguration after LGM; the fact that the only one strictly Andean intraspecific lineage appears to have been persistently distributed at this mountain range since LGM, strongly agreed with this hypothesis. Assuming that this process may have repeated across several glacial/interglacial transitions during Pleistocene, allows us to hypothesize that climate change and habitat shifts played an important role in the intraspecific diversification of mammals distributed in the mountain ranges of central Chile.

Keywords

Andean Cordillera, Coastal Cordillera, central Chile, sigmodontine mice, population disjunction, Last Glacial Maxima, niche modelling.

INTRODUCTION

The Pleistocene is characterized by worldwide climatic changes associated with glacial cycles, forcing species to shift their ranges, and subsequently impacting the structure of their populations. Under glacial-dominated scenarios, species at higher latitudes might have experienced strong demographic and genetic changes in their populations (Hewitt, 2004). In boreal communities, organisms contracted to refugia during glacial maxima, and then colonized or expanded into newly available habitats after glacial retreat (e.g. Hewitt, 2000; Lessa *et al*., 2003). In South America, Pleistocene glacial events would have had severe effects on populations associated with Andean mountains, where ice sheets and permafrost were focused on the southern cone (Clapperton, 1993; 1994; Hollin & Schilling, 1981; Mercer, 1983). Populations inhabiting these latitudes would have suffered local extinctions, expansions and retractions following the Quaternary glacial oscillations (Brown & Lomolino, 1998; Hewitt, 2000; Viulleumier, 1971).

Montane regions are of particular interest when assessing species' responses to historical climate oscillations because they can cause favorable environments for a species to shift, contract, or expand along not only elevational (Hewitt, 2000; 2004) but also latitudinal gradients (Guralnick, 2007). During climatic fluctuations, mountain populations may experience alternating periods of isolation and connectivity, with for example, range expansion during glacial periods and range contractions during warmer interglacials (Brown, 1971; Hewitt, 1999; Knowles, 2000; Patton & Smith, 2002; Provan & Bennet, 2008; Rowe *et al*., 2004).

Montane environments are a major component of the Chilean biogeography, particularly in central Chile where a Mediterranean ecosystem is located along the western margin of the Andes between 30-37˚ S (Arroyo *et al.*, 1994). This ecosystem is conformed by highly heterogeneous vegetation mosaic and major vegetation types are dry, xerophytic thorn scrub dominated by summer deciduous shrubs and succulents. The mesic communities of this ecosystem are dominated by evergreen sclerophyllous trees in the coastal and Andean foothills, and the forests are dominated by winter-deciduous trees in the southern edge of the region. The southern border of the Mediterranean ecoregion is the Bio-Bío River (37˚ S), whereas the northern limit is the Atacama Desert in the Copiapó region (27˚ S).

Biogeographers, when intending to explain disjunct patterns in species distribution on the Cordillera de los Andes and the Cordillera de la Costa (that run in parallel along the country), hypothesize that the disjunction would have occurred by downward shifts of mountaintop habitats during the glaciation events of the Pleistocene (Villagrán & Armesto, 2005), with a subsequent shift upwards during postglacial. Geological and glaciological data on the Last Glacial Maximum (LGM) during Pleistocene times, demonstrated that about two thirds of the Temperate and Patagonian forests were reached by glaciers (Hollin & Schilling, 1981; Denton, 1999; Heusser *et al*., 1999). Towards the north, ice masses advanced throughout the Cordillera de los Andes, and in central Chile descended to around 1,100-1,300 m (Clapperton, 1990; 1994; Rabassa & Clapperton, 1990). These ice masses triggered a local drop of temperatures of about 6-7˚ C and an increase in the rainfall (Heusser, 1983; Graf, 1994; Lamy *et al*., 1999). As a consequence, the Andean vegetational belts shift downwards, to the central valley depression (Darwin, 1859; Simpson, 1983; Heusser, 1990; Villagrán, 2001; Villagrán, *et al*. 2004). Following glacial cycles, a warmer climate prevailed with a subsequent shift of the vegetational belts upwards not only to the Andes, but also to Coastal altitudes. These events created true "biogeographic islands" at different localities on the top of the Cordillera de la Costa in central Chile, now hosting disjunct biota whose main ranges are at similar altitudes in the Andes.

To date, there are no studies on those habitat shifts of biota between both mountain systems in Mediterranean Chile from a genetic perspective. Most studies have been focused on vegetation showing the floristic affinities between the high-andean biotas of the Cordillera de la Costa and Cordillera de los Andes (García, 2006; Romero & Teiller, 2003), and probable terrestrial corridors from the Andes to coastal mountaintops due to the descent of temperatures and vegetational habitats during the last glaciation (García, 2006). However, there are some studies using molecular tools for some of the coniferous species of the southern flora of Chile (37-43˚ S), particularly for the coastal mountaintops that have a distribution that is mainly Andean (i.e., *Araucaria araucana* [pehuén]; *Fitzroya cupressoides* [the "alerce"]; *Austrocedrus chilensis* ["ciprés de la cordillera"] (Villagrán & Armesto, 2005). In all these case studies a marked fragmentation of coastal areas populations is reported with strong genetic segregation of populations between the Coast and Andean taxa (Allnutt et al. 1999, Premoli et al. 2000, Marchelli & Gallo 2006)).

The major goal of this paper was to investigate the relationships between small mammal's genetic structure and altitudinal species' distribution across the Andes and Coastal mountaintops of central Chile, and how that relationships may have changed due to altitudinal shifts of plant biota during and after the Last Glacial Maximum (LGM) of the Pleistocene. To that goal, we used two

sigmodontine rodent taxa as study models, inhabitant of both mountain ranges: *Phyllotis darwini* and *Abrothrix olivaceus*. *Phyllotis darwini* is an endemic species of Mediterranean Chile and altitudinally it is found between the coast up to 2000 m (Iriarte, 2008). *A. olivaceus* has a wide distributional range, from southern Peru downward to the Patagonia of Chile and Argentina, and altitudinally it is also found up to 2000 m (Rodríguez-Serrano *et al*., 2006; Spotorno *et al*., 2001). Therefore, the major hypothesis to evaluate in this paper is that during LGM populations of both *P. darwini* and *A. olivaceus* experienced altitudinal shifts due to displacement of habitats from the Andes to the Coast. Current distribution of intraspecific lineages must have been largely determined by postglacial climate changes and habitat reconfiguration. Hypothetically, the contraction events during postglacial may have leave remnants of these two rodent species populations on the Coastal Cordillera of central Chile, leaving disjunct populations on the mountaintops of the Cordillera de la Costa and Cordillera de los Andes in central Chile. We analyzed the phylogenetic and population structure, climatic niche and distributional shifts since LGM on the Andean and Coastal populations for the two above sigmodontine rodent species. We used mitochondrial and nuclear markers in a phylogeographic approach.

MATERIALS AND METHODS

Study area and taxon sampling

For the purposes of this study we sampled a complex of localities between the Aconcagua and Metropolitana of Santiago regions encompassing Coastal, valley, pre Andean and Andean areas, located between 32 and 33˚ S in Mediterranean Chile (see Fig. 1 for the study area, and Table 1 for a detailed list of localities sampled). A total of 14 localities were sampled in central Chile of which four were Coastal localities (Cerro La Campana, Cerro El Roble, Altos de Chicauma, Altos de Cantillana), 6 were central valley localities (Villa Alemana, Rinconada de Maipú, Melipilla, Rabuco, La Florida and Paine), and 4 were Andean localities (Farellones, Campos Ahumada, San Carlos de Apoquindo and Cajón del Maipo). A total of 141 mice were trapped in all these localities of which 79 were *Abrothrix olivaceus* and 62 were *Phyllotis darwini*. Rodent trapping was performed with Sherman traps (8 x 9 x 23 cm) using a mixture of oat and canned fish as bait. For each specimen the heart, kidney, spleen, liver, and lung was extracted and stored in liquid nitrogen.

Specimens were sacrificed in the field via cervical dislocation previously anesthetized using ketamine. We followed established safety guidelines for small mammal captures and processing according to the Center for Disease Control and Prevention (CDC) protocols (Mills *et al*., 1995), American Society of Mammalogists (ASM) safety guidelines for mammalogists from Hantavirus (Kelt *et al*., 2010), ASM guidelines for the use of wild mammals in research (Sikes *et al*., 2011), and the Bioethical Protocols established from the Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

PCR and sequencing protocols

DNA was extracted from frozen liver samples treated with the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin). We amplified via PCR a region of 486 and 574 bp of the mitochonfrial DNA (mtDNA) control region for *A. olivaceus* and *P. darwini*, respectively. We amplified 79 specimens of *A. olivaceus* and 62 of *P. darwini* using primers LBE08 and 12S1 (Rodríguez-Serrano *et al*., 2006 for *A. olivaceus*), and primers 283F and 282R for *P. darwini* (Bacigalupe *et al*., 2004). In addition, we amplified a region of 680 bp for *A. olivaceus* and 584 bp for *P. darwini* of the intron 7 of the nuclear b-fibrinogen gene (FGB) for 30 specimens of *A. olivaceus* and 23 *P. darwini* using primers β17-mammL and βfib-mammU (Matocq et al. 2007; see Appendix 1 for FGB gene sequenced localities). The thermal cycle to amplify the *A. olivaceus* control region followed the protocol used in Rodríguez-Serrano *et al*. (2006), whereas the thermal cycle to amplify the *P. darwini* control region was: initial denaturation for 7 min at 95˚ C, followed by 30 cycles of 94˚ C (30 s), 58˚ C (15 s), and 72˚ C (1 min 15s). A final extension at 72˚ C for 4 min terminated the reaction. The thermal cycle used to amplify the FGB gene for both species was performed using the following protocol: initial denaturation for 5 min at 94˚ C, followed by 30 cycles of 94˚ C (1 min), 64˚ C (15 s), and 72˚ C (40 s). The final extension was at 72˚ C for 4 min. Double- stranded polymerase chain reaction products were purified with Qiaquik (Qiagen, Valencia, California). Cycle sequencing (Murray, 1989) was performed using primers 14724, MVZ14, and 15162 (Irwin *et al*., 1991) for Cytb, and b17- mammL and bfib-mammU for FGB, labeled with the Big Dye Terminator kit (Perkin Elmer, Norwalk, Connecticut). Sequencing reactions were analyzed on an Applied Biosystems Prism 3100 automated sequencer (Applied Biosystems, Foster City, California). Sequences were aligned using the CLUSTAL_X program (Thompson *et al*., 1997) and by eye. All sequences have been deposited in GenBank under

accession numbers GU564005–GU564084 and HM004435 for the control region and GU564085–GU564113 for the FGB.

Phylogenetic Analyses

Phylogenetic analyses were conducted using maximum likelihood (ML) performed with the Treefinder version of October 2008 (Jobb 2008). We selected the best-fitting model of nucleotide substitution using the corrected Akaike Information Criterion (AICc—Akaike 1974) in Treefinder. Support for the nodes was evaluated with 1000 bootstrap replicates (Felsenstein, 1985). For control region sequences the AICc identified the GTR + I + Γ model (Tavaré 1986) as the best model of base substitution. The proportion of invariable sites value was 0.5520, and the gamma shape parameter was = 1.7661. The proportions of nucleotides were A = 0.2806, C = 0.2867, G = 0.1287, and T = 0.3038. For the concatenated sequences the AICc identified the GTR + Γ (Tavaré 1986) as the substitution model. The gamma shape parameter was 0.1116, and the proportions of nucleotides were A = 0.3003, C = 0.2306, G = 0.1504, and T= 0.3185. Sequences also were analyzed in a Bayesian framework to estimate the posterior probabilities of phylogenetic trees. Ten million phylogenetic trees were generated, sampling every 1,000 trees to assure that successive samples were independent. The first 1000 trees of the sample were removed to avoid including trees before convergence of the Markov Chain. Given that we used two independent molecular markers, we applied a general likelihood-based mixture model (MM) as described by Pagel & Meade (2004, 2005), based on the general time-reversible (GTR) model (Rodríguez *et al*., 1990) of sequence evolution. This model accommodates cases in which different sites in the alignment evolved in qualitatively distinct ways but does not require prior knowledge of these patterns or partitioning data. These analyses were conducted using the Bayes Phylogenies software (http://www.evolution.rdg.ac.uk/SoftwareMain.html). To find the best mixture model of evolution we estimated the number of GTR matrices by using a reversible-jump Markov Chain Monte Carlo method (RJMCMC—Pagel & Meade, 2006). The RJMCMC visits the different mixtures of GTR matrices in proportion to their posterior probabilities, "jumping" from simple to complex models or vice-versa, making a direct estimate of the support of 1GTR, 2GTR, 3GTR, and so on. Only the combination of matrices with the fewest number of parameters that significantly increased the likelihood was used (1GTR + Γ for cytochrome *b* data; 2GTR + Γ for concatenated data) to compute a 50% majority rule consensus tree. The percentage of samples that recover any particular

clade on this tree represents the posterior probability of that clade; these are the p values, and p ≥ 95% was considered evidence of significant support for a clade (Huelsenbeck & Ronquist, 2001).

Population genetic and demographic analyses

We used the DnaSP v 5.10.01 software to describe the genetic diversity in all groups and the complete data set. We calculated the number of haplotypes (Nh), the haplotype diversity (Hd), the nucleotide diversity pi (the average number of pairwise nucleotide differences per site, and the segregating sites (S). We also assessed demographic history of the mountaintop groups by performing Fu's Fs neutrality test statistics [Fu, 1997], and Tajimas's D test [Tajima, 1989] testing the significance of the statistics from 10,000 simulated samples (Table 1) using DnaSP 5.10.01 [Librado & Rozas, 2009]. To evaluate the presence of population structure for each species, we used the program GENELAND v. 1.0.7 [Guillot *et al*., 2008] in the R-Package [Ihaka & Gentleman, 1996], which implements a population statistical model with Bayesian inference in a set of georeferenced individuals with DNA sequences data (http://www2.imm.dtu.dk/~gigu/Geneland/#). This model's objective is to infer and locate the genetic discontinuities between populations of geo-referenced genotypes, considering the uncertain localization of the sampled individuals. The number of clusters was determined by running MCMC (Markov chain Monte Carlo) iterations five times, allowing *K* (i.e., the most probable number of populations) to vary, with the following parameters: 5×10^6 MCMC iterations, maximum rate of the Poisson process fixed to 100 (this is the default value of the software). The minimum K = 1, maximum K = 10 (values that allow us to explore a wide potential number of populations, and considering the maximum spatial subdivision in the latitudinal range. The maximum number of nuclei in the Poisson-Voronoi tessellation was fixed to 300 (3 x maximum rate was suggested by Guillot *et al*., (2005). After inferring the number of populations in the data set from these five runs, the MCMC was run 30 times with *K* fixed to the inferred number of clusters, with the other parameters the same as above. The mean logarithm of the posterior probability was calculated for each of the 30 runs and the posterior probability of population membership for each pixel of the spatial domain was then computed for the three runs with the highest values.

To establish the relationships between haplotypes, we constructed a haplotype network using the Neighbor-Net (Bryant, 2004) distances transformation and equal angle splits transformation (Dress & Huson, 2004). Splits computed from the data are represented as parallel

edges rather than single branches, allowing visualization of ambiguous and conflicting signals in the data set providing an implicit representation of evolutionary history (Huson, 2006).

Climatic niche models

Distribution models .- We modeled the climatic niche of each intraspecific lineage to approximate the whole species' current distribution, and its distribution during the LGM under the assumptions that: (1) climate is an important factor driving the species' distribution; (2) the climatic niche of species remained conserved between the LGM and present time, and (3) overlapped lineage's distribution ranges will approach the whole species geographic range. The latter assumption was tested by overlapping distribution models of each intraspecific lineage in order to approach the full species distributional range, as the sum of ranges estimated for each lineage. The resultant distributional range was roughly contrasted with another model built for the whole species without considering phylogenetic structure.

The climatic niches were reconstructed using the methodology of ecological niche modeling, where environmental data are extracted from occurrence records and random points (represented by geographic coordinates). Habitat suitability was evaluated across the landscape using program specific algorithms (Elith *et al.*, 2006). The current models were then projected on the climatic reconstructions of the LGM. For occurrence records, we used our unique sampling localities. In addition to full geographic distribution models for each species, we built climatic models for each major lineage recovered in the intraspecific phylogenies following the same approach. As a test of consistency we overlapped the lineage distribution models for the lineages of each species, to compare it to the full species distribution models.

The current climate was represented by bioclimatic variables from the WorldClim dataset v. 1.4 (http://www.world clim.org/; Hijmans *et al.*, 2005) that are derived from monthly temperature and precipitation data, and represent biologically meaningful aspects of local climate (Waltari *et al.*, 2007; Jezkova *et al.*, 2009). For environmental layers representing the climatic conditions of the LGM, we used ocean–atmosphere simulations (Harrison, 2000) available through the Paleoclimatic Modelling Intercomparison Project (Braconnot *et al.*, 2007). These reconstructions of the LGM climate are based on simulated changes in concentration of greenhouse gases, ice sheet coverage, insulation and topography (caused by lowering sea levels). We used two models that have been

previously downscaled for the purpose of ecological niche modeling (Waltari *et al.*, 2007): Community Climate System Model v. 3 (CCSM; Otto-Bliesner *et al.*, 2006) and the Model for Interdisciplinary Research on Climate v. 3.2 (MIROC; Hasumi & Emori, 2004). The original climatic variables used in these models have been downscaled to the spatial resolution of 2.5 min under the assumption that changes in climate are relatively stable over space (high spatial autocorrelation) and were converted to bioclimatic variables (Peterson & Nyári, 2007).

Climatic niche models were built in the software package MAXENT v. 3.2.1 (Phillips *et al.*, 2006), a program that calculates relative probabilities of the species' presence in the defined geographic space, with high probabilities indicating suitable environmental conditions for the species (Phillips *et al.*, 2004). Trapping coordinates of each individual captured for DNA extraction were used as presence points. We used the default parameters in MAXENT (500 maximum iterations, convergence threshold of 0.00001, regularization multiplier of 1, and 10 000 background points) with the application of random seed and logistic probabilities for the output (Phillips & Dudik, 2008). We masked our models to four altitudinal categories resuming both, the abrupt altitudinal clines characteristic of central Chile, and some known altitudinal distribution limits for several vertebrate taxa in this area (Fuentes & Jaksic, 1979). This procedure was conducted because reducing the climatic variation being modeled to that which exists within a geographically realistic area improves model accuracy and reduces problems with extrapolation (Pearson *et al.*, 2002; Thuiller *et al.*, 2004; Randin *et al.*, 2006). We ran 10 replicates for each model, and an average model was presented using logistic probability classes of climatic niche suitability. The presence–absence map was determined using the 'maximum training sensitivity plus specificity logistic threshold' where the omission error of all occurrence records is set to zero (i.e., locations of all occurrence records are predicted as 'suitable'). Nevertheless, we arbitrary defined a second threshold as the 50% highest logistic probability values observed between the maximum training sensitivity plus specificity logistic threshold and the maximum observed logistic value, in order to depict the areas with highest probability of suitability. We used the receiver operating characteristic for its area under the curve (AUC) value to evaluate the model performance (Fielding & Bell, 1997; Raes & ter Steege, 2007). AUC values range from 0.5 for a random prediction to 1 for perfect prediction (Phillips *et al.*, 2004).

RESULTS

As we mentioned in the former section, for the d-loop we sequenced 79 specimens of *A. olivaceus* of which 27 were from the Andes, 29 from the valley and 23 from the coast recovering 20, 10 and 14 polymorphic sites for each of the sequenced sites; and 12, 7 and 9 haplotypes,

respectively. On the other hand, we sequenced 62 specimens of *P. darwini* of which 27 were from the Andes and 35 from the coast obtaining 59 polymorphic sites (S) for the Andean specimens and 51 for the coastal forms, and 22 and 13 haplotypes, respectively. The haplotype diversity (Hd) for *A. olivaceus* was 0.895 in the Andes, 0.603 in the Valley and 0.727 in the Coast; for *P. darwini* was 0.983 in the Andes and 0.780 for the Coast. The nucleotide diversity (pi) of *A. olivaceus* was 0.013, 0.003 and 0.004 for the Andes, the Valley and the Coast respectively*;* whereas that of *P. darwini* was 0.037 and 0.023 for the Andes and for the Coast respectively. Fu's test values for *A. olivaceus* were significantly different from zero for the Valley (-1.475) and the Coast localities (-3.041), indicating population expansion, whereas for the Andes it was not significantly different from zero suggesting a population in equilibrium (-0.654). Fu's neutrality test statistics for *P. darwini* was negative and significantly different from zero for the Andes (- 3.12), whereas for the Coast was positive (3.437) and significantly different from zero, indicating that the null hypothesis of population equilibrium is rejected in favor of a population expansion.

The d-loop based intraspecific phylogeny for *A. olivaceus* is similar for both, maximum likelihood and Bayesian analyses, thus we show a single tree (Fig. 2). For this species we observed a well-supported split between two major clusters. One of them is constituted of haplotypes sampled exclusively at Andean localities (e.g., Farellones, San Carlos de Apoquindo; lineage A). The other major group mostly included haplotypes sampled at central valley and coastal localities (lineage B) (Fig. 2) mixed with some haplotypes sampled at the Andes, as for example the localities of Farellones and San Carlos de Apoquindo (Andean). However in the Andean haplogroup (lineage A) we did not obtain any coastal haplotype for *A. olivaceus* (Fig. 2). As for *P. darwini*, we also recovered a well-supported dichotomy of two differentiated phylogroups, although we could not recognize any of the clusters strictly associated to a specific mountain range. In fact, the largest cluster (Fig. 3, lineage A) reunited coastal (e.g., Cantillana, El Roble, Chicauma) and Andean localities (e.g., Farellones, Campos Ahumada), as well as for lineage B. Nevertheless, *P. darwini's* lineage A is distributed in both mountain ranges. It is important to notice that this lineage is distributed exclusively in localities above 1500 m altitude; this pattern has been previously reported for the species with completely different samples and using only the D-loop mithochondrial marker (Gutiérrez-Tapia & R.E. Palma, in prep.).The neighbor-net analysis, on the other hand, showed similar patterns of divergence to that of intraspecific phylogenies between the Coastal (blue) and Andean (red) haplogroups both for *A. olivaceus* and *P. darwini* (Fig. 4 and Fig. 5, respectively).

A similar topology to that of d-loop was obtained for *Abrothrix olivaceus* when analyzing phylogenetically the concatenated d-loop and FGB sequences (Fig. 6), in which it is clear the

dichotomy between strictly Andean haplogroup and a mixed haplogroup. The combined d-loop/FGB phylogenetic analysis (likelihood and Bayes) for *P. darwini* showed a similar topology to that obtained with d-loop, recognizing two well supported clusters that combined DNA sequences from the coast and the Andes, with lineage A distributed above 1500 m altitude (Fig. 7).

The results of Geneland analyses recovered two clusters in each species, *A. olivaceus* and *P. darwini* (Fig. 8 and Fig. 9, respectively). Cluster 1 for *A. olivaceus* suggested that Andean localities of Farellones and Campos Ahumada constitute a single population with high probability values as it is shown through the posterior probability isocline (Fig. 8). Cluster 2 for the same species suggested that coastal areas such as Rabuco, La Campana and Villa Alemana belong to a panmictic unit together with La Florida from the Andes. For *Phyllotis darwini*, on the other hand, cluster 1 shows that Andean populations of El Canelo, San Carlos de Apoquindo and Farellones seem to have constituted a single genetic unit along with populations of Rabuco and La Campana in the Coast, despite being currently distributed in disjunction. Whereas for cluster 2 the coastal mountaintop populations of Chicauma, Cantillana and El Roble seem to have formed a single genetic unit with Andean populations of Campos Ahumada and Farellones (Fig. 9). Current disjunct distribution could be attributed to recent fragmentation.

Distribution models

In order to test the assumption that overlapped lineage distribution models may approximate whole species distribution models, we compared our estimations of each species' distribution range at present from i) all trapping localities, considering whole species as single distributional unit (data not shown), and ii) submodels for trapping localities assigned to different intraspecific lineages as independent distributional units (Fig 10, Tables 1 and 2). The results show that whole species' range models are good approximations of the observed distribution range for each species, and also with high model performance (AUC, Table 1). Overlapped lineage distribution models in *P.darwini* performs as well as does the whole species model; in the case of *A. olivaceus*, overlapped lineage models performs even better than the whole species range model. Consequently, both model approaches (whole species and overlapped lineage's ranges) are good and consistent

approximations of current species´ geographic ranges (considering the whole species' range as the portion of the distribution of *A. olivaceus* and *P. darwini* assessed in this work).

Range dynamics

Phillotis darwini

Distribution model at current climatic conditions for this species shows that lineage B has suitable areas across the Andes between 32°S and 34°S, and in some spots of the coastal mountain range within the same latitude; the valley is also suitable for this lineage between 32°S and 33°S. The distribution model for *P. darwini*'s lineage A is surprisingly well defined across the Andes between 27°S and 35°S; this lineage also displays suitable spots across the coastal mountain range between 31°S and 34°S, with high logistic probability values (Fig. 10, red areas). Lineage A has also suitable areas in the points where both, Andean and coastal mountain ranges are very close to each other and the valley becomes narrow.

Both distribution models for lineage B at LGM (CCSM and MIROC, Fig. 10) shows that latitudinal distribution was approximately the same that at present, but high altitude spots at the Andes were absent at those climatic conditions; there is some disagreement between both models: according to the CCSM model, lineage B distribution at the valley and the coastal mountain range was approximately the same that at current conditions. On the other hand, MIROC based distribution model displays a relictual distribution for lineage B during LGM (Fig. 10), restricted to a narrow low altitude fringe at the Andean border, and some isolated populations at coastal mountain range at 32°S and 34°S. On the other hand, differences between current and past distribution models for lineage A in *P. darwini* are far more dramatic: CCSM and MIROC based models shows that the broad Andean distribution observed at present was almost absent during LGM, when this lineage had notoriously expanded its distribution towards the valley and coastal mountain range (northern

distribution limit for the species could have been located at 32°S at LGM, five latitude degrees south from current northern limit).

Abrothrix olivaceus

Distribution model at current climatic conditions for this species shows that lineage B has a broad suitable distribution area between 31°S to 35°S, at the Andes, the valley and the coastal mountain range. Meanwhile, lineage A displays suitable areas at the Andes between 32°S-34°S, and some suitable spots in the coastal mountain range (nevertheless, all individuals assigned to this lineage have been sampled at Andean localities).

Hypothesized distribution at LGM for lineage B is almost identical to its current distribution according to both, CCSM and MIROC based distribution models (Fig. 10). A very similar behavior is observed for the "Andean" lineage A; the only disagreement occurs in MIROC based distribution models, which shows a smallest suitable area for this lineage at LGM, but with very similar latitudinal and altitudinal distribution. According to this model, it is possible that *A. olivaceus*'s lineage A may have been restricted to a narrow low altitude fringe at the Andean border.

Consequently, the distribution of both lineages in *A. olivaceus* has remained relatively constant since LGM to present, with probably small postglacial expansions towards the Andes; it is possible that lineage A have had remained distributed exclusivey in Andean localities. On the other hand, *P. darwini*'s lineage A has notouriously expanded its distribution northwards through the Andean mountain range, and to suitable areas in the valley, and in the coastal mountain range has contracted its distribution since LGM until present day, leaving just some isolated populations at the top of the Coastal Cordillera.

DISCUSSION

Our results exhibited an evident split of haplogroups for both species of sigmodontines of the Andes and the Coastal Cordillera in the study area. For *Abrothrix olivaceus* we observed that one of the haplogroups is strictly restricted to Andean localities (lineage A), while the other is distributed in both mountain ranges, and also in some valley and coastal localities (lineage B). This was not the case for the other studied species *Phyllotis darwini*, for which we recovered two haplogroups, both of them distributed in the Andes and in the Coastal mountain ranges. Nevertheless, the geographical segregation of haplogroups within the latter species is clear: one haplogroup is distributed in the valley and both mountain ranges (lineage B), whereas the other (lineage A) is strictly restricted to localities above 1500 m altitude, across both mountain ranges but with a broad latitudinal extension across the Andean mountain range.

The first cluster obtained for *A. olivaceus* with Geneland analysis grouped sequences from the Andean populations of Campos Ahumada and Farellones, whereas the second cluster obtained for this species joined sequences from the Andes, central valley and coastal localities; that is to say, lineages inside *A. olivaceus* are currently panmictic units. For *P. darwini* on the other hand, cluster one grouped localities from the Andes and the Coast, pattern that was also recovered for cluster 2 that joined Andean populations of Farellones and Campus Ahumada with those of the El Roble, Cantillana and Chicauma at the Coast. This strongly disjunct distribution of populations suggest that those lineages were panmictic units which have been recently fragmented, but probably still keeps some degree of genetic flux.

We suggest that the biogeographic mechanism that may have triggered the dynamic range shift in the recent evolutionary history of both *P. darwini* and *A. olivaceus* was the downwards displacement of the Andean vegetational belts towards the valley, as a consequence of the 7° C drop of temperature driven by the ice advance throughout the Andes of central Chile in the LGM (Clapperton, 1990; 1994). Our results thus suggest that mountain populations of *P. darwini* and *A. olivaceus* moved between both cordilleras, and that these movements might have been triggered by the glaciation events that affected the Andes during the glaciation cycles of the Pleistocene. Glaciations may have allowed Andean populations to be refuged at low altitudes in the Andes mountains (e.g., *A. olivaceus*' lineage A at San Carlos de Apoquindo) and/or in areas free of ice in

the Coastal Cordillera. But what evidence do we have of these movements and when these might have occurred since during the Pleistocene several glaciation cycles have been reported?.

Niche modeling analyses for both species suggest that there has been lineages with persistant distribution at valley and coastal mountain ranges wich have slightly expanded its distribution towards Andean mountain ranges after glacial retreat (*P. darwini*'s lineage B and *A. olivaceus*'s lineage B), whereas other lineages have dramatically expanded their range exclusively across mountain ranges (*P. darwini*'s lineage A). The only lineage which apparently has not move between mountain ranges since LGM to present is *A. olivaceu*s's lineage A, which is also the only intraspecific lineage without mixed haplotypes between coastal and Andean mountain ranges; in addition is currently a panmictic unit.

The fact that *A. olivaceus* displays an strictly Andean haplogroup, and another lineage with broad distribution in both mountain ranges, suggests that the mixed haplogroup is the result of range displacements between mountain ranges, while the Andean haplogroup appears to have remained restricted to the Andes, even with relictual distribution during glacial cycles (as is suggested by its hypothesized distribution during LGM according to MIROC based distribution model). On the other hand, both linneages inside *P. darwini* are distributed at both mountain ranges (one of them strictly restricted to elevations above 1500 m, with disjunct distribution along both cordilleras). In adittion, lineages in this species displayed the largest distributional shifts from LGM until current conditions, with a high altitude lineage which has dramatically expanded its distribution across mountain ranges. Therefore, we hypothesize that *P. darwini*'s current distribution has been determinated by at least the last glacial cycle (differential postglacial colonization for each intraspecific lineage), and the origin of its lineages is probably related to ancient range shifts across mountain ranges.

Why do we have different biogeographic patterns on the mountaintops for the two species of sigmodontine rodents that coexist in central Chile?. Our results showed that *Phyllotis darwini* shares more haplotypes between both mountain systems compared with the other studied species *A. olivaceus*, which possess one lineage that remained in the Andes, even during LGM. In general, the former species exhibited higher genetic variability as expressed in the number of polymorphic sites and haplotype numbers if compared to *A. olivaceus*. In fact, *Phyllotis darwini* characterizes for being one of the most ubiquitous in the semiarid and arid regions of northern and central Chile and appears to be capable of seasonally adjusting its resistance to desiccation utilizing seeds and

succulents (Meserve & Glanz, 1978; Meserve & Le Boulengé, 1987). In contrast, *A. olivaceus* characterizes for preferring habitats with less shrub and greater herbaceous cover (Meserve, 1981). A phylogeographic study on *A. olivaceous* recovered a structured pattern, suggesting local adaptation of populations along their range from semiarid, to Mediterranean, to forest environments in the southern part of the country (Rodríguez-Serrano *et al*., 2006).

Thus, our hypothesis to explain current patterns on mountaintop populations for both species of sigmodontine mice in central Chile would rest on historical events as the LGM (and probably former glacial/interglacial transitions) that would have triggered the descent of populations from the Andes to lower elevations and refuge areas in Coastal Cordillera as suggested by our phylogeographic analyses. Further movements of populations backwards after glacial retreats may have followed, leaving, in some cases, population isolates on the mountaintops of the Coastal Cordillera. These "mountain island isolates" occurring mainly at the valley and coastal cordillera during LGM, may have recolonized the Andean mountains range after glacial retreat, explaining the current pattern of haplotype admixture but current disjunct distribution across both mountain ranges. The fact that the only lineage (*A. olivaceous* A) which remained associated to a single mountain range through the last glacial/interglacial transition is the only one which lacks of haplotype admixture between mountain ranges, strongly agreed with this hypothesis.

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Figure Captions.

Figure 1. Map showing the localities sampled in central Chile, from the coast, central valley and Andean areas.

Figure 2. Bayesian and Maximum likelihood tree based on d-loop sequences, representing the intraspecific relationships of *Abrothrix olivaceus* from central Chile mountaintop and lowland areas. Numbers on the nodes represent the posterior probability and 1000 bootstrap support values.

Figure 3. Bayesian and Maximum likelihood tree based on d-loop sequences, representing the intraspecific relationships of *Phyllotis darwini* from central Chile mountaintop and lowland areas. Numbers on the nodes represent the posterior probability and 1000 bootstrap support values.

Figure 4. Neighbornet of d-loop sequences of *Abrothrix olivaceus* haplotypes. Labels for haplotypes represent the following zones: red: Andean areas, blue: coastal areas and green lowland areas.

Figure 5. Neighbornet of d-loop sequences of *Phyllotis darwini* haplotypes. Labels for haplotypes represent the following zones: red: Andean areas, blue: coastal areas and green lowland areas.

Figure 6. Bayesian and Maximum likelihood tree of the concatenated d-loop and FGB sequences, representing the intraspecific relationships of *Abrothrix olivaceus* from central Chile mountaintop and lowland areas. Numbers on the nodes represent the posterior probability and 1000 bootstrap support values.

Figure 7. Bayesian and Maximum likelihood tree of the concatenated d-loop and FGB sequences, representing the intraspecific relationships of *Phyllotis darwini* from central Chile mountaintop and lowland areas. Numbers on the nodes represent the posterior probability and 1000 bootstrap support values.

Figure 8. GENELAND analyses with posterior probability isoclines denoting the extent of genetic landscapes for the two clusters recovered in *Abrothrix olivaceus*. Coastal and Andean mountaintops are recovered in the figure. To facilitate interpretation, GENELAND output has been cropped, re-scaled and superimposed over the map of central Chile where this study was conducted for *A. olivaceus*. Black dots represent localities analyzed in this study. Regions with the greatest probability of inclusion are indicated by white, whereas diminishing probabilities of inclusion are proportional to the degree of coloring.

Figure 9. GENELAND analyses with posterior probability isoclines denoting the extent of genetic landscapes for the two clusters recovered in *Phyllotis darwini*. Coastal and Andean mountaintops are recovered in the figure. To facilitate interpretation, GENELAND output has been cropped, re-scaled and superimposed over the map of central Chile where this study was conducted for *P. darwini*. Black dots represent localities analyzed in this study. Regions with the greatest probability of inclusion are indicated by white, whereas diminishing probabilities of inclusion are proportional to the degree of coloring.

Figure 10. Lineage distribution models. The figure shows the species distribution models for present and two LGM climatic models (rows). Models were built independently for *P. darwini* and *A. olivaceus* intraspecific lineages (columns). Yellow represents suitability areas for lineages according to the maximum training sensitivity plus specificity logistic threshold; red areas represent suitability areas according to an arbitrary restrictive threshold, defined as the 50% highest value observed between the maximum training sensitivity plus specificity logistic threshold, and the maximum logistic probability value for each model.

Table legends.

Table 1. AUC average values for each distribution model.

Table 2. General description of hypothesized distribution with latitudinal extension and orographic characteristics in suitable areas. Rows are intraspecific lineages and files represent climatic models for current conditions (current) and LGM conditions (CCSM and MIROC). The last row indicates if a lineage has an stable distribution since LGM until present day, or its distribution range has changed.

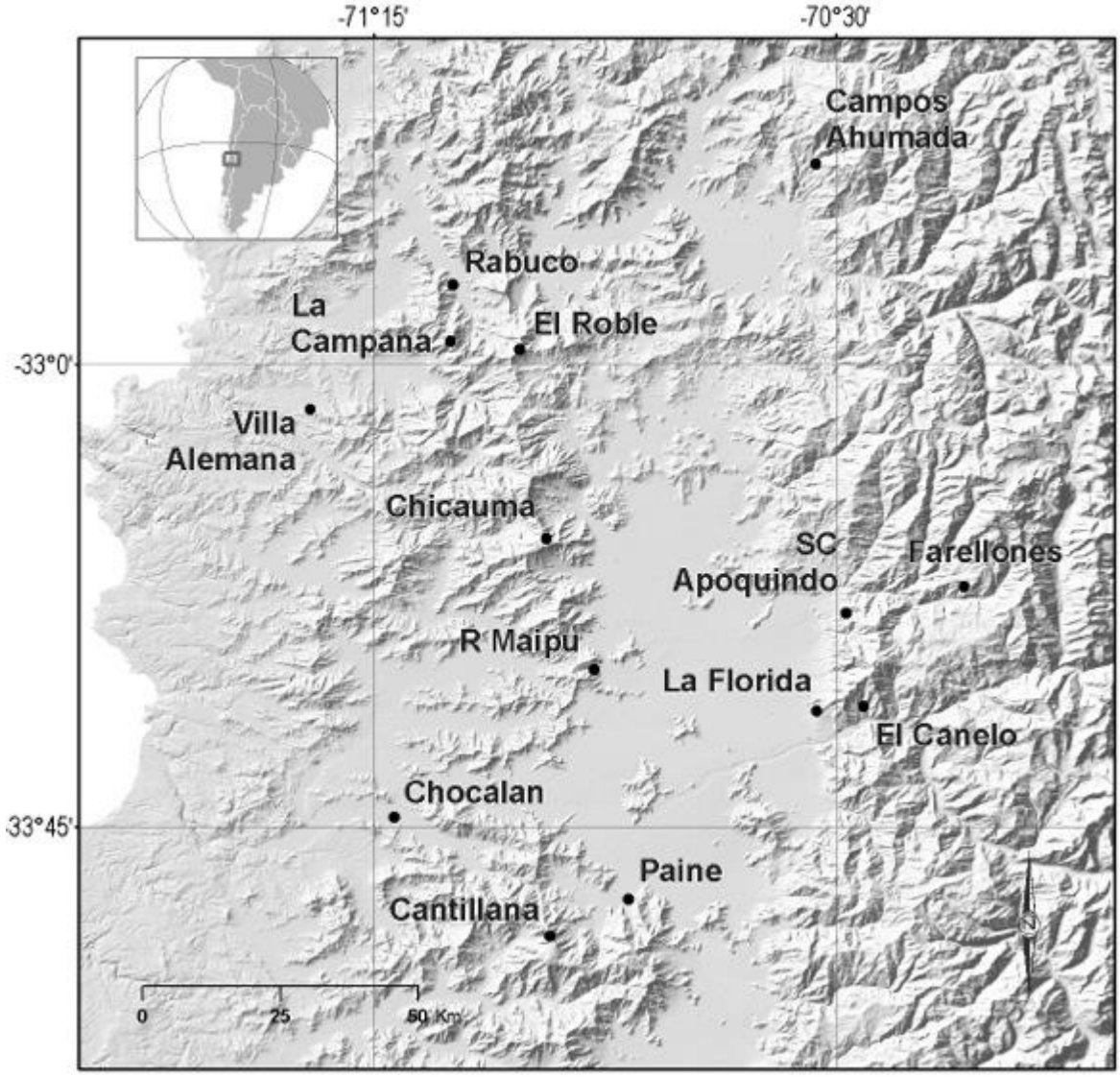
Table 1

MaxEnt Model	AUC Average	AUC stdv.
Phyllotis darwini whole specie´s range	0.971	0.022
Phyllotis darwini A	0.970	0.050
Phyllotis darwini B	0.969	0.023
Abrothrix olivaceus whole specie´s range	0.875	0.046
Abrothrix olivaceus A	0.916	0.028
Abrothrix olivaceus B	0.916	0.086

Table 2.

	Phyllotis A	**Phyllotis B**	**Abrothrix A**	**Abrothrix B**
Present	28°S-35°S (Mainly at Andes; some populations at coastal cordillera between 32°S-34°S)	**32°S-34°S** (Andes and discontinuous distribution at valley and coastal mountain range)	32°S to 34°S (Mainly at Andes; some suitable spots at the coastal mountain range)	**31°S-35°S** (Andes, valley and coastal mountain range)
CCSM	32°S-34°S (Valley, Andes and coastal mountain ranges)	**32°S-34°S** (Andes and discontinuous distribution at valley and coastal mountain range)	32°S - 34°S (Andes, valley and coastal mountain range. Continual distribution)	**31°S-35°S** (Andes, valley and coastal mountain range)
MIROC	32°S-34°S (Valley, Andes and coastal mountain ranges)	**32°S-34°S** (Andes and discontinuous distribution at valley and coastal mountain range)	32°S - 34°S (Isolated suitable spots, mainly in low altitude localities at Andes)	**32°S-34°S** (Andes, valley and coastal mountain range)
	Range Dynamics	Range Stability	Range Dynamics	Range Stability

Figure 1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Rabuco
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El Roble
Villa Alemana
Chicauma
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R Maipu
La Florida
El Canelo
Chocalan
Paine
Cantillana
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25
50 Km

Figure 2

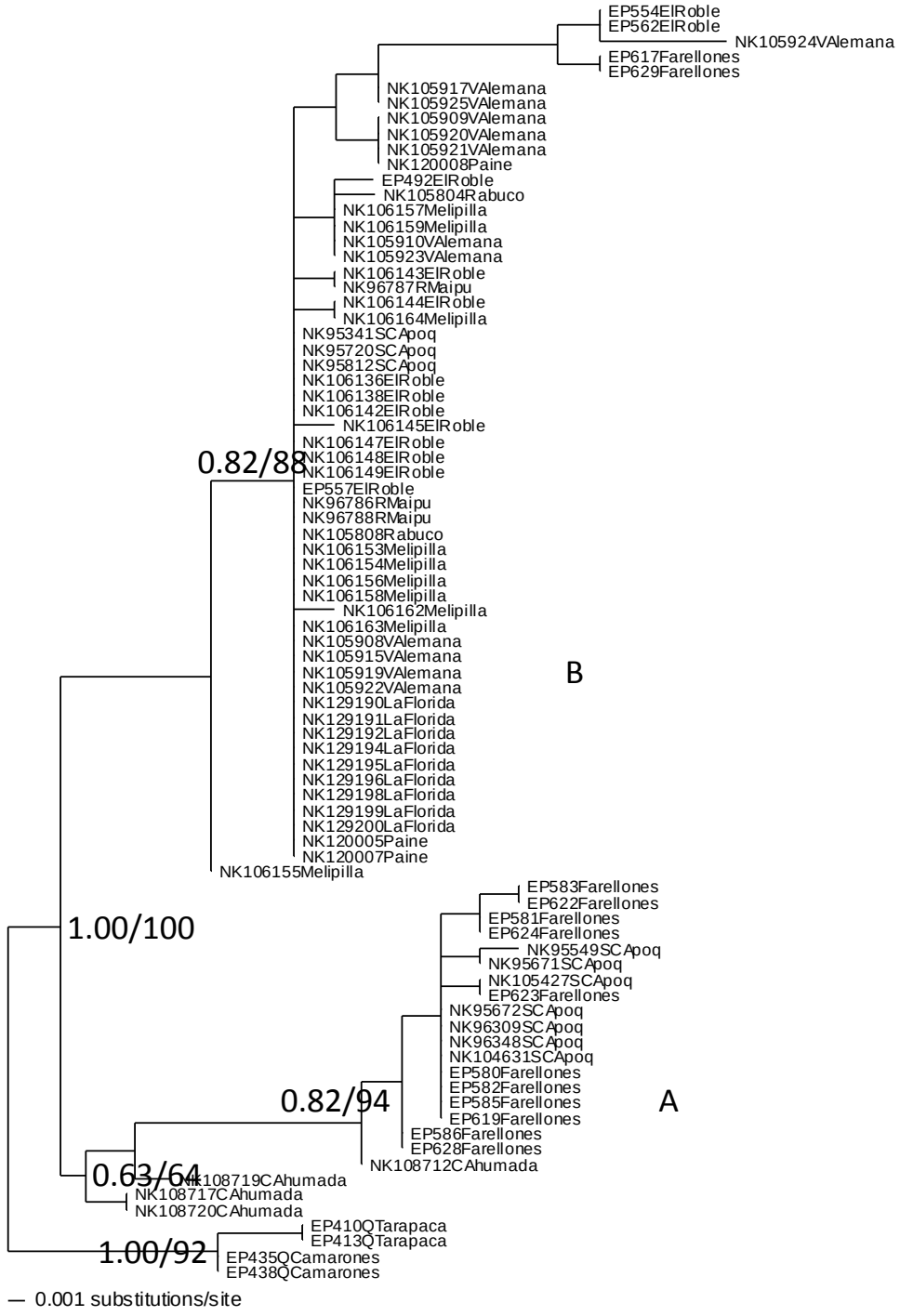
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Figure 3

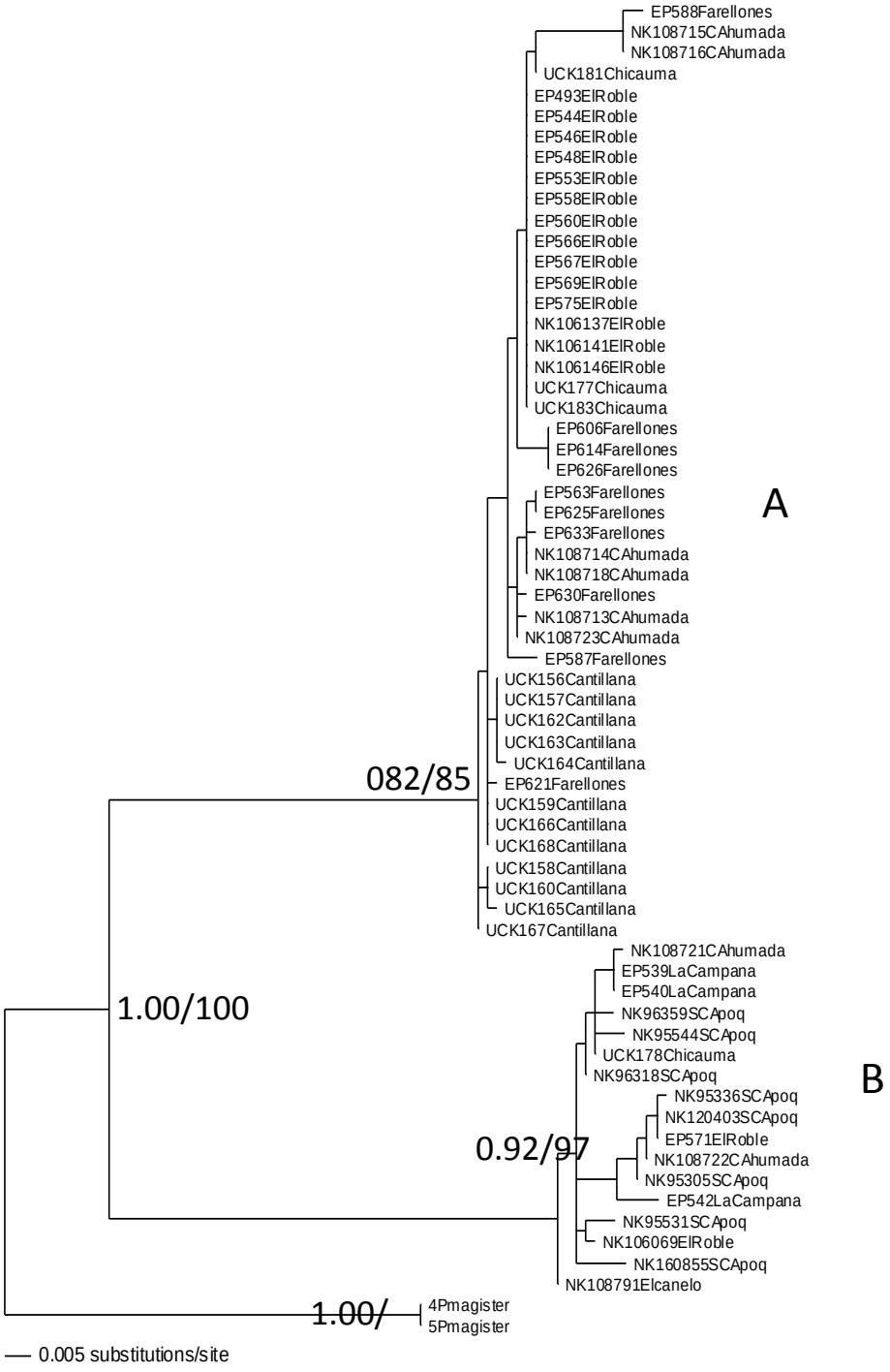
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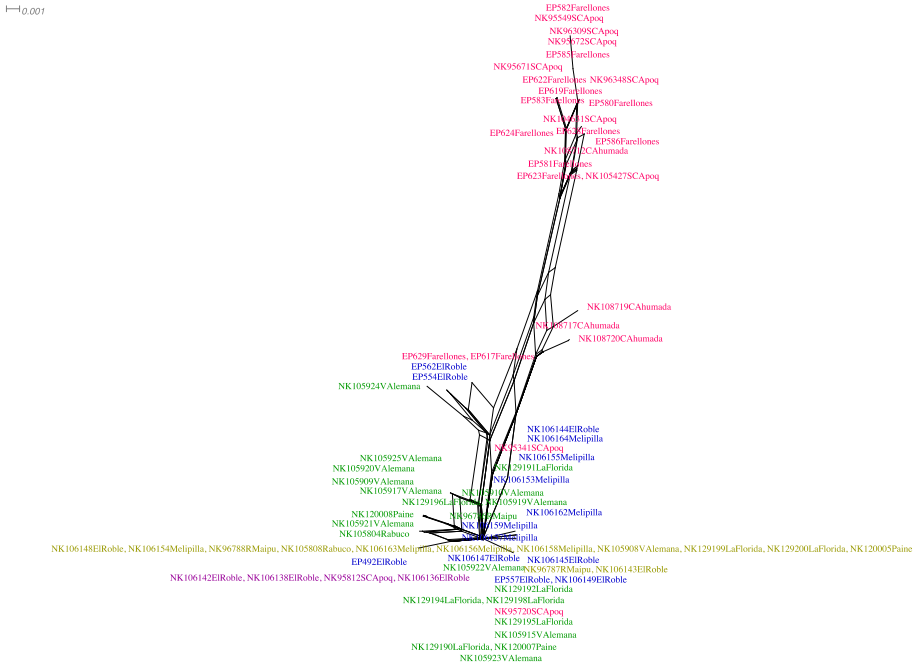
Figure 4



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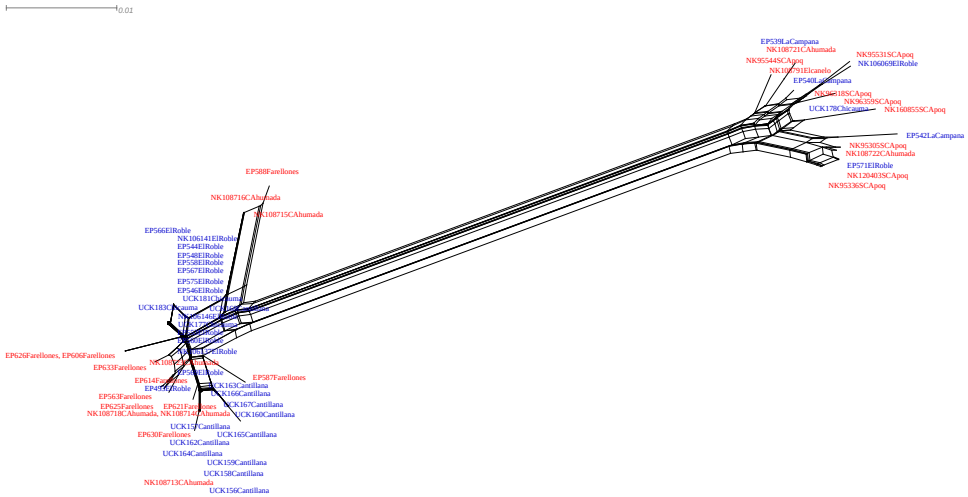
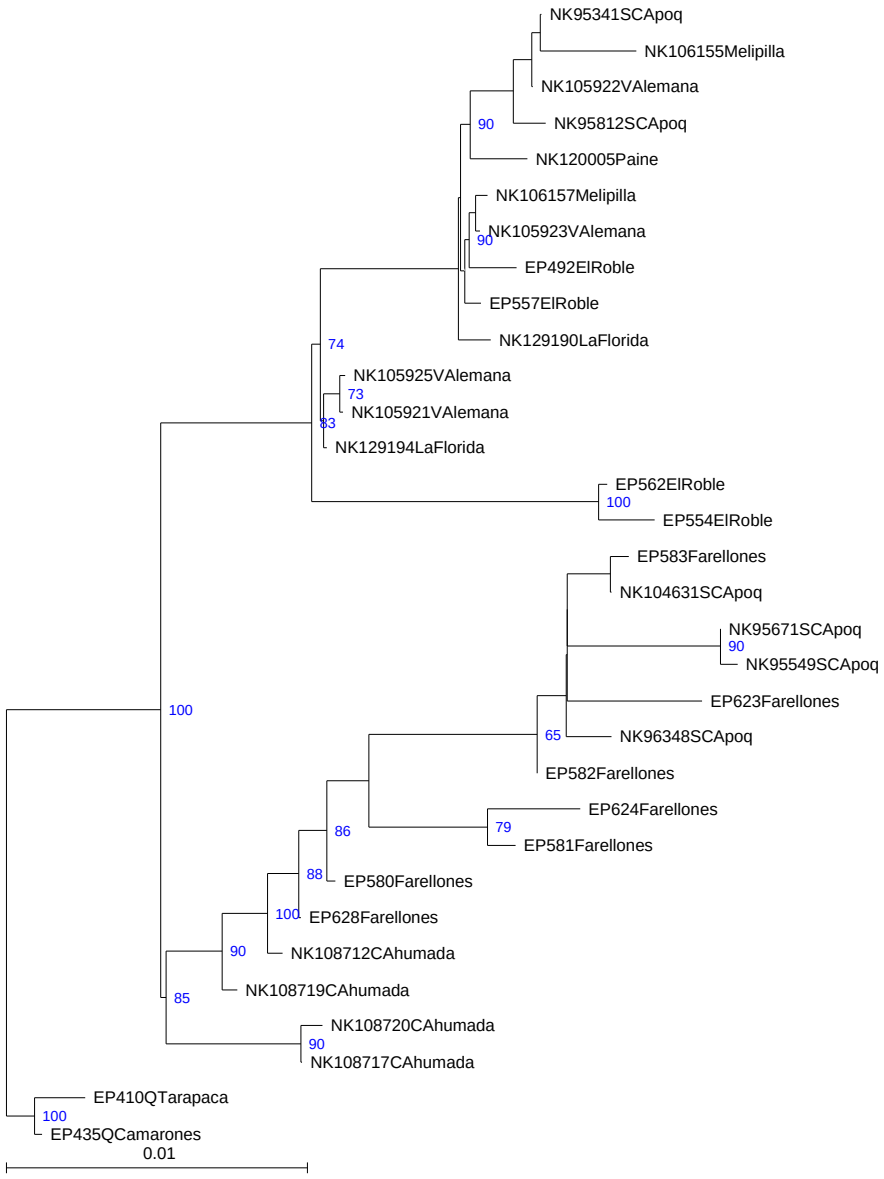


Figure 6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NK105921VAlemana
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100
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EP583Farellones
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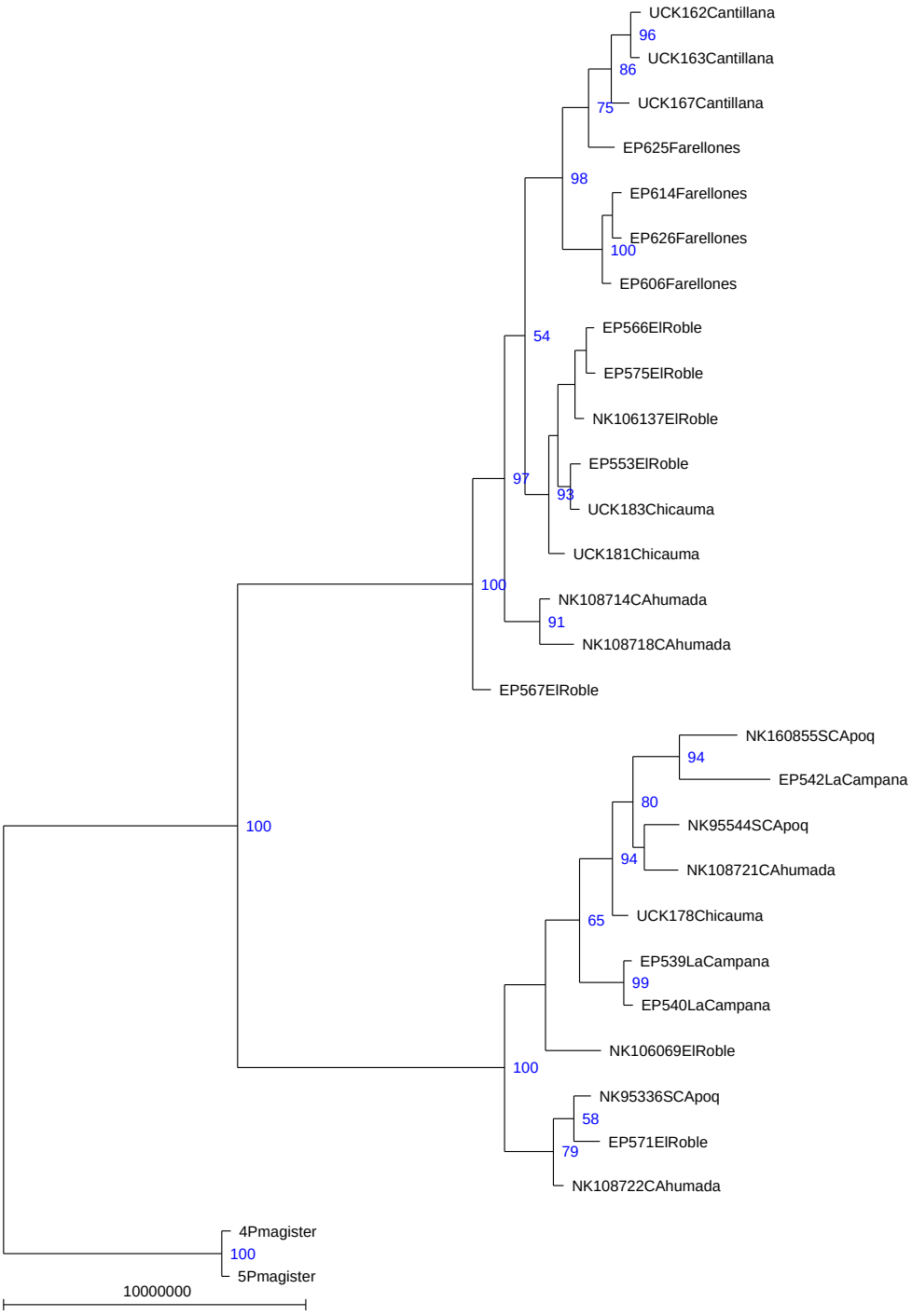
Figure 7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5Pmagister
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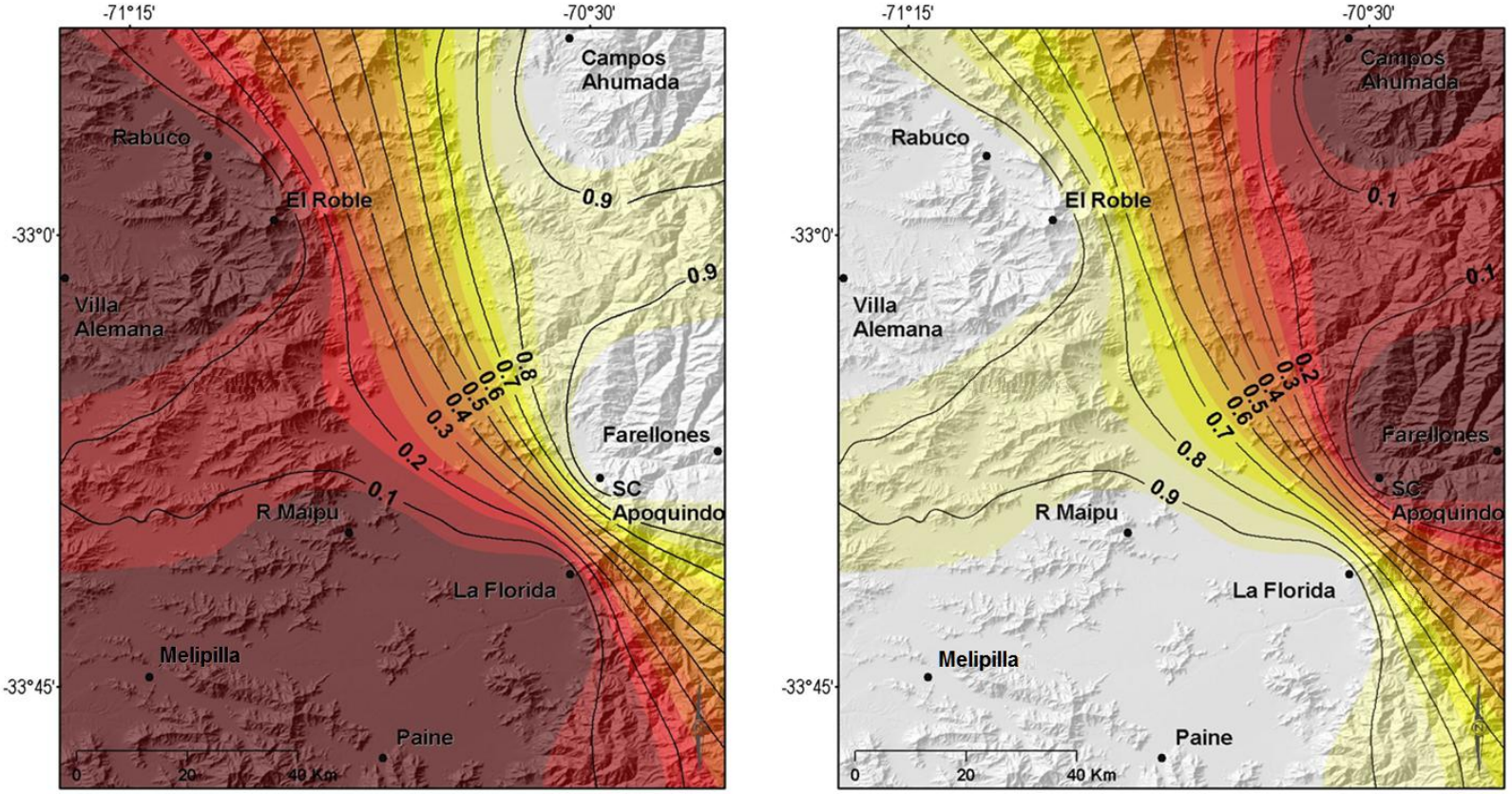
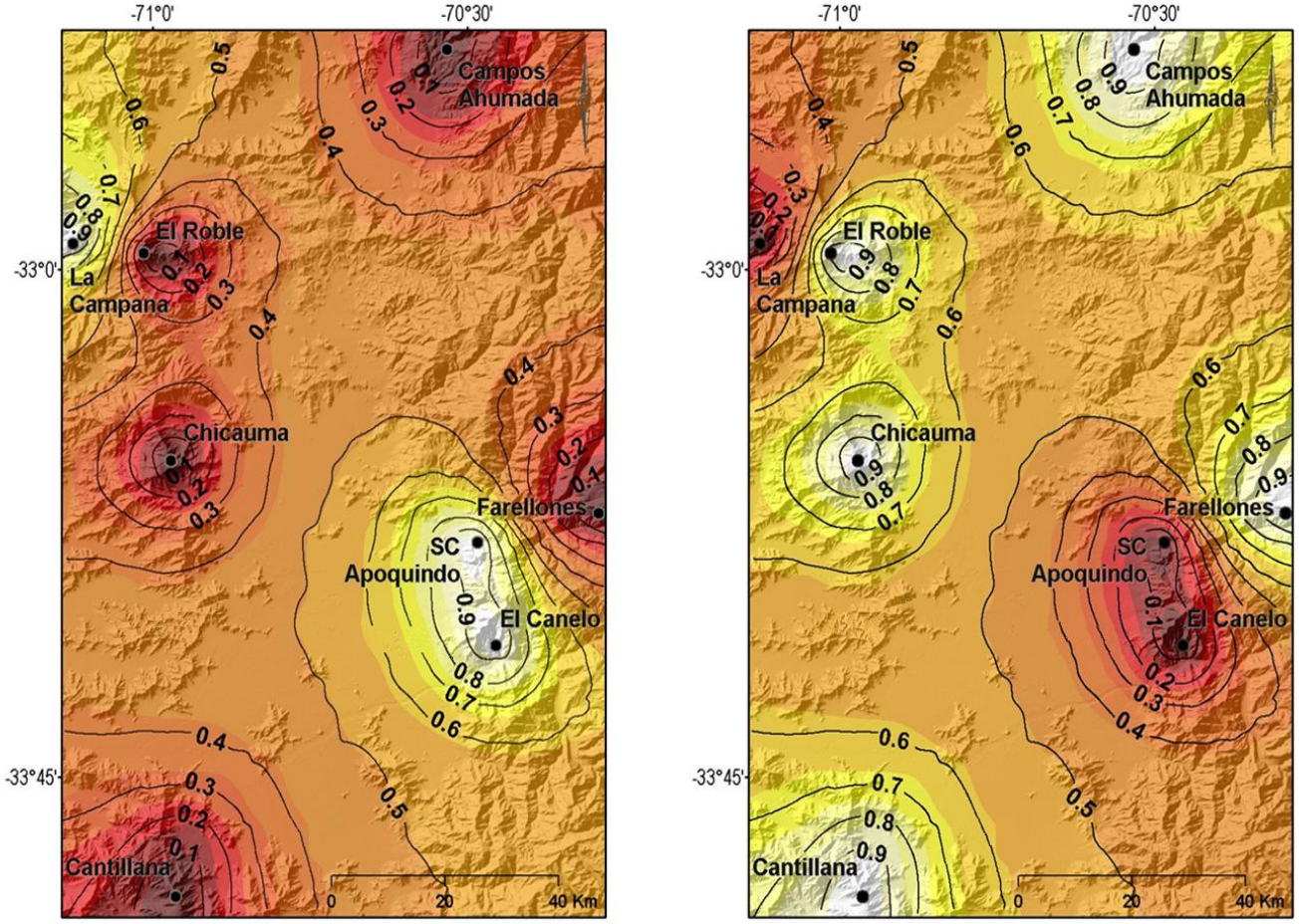
Figure 8



Cluster 1

Cluster 2

Figura 9



Cluster 1

Cluster 2

Figure 10

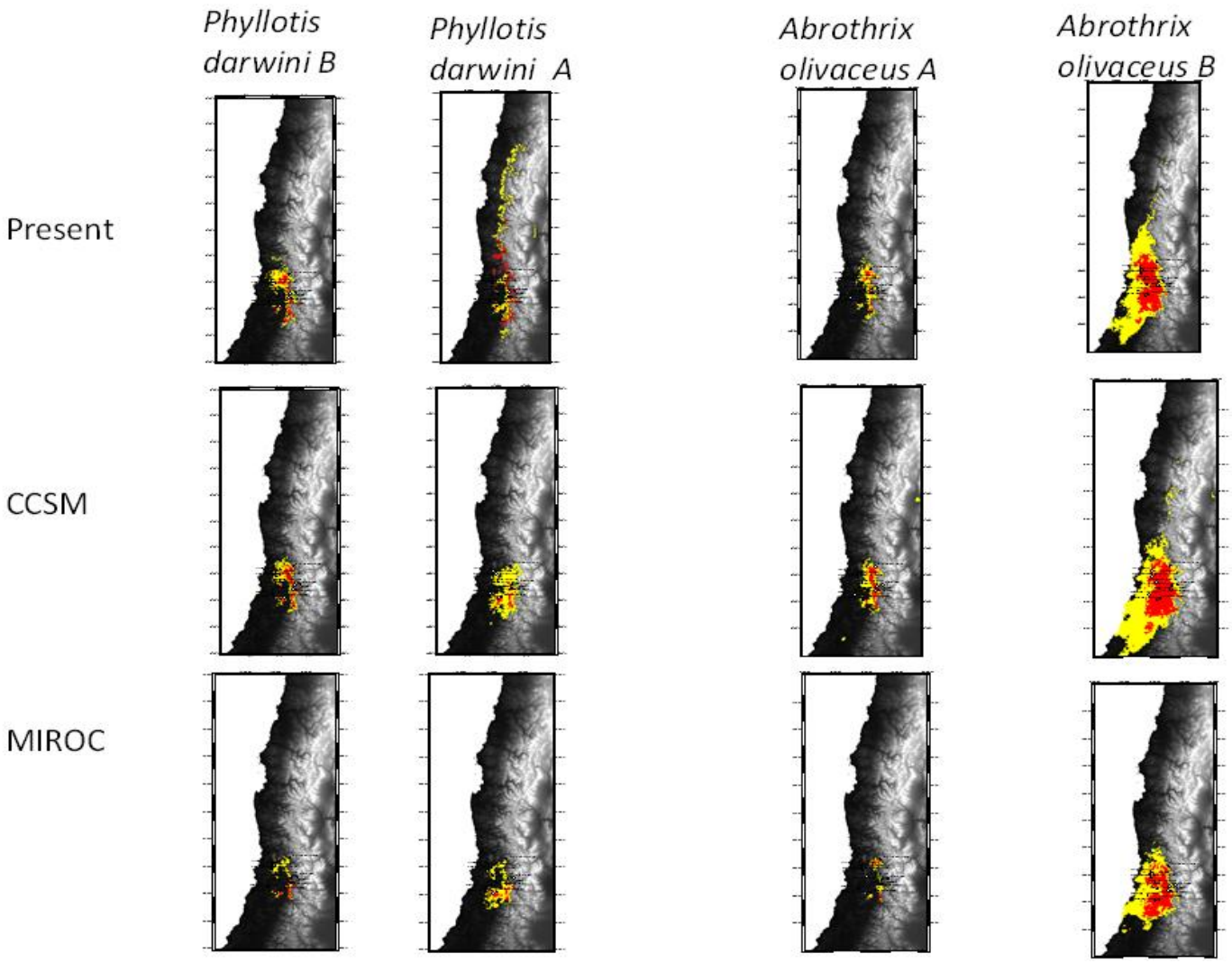
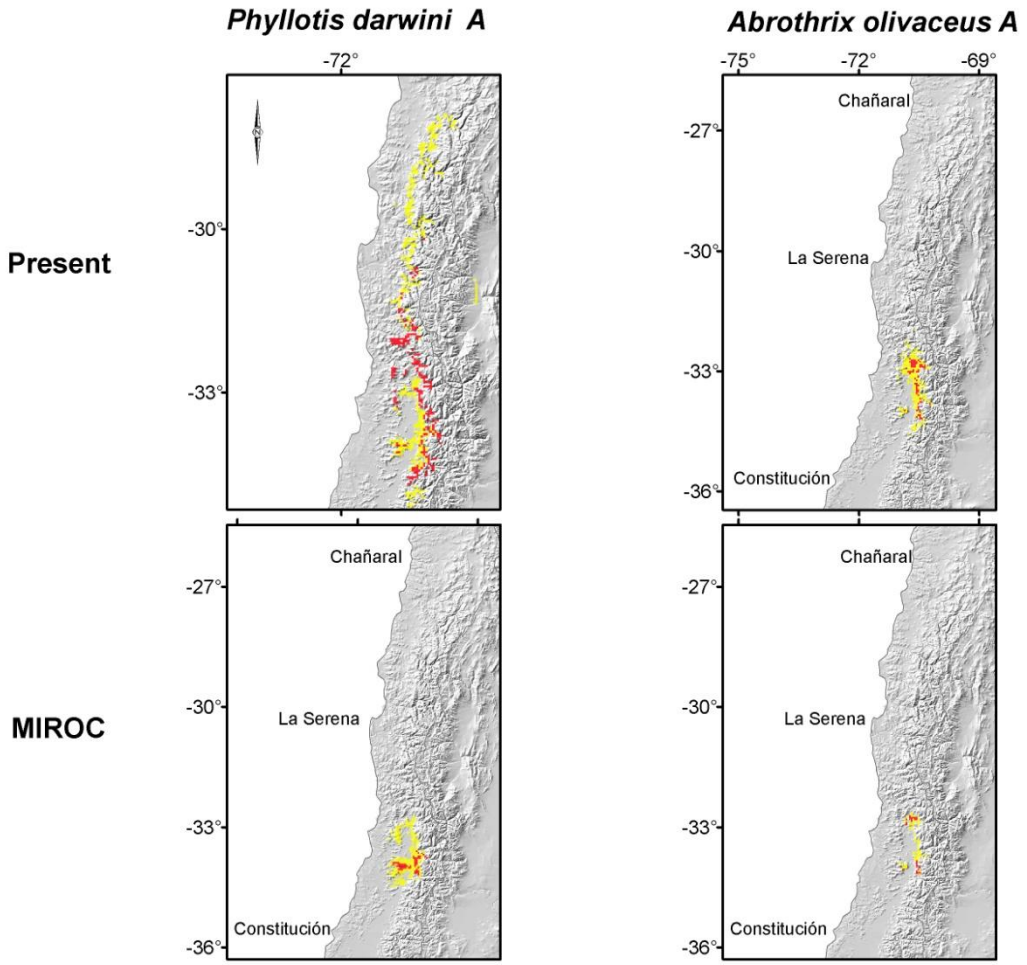

Phyllotis darwini B
Phyllotis darwini A
Abrothrix olivaceus A
Abrothrix olivaceus B
Present
CCSM
MIROC

Fig 10.1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Abrothrix olivaceus A
Present
MIROC
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-75°
-69°
-27°
-30°
-33°
-36°
Chañaral
La Serena
Constitución

Comparative phylogeography of vertebrates in the hotspot of central Chile: influence of the Last Glacial Maximum on the distribution of intraspecific lineages.

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Abstract

Distributional responses to climate change after the Last Glacial Maximum (LGM) , in currently endangered areas as biodiversity hotspots, are an appropriate model to search for mechanisms involved in the current threat of cryptic biodiversity loss in a global change scenario. In this work, we investigated the past distributional responses of four vertebrate species, endemic to the central Chile hotspot. We combined current and LGM distribution models with phylogenetic information at the intraspecific level to test alternative biogeographic scenarios. Our main conclusion is that lineages inside species have had independent distributional responses often associated with differential recolonization of mountain ranges and lowlands, and also stable and dynamic distribution for lineages within the same species. We have also identified an area around 33°S and 36°S which have hypothetically behaved as a refuge for cryptic biodiversity through several glacial/interglacial transitions. In current interglacial conditions, mountain ranges at central Chile are important biological corridors which may be considered a conservation priority,

while coastal cordillera around 33- 36°S is an important cryptic biodiversity reservoir through major climatic oscillations.

Introduction

It is well known that climate regime may affect species distributions and therefore future climate change could potentially induce geographical range dynamics such as contraction, expansion or geographical range shifts (Walther *et al.*, 2002; Pearson & Dawson, 2003; Summers *et al.*, 2012). It is also agreed that the climatic impact on species distribution can be extrapolated in community and ecosystem shifts (Walther *et al.*, 2002; Hamann & Wang, 2006). For these reasons, the species level might be critical for conservation issues in a global climate change scenario (GCC).

At the species level, climate change is expected to affect the geographical range mainly through physiological restrictions as temperature and precipitation tolerances in conjunction with species dispersal abilities (Walther *et al.*, 2002). Altogether, those factors may determine species ability to keep up with climate change. From an ecological perspective, expected species responses to climate change are i) to tolerate or spread in the new climatic scenario (either by physiological tolerance or phenotypic plasticity) ii) to change its distribution in order to catch up the new climate regime iii) to go extinct (Pettorelli, 2012). From an evolutionary perspective, range shifts may change the distribution of genetic diversity and range contractions will most likely reduce genetic diversity (Alsos *et al.*, 2012; Pauls *et al.*, 2012). Nevertheless, the emphasis on the role of evolution in species responses to climate change has been usually focused on evolutionary

adaptations and the relationship between species' adaptation speed and climatic change rate (Hoffmann & Sgrò, 2011).

It has been recently emphasized about the need to consider GCC effects on biodiversity below the species level, other than the usually advocated adaption potential and phenotypical plasticity issues. There exists a severe lack of studies on GCC effects on biodiversity, at the level of intraspecific genetic diversity. This is highly evident if we compare the vast amount of publications at the ecosystem, community and species levels, with approaches below species level (Fraser & Bernatchez, 2001) despite the general agreement on the importance of Evolutionary Significant Units (ESUs) for conservation planning. On this line of arguments, (Bálint *et al.*, 2011) demonstrated that species with strong population genetic structure will face massive losses of diversity at the intraspecific level in a GCC scenario. That is to say morphospecies based estimation on GCC impacts on biodiversity will severely underestimate cryptic diversity loss, therefore conservation strategies based solely on above species organization levels might be an oversimplified approach.

For the above reasons, it might be critical for future conservation planning not just to quantify the amount of cryptic diversity at risk (i.e. local genetic variants with adaptative potential, fitness related variability, or intraspecific lineages with a characteristic distribution), but to also understand what could be the ecological and evolutionary responses of ESUs below the species level to GCC. There exist a substantial number of publications trying to understand the relationship between species' evolutionary and ecological responses to climate change, mainly through phylogeographic information and species distribution models (SDMs) (Carstens & Richards, 2007; Waltari *et al.*, 2007;

Kozak *et al.*, 2008; Provan & Bennett, 2008; Cordellier & Pfenninger, 2009; Marske *et al.*, 2009; Waltari & Guralnick, 2009; Buckley *et al.*, 2010; Lim *et al.*, 2010; Allal *et al.*, 2011; Eckert, 2011; Gugger *et al.*, 2011; Marske *et al.*, 2011; Svenning *et al.*, 2011; Marske *et al.*, 2012; Qi *et al.*, 2012). However, approaches using intraspecific phylogenetic information to build lineage distribution models (lineages below species level) are less frequent. Even though this approach has been used with success in systematics for species delimitation and cryptic speciation issues (Raxworthy *et al.*, 2007; Rissler & Apodaca, 2007; Engelbrecht *et al.*, 2011; du Toit *et al.*, 2012; Florio *et al.*, 2012), the need to discern lineage-specific ecological responses to GCC at the intraspecific level (and its potential applicability in conservation strategies, as a measure of distributional shifts experimented by cryptic phylogenetic lineages) is a relatively new issue in the scientific literature. Several efforts have been recently made on this concern: by using SDMs and genetic diversity measures Schorr *et al.*, (2012) concluded that this integrated approach may change the distributional history inferred from phylogeographic information at the species level. Studying the relationship between lineage formation and variation in the ecological niche in the *Peromyscus maniculatus* species group Kalkvik *et al.*, (2011) demonstrated that the majority of genetic lineages within species do occupy distinct environmental niches. In another integrated approach using SDMs and phylogeography Fontanella *et al.*, (2012) concluded that two main haploclades in the lizard *Liolaemus petrophilus* shared different distributional responses to climate change during the Pleistocene. Those findings suggested that geographical range shifts triggered by climatic shifts might be complex if the species share phylogenetic structure, and some degree of intraspecific niche divergence related to cryptic phylogenetic lineages. Therefore, realistic predictions of range shifts for future climate change scenarios should consider phylogenetic information to perform lineage-

specific distribution models, because species might not necessarily respond as a simple ecological unit to future GCC.

Among the world biodiversity hotspots, the African "Cape Floristic Region" (CFR) is one of the better known with respect to the evolutionary origin of its biota (Verboom *et al.*, 2009a). In fact, a phylogeographic study in the genus of dwarf chameleons *Bradypodium* (Fitzinger, 1843) suggested that there was a concerted phylogeographic pattern between species of this genus, and between these and two other endemic lizards associated to the emergency of the Cape Flora (Tolley *et al.*, 2006; Tolley *et al.*, 2009). At he same time, the Cape Flora may display a complex diversification history with ancient and recent speciation events, probably caused by a combination of climatically induced fragmentation and adaptative radiation (Verboom *et al.*, 2009b). On the other hand, a comparison of the phylogeographic pattern conducted in six taxonomic groups, endemic to the "California Floristic Province" hotspot, concluded that genetic structure of these taxa show major splits highly consistent across most taxa (Calsbeek *et al.*, 2003). The latter authors concluded that diversification can be spatially and temporally explained by the climatic and geographic history of the California hotspot. Finally, in the "Brazilian Atlantic Forest" hotspot, Carnaval *et al.*, (2009) demonstrated that climatic stability in Pleistocene refugia is a good predictor of the current genetic diversity within this hotspot. Altogether those evidences suggest that biodiversity hotspots may be appropriate systems to study the relationship between climate dynamics and genetic differentiation in currently endangered areas.

Within the former scenario, we have chosen four vertebrate species (see below) endemic to the biodiversity hotspot of central Chile focused 1) to evaluate how vertebrate species have responded to climate change in a currently endangered area (there is a strong

need of that information for future conservation planning); 2) to evaluate the genetic structure of species in the Chilean hotspot, since this feature may be characteristic of the region due to its particular evolutionary and geo-climatic history. To those goals we reconstructed intraspecific phylogenies for each taxa to characterize cryptic lineages, and we built lineage distribution models at present and paleodistribution models at LGM to assess the existence of lineage-specific distributional responses to climate change, by using a climatic niche approach. In addition, we integrated phylogenetic information with lineage distribution models into alternative biogeographic scenarios, and we tested the alternative hypothetical scenarios in a comparative phylogeographic framework. For this purpose, simulations of the expected distribution of the number of deep coalescens were implemented. Our main objective is to characterize the distributional responses of species and cryptic intraspecific lineages to climate change after LGM, in order to understand how cryptic diversity may affect our criteria for delimiting Evolutionary Significative Units (ESUs) for conservation planning, in an endangered area as a biodiversity hotspot.

To evaluate the above proposed goals we have chosen four vertebrate species endemic to the biodiversity hotspot of central Chile. The criteria used to choose the appropriate vertebrate taxa to test the hypothesis in the central Chile hotspot were i) taxa should be endemic to the region of interest ii) the set should represent a broad spectrum of terrestrial vertebrate taxa. Accordingly, the selected taxa were: 1) the sigmodontine rodent *Phyllotis darwini* 2) the didelphid marsupial *Thylamys elegans* 3) the iguanid lizard *Liolaemus monticola* 4) and the anuran toad *Rhinella arunco*.

Methods

Study area and main distributional hypothesis Fifty percent of all endemic species of vertebrates in Chile occur in an area that does not exceed 16% of its land area (Simonetti 1999): This region has been identified as the globally significant 'biodiversity hotspot of central Chile' (Myers et al. 2000). Understanding the processes responsible for this biodiversity pattern is an important concern in the context of global change. The central Chile Sub-region extends between 28°S to 36°S (Morrone 2006), which approximately matches Myers (2000) definition. In this work, we chose vertebrate species endemic to the hotspot to assess the effects of GCC after one glacial maximum on the geographic distribution of lineages, in order to construct a potentially more general model of intraspecific responses to climate change in a hotspot area. In addition, this information will be very valuable in order to understand what should be the relevant implications of considering cryptic diversity in conservation strategies planning, compared to approaches based only in species or ecosystem level, and therefore to improve conservation strategies by using more realistic assumptions on distributional responses to GCC. We expect that potential cryptic lineages inside vertebrate species might be associated to the altitudinal gradient given by the Andes, the valleys, and the Coastal Cordillera. The latter topography constitutes the most important geographic features in the central Chile hotspot. We also expect that the specie's geographic range history may display signatures of one of the most important geo-climatic events in central Chile: the Last Glacial Maximum (LGM). This event was chosen to test the hypothesys because in addition to being one of the greatest episodes of recent climate change (represents the opposite climatic extreme from the current interglacial period) it is known that Quaternary glaciations in general have been one

of the most important factors in determining the current genetic structure of many populations, species and communities (Hewitt, 2000). Even though central Chile was not extensively covered by ice sheets at LGM, the glacial advanced northward through the Cordillera de los Andes (Clapperton, 1990; Clapperton, 1994) descending to about 1,100 m with a subsequent drop in the temperature and an increased rainfall in the whole region (Heusser, 1983; Heusser, 1990; Lamy *et al.*, 1999). The palinological evidence has demonstrated that high Andean vegetational belt shifts downwards the valley during the glacial advance, and then move upwards, reaching its original high altitude distribution after glacial retreat. This vegetational shift may have given rise to the existing biogeographical insulas of Andean vegetation in both, the Andes and the Coastal cordilleras of central Chile (Darwin, 1859; Simpson, 1983; Heusser, 1990; Villagrán & Armesto, 1991; Villagrán & Hinojosa, 2005). In addition, it has been suggested that lizards and rodent's relative species diversity in central Chile is explained by different speciation modes in both groups, as a result of differential interaction between mountain geography, Quaternary glaciations, and ecological features in both groups (Fuentes & Jaksic, 1979). We have previous evidence which suggests that the rodent *Phyllotis darwini* does not behave as a simple distributional unit to climate change after LGM, but is composed of two cryptic lineages which have expanded its range following an strict altitudinal segregation (valley and mountain ranges) after glacial retreat, and it's phylogeographic structure pattern accommodates more to the "lizard speciation mode" rather than the "rodent speciation mode" postulated by Fuentes & Jaksic 1979 (Gutiérrez-Tapia & R.E. Palma, in prep.). Those findings had lead us to hypothesize that species with intraspecific phylogenetic structure may not behave as distributional units to climate change, but as a mosaic of independent distributional responses by each cryptic lineage.

Specimens and molecular markers All specimens and sequences used in this paper come from previous work in molecular phylogeography of the central Chile hotspot, part of previous studies of our research group. For *Phyllotis darwini* we used mitochondrial D-loop sequences from 68 individuals, 412 bp and *f*our specimens of *P. magister* were used as outgroups (Gutiérrez-Tapia & Palma, in prep.). For *Thylamys elegans* we used 69 mitochondrial cytochrome b sequences, 983 bp, and we used the sister taxa *T. pallidior* and *T. tatei* as outgroups (Boric-Bargetto, in prep.). For *Liolaemus monticola* we used 45 mitochondrial cytochrome b sequences, 700 bp, using *L. nigroviridis, L. tenuis* and *L. pictus* as outgroups (Torres-Pérez *et al.*, 2007). Finally, for *Rhinella arunco* we used 163 mithochondrial D-loop sequences, 899 bp (Vasquez *et al.*, 2013). Since this study constituted a multi-taxa approach, we considered that using a single molecular marker per species would be suitable to characterize the general distributional responses among taxa. On the other hand, coalescent simulations of molecular evolution inside each species give support to our species trees. Individual sampling roughly represents all species' geographic ranges. The full list of specimens, geographic cooordinates and GeneBank accession numbers is given in Appendix I.

Intraspecific Phylogeny For phylogenetic reconstruction, sequences from each species were transformed into haplotypes to simplify the analysis, but data about haplotype frequency was not take into account in this work.

The Markov Chain Monte Carlo (MCMC) method within a Bayesian framework (hereafter BMCMC) was used to estimate the posterior probability of phylogenetic trees. The MCMC

procedure ensures that trees are sampled in proportion to their probabilities of occurrence under the model of gene-sequence evolution. Approximately 10,000,000 phylogenetic trees were generated using the BMCMC procedure, sampling every 1000^{th} trees to ensure that successive samples were independent. The first 50 trees of the sample were removed to avoid including trees sampled before convergence of the Markov Chain. We used a general likelihood-based mixture model (MM; Pagel & Meade, 2004), based on the general time-reversible (GTR) model of gene-sequence evolution to estimate the likelihood of each tree. This model accommodates cases in which different sites in the alignment evolved in qualitatively distinct ways, but does not require prior knowledge of these patterns or partitioning of the data. These analyses were conducted using the software BayesPhylogenies, available at the website http://www.evolution.rdg.ac.uk/SoftwareMain.html. In order to find the best mixture model of gene-sequence evolution, we obtained the likelihood of the trees by first using a GTR matrix plus the gamma distributed rate heterogeneity model (1GTR + G) and then continuing to add up to five GTR + G matrices were determined. For the posterior analyses, only the combination of matrices with the fewest number of parameters that significantly increased the likelihood was used. Posterior probabilities for topologies were assessed as the proportion of trees sampled after burn-in, in which that particular topology was observed.

Distribution models .- We modeled the climatic niche of each intraspecific lineage to approximate the whole species' current distribution, and its distribution during the LGM under the assumptions that: (1) climate is an important factor driving the species' distribution; (2) the climatic niche of species remained conserved between the LGM and

present time, and (3) overlapped lineage's distribution ranges will approach the whole species geographic range. The latter assumption was tested by overlapping distribution models of each intraspecific lineage to approach the full species distributional range, as the sum of ranges estimated for each lineage. The resultant distributional range was roughly contrasted with another model built for the whole species without considering phylogenetic structure.

The climatic niches were reconstructed using the methodology of ecological niche modeling, where environmental data are extracted from occurrence records and random points (represented by geographic coordinates). Habitat suitability was evaluated across the landscape using program specific algorithms (Elith *et al.*, 2006). The current models were then projected on the climatic reconstructions of the LGM. For occurrence records, we used our unique sampling localities. In addition to full geographic distribution models for each species, we built climatic models for each major lineage recovered in the intraspecific phylogenies following the same approach. As a test of consistency we overlapped the lineage distribution models for the lineages of each species, to compare it to the full species distribution models.

The current climate was represented by bioclimatic variables from the WorldClim dataset v. 1.4 (http://www.world clim.org/; Hijmans *et al.*, 2005) that are derived from monthly temperature and precipitation data, and represent biologically meaningful aspects of local climate (Waltari *et al.*, 2007; Jezkova *et al.*, 2009).

For environmental layers representing the climatic conditions of the LGM, we used ocean–atmosphere simulations (Harrison, 2000) available through the Paleoclimatic Modelling Intercomparison Project (Braconnot *et al.*, 2007). These reconstructions of the LGM climate are based on simulated changes in concentration of greenhouse gases, ice

sheet coverage, insulation and topography (caused by lowering sea levels). We used two models that have been previously downscaled for the purpose of ecological niche modeling (Waltari *et al.*, 2007): Community Climate System Model v. 3 (CCSM; Otto-Bliesner *et al.*, 2006) and the Model for Interdisciplinary Research on Climate v. 3.2 (MIROC; Hasumi & Emori, 2004). The original climatic variables used in these models have been downscaled to the spatial resolution of 2.5 min under the assumption that changes in climate are relatively stable over space (high spatial autocorrelation) and were converted to bioclimatic variables (Peterson & Nyári, 2007).

Climatic niche models were built in the software package MAXENT v. 3.2.1 (Phillips *et al.*, 2006), a program that calculates relative probabilities of the species' presence in the defined geographic space, with high probabilities indicating suitable environmental conditions for the species (Phillips *et al.*, 2004). Trapping coordinates of each individual captured for DNA extraction was used as presence points. We used the default parameters in MAXENT (500 maximum iterations, convergence threshold of 0.00001, regularization multiplier of 1, and 10 000 background points) with the application of random seed and logistic probabilities for the output (Phillips & Dudik, 2008). We masked our models to four altitudinal categories resuming both, the abrupt altitudinal clines characteristic of central Chile, and some known altitudinal distribution limits for several vertebrate taxa in this area (Fuentes & Jaksic, 1979). This procedure was conducted because reducing the climatic variation being modeled to that which exists within a geographically realistic area improves model accuracy and reduces problems with extrapolation (Pearson *et al.*, 2002; Thuiller *et al.*, 2004; Randin *et al.*, 2006). We ran 10 replicates for each model, and an average model was presented using logistic probability classes of climatic niche suitability. The presence– absence map was determined using the 'maximum training sensitivity plus

specificity logistic threshold' where the omission error of all occurrence records is set to zero (i.e., locations of all occurrence records are predicted as 'suitable'). Nevertheless, we arbitrary defined a second threshold as the 50% highest logistic probability values observed between the maximum training sensitivity plus specificity logistic threshold and the maximum observed logistic value, in order to depict the areas with highest probability of suitability. We used the receiver operating characteristic for its area under the curve (AUC) value to evaluate the model performance (Fielding & Bell, 1997; Raes & ter Steege, 2007). AUC values range from 0.5 for a random prediction to 1 for perfect prediction (Phillips *et al.*, 2004)

Alternative biogeographical scenarios, coalescent simulations and hypothesis testing

Phylogenetic grouping of mithocondrial haplotypes inside species was used to delimitate lineages for distribution and paleodistribution models. Past and present distribution for each intraspecific lineage was compared whiting and between species. Altogether, this information was summarized into hypothetical population genealogies. Those hypotheses were tested in an integrative framework following the general protocol described in (Carstens & Richards, 2007; Richards *et al.*, 2007). This approach can be roughly described as follows: i) To integrate distributional and phylogenetic information into explicit hypothesis about genealogic relationships (and divergence times) between populations within species ii) to perform coalescent simulations of molecular evolution restricted to those hypothetical genealogies iii) to summarize simulated genealogies by computing the number of deep coalescents (nDC) in each iteration; this statistic is used to construct the expected null distribution of nDC under each hypothetical biogeographic scenario iv) to test hypotheses by comparing the observed nNDC in the phylogenetic reconstruction with

the expected null distribution of nDC of each alternative hypotheses. If the observed nDC is less or equal than 5% of the expected nDC distribution, that particular biogeographic scenario can be rejected. Coalescent simulations of DNA evolution for each species and hypothesis were performed in the software MESQUITE 2.75 (Maddison & Maddison, 2011). One hundred coalescent simulations were performed to construct the expected null distribution of nDC for each hypothesis. The same number of characters and also the same molecular evolution parameters estimated in phylogenetic reconstruction were used for DNA sequence evolution simulations.

Two main modifications to the protocol described by Richards et al. were made: First, as we knew a priori that phylogeographic patterns of interest were pseudo-congruents (i.e. similar topology and distribution, but different divergence time), we did not build the general genealogical hypothesis with explicit divergence time or number of generations; instead we used the maximum observed branch length for each haplogroup, because we were more interested in the effect of long term distributional patterns on genealogy (i.e. species tree) rather than specific historical events. Second, we sacrificed realism in coalescent simulations regarding the effective population size parameter (Ne), because when we introduced skyline estimations of Ne (Ho & Shapiro, 2011) in coalescent simulations, null distributions of nDC were incommensurable with observed nDC. Therefore, we defined the Ne in simulations as the minimum value allowed by MESQUITE in all coalescent simulations.

Results

Intraspecific Phylogenies

General topology is similar among species' phylogenetic trees, except for *Rhinella arunco* which shows no intraspecific phylogenetic structure (very low posterior probability values for internal nodes Fig. 1). All other species display an intraspecific split between two major phylogroups (A and B; Fig. 1), with one of the major groups divided into two subgroups (A1 and A2 subgroups Fig. 1). There is one supported subgroup within phylogroup B for *P. darwini*, but this whole B group has a wide and almost continuous distribution across the species' latitudinal range. Posterior probability values for the nodes which define this pattern is high (values between 0.94 to 1.0; Fig 1).

Distribution models

To test the assumption that overlapped lineage distribution models may approximate whole species distribution models, we compared our estimations of each species' distribution range at present from i) all trapping localities, considering whole species as single distributional units (data not shown), and ii) models with trapping localities assigned to different intraspecific lineages as independent distributional units (independent lineage distribution models) (Fig 7, Tables 1 and 2). The result shows that whole species' range models are good approximations of the observed distribution range for each species, and also with high model performance (AUC, Table 1), whereas overlapped lineage distribution models slightly over-predict the northern distribution of *Phylloti darwini*. Both model

approaches (whole species and overlapped lineage's ranges) are good and consistent approximations of species´ current geographic ranges.

Range dynamics

Phyllotis darwini Distribution model for lineage B encompasses the whole distributional range of *P. darwini* (Fig 2, table 2) and slightly over-predicts its southern distributional range until 37° S. Interestingly, when considering an arbitrary high threshold (50% highest logistic probability value, red areas in Fig. 2) the predicted distribution range is mainly restricted to lowlands in the valley and coast. On the other hand, distribution model for lineage A shares lower AUC value and clearly over-predicts the observed distribution of this phylogroup (sampled at disjunct localities across both mountain ranges). Nevertheless, when considering our arbitrary high threshold, the estimated distribution for lineage A is surprisingly well delimited and restricted almost exclusively to Andean mountain ranges (some populations in coastal mountain range) above the 1500 m elevation limit detected for this phylogroup in a previous work (Gutiérrez-Tapia et al. in prep). This mountain-restricted lineage occurs between 25°S and 36°S.

In summary, overlapped lineage distribution models for *P. darwini* has slightly worst performance and some over-prediction compared to the whole species distribution model, but when considering our arbitrary 50% highest logistic probability threshold, lineage distribution models reproduced very accurately the altitudinal pattern reported for both phylogroups at present, with phylogroup A currently distributed across mountain ranges above 1500 m, and phylogroup B mainly distributed in the lowlands and low elevation

mountain belts. This information is missed in the whole species distribution range model (data not shown).

Past lineage distribution estimated by both LGM models is conflicting (CCSM and MIROC, Fig. 2, table 2): the CCSM model predicts a distributional gap during LGM for both phylogroups, but MIROC based distribution models predicts that both phylogroups were restricted to the southern portion of the current distributional range. Nevertheless, both models consistently predicted the area between 31°S-35°S as suitable for both phylogroups during LGM. This latitudinal distribution dynamics must be considered with caution because downscaled climatic data may not represent local geographic complexity with accuracy.

It is important to emphasize that altitudinal particularities reported for both lineages at present were already established during LGM: lineages A and B might have been restricted to approximately the same latitude, but only lineage A displayed suitable areas at the Andean mountain range during LGM (Table 2), which also has been sampled mainly in the Andes and above 1500 m. at present.

Thylamys elegans This species possess two main intraspecific lineages with essentially allopatric distribution at present. Our model suggests that northern phylogroup A is distributed from 30°S to 34°S by the coast, the valley and mountain ranges, whereas southern phylogroup B ranges from 33°S to 36°S at the coast, the valley and mountain ranges, and even until 37°S across coastal mountain ranges (Fig. 2, Table 2).

MIROC and CCSM past distribution models consistently shows a more restricted distribution range for phylogroup A which may have been confined to 31°S to 33°S during

LGM, and its altitudinal distribution at Andean mountain ranges appears to have also retracted to lower elevations. Southern phylogroup B on the contrary, had apparently retained its distributional range between 33°S to 36°S at the coast, the valley and mountain ranges since LGM, according to both MIROC and CCSM based distribution models (Fig. 2, Table 2) .

Liolaemus monticola Our distribution models at present describes surprisingly well the altitudinal distribution of the species, which is known to be currently distributed above 300 m. until 2200 m. in both, coastal and Andean mountain ranges (Mella, 2005). The model displays very little over-prediction in lowlands for both phylogroups. Phylogroup A is distributed from 32° S to 34° S according to the model for current climatic conditions; there is a slight over-prediction in the lowlands, but as in *Phyllotis darwini ´*s phylogroup A, red areas (which correspond to our arbitrariy threshold of 50% highest logistic probability values) are a good descriptor of the mountain-range-restricted distributional pattern in this species. The same altitudinal pattern is truth for the southern phylogroup B, but distributed between 33° S and 36° S mainly in the Andes and some populations at the coastal mountain range (Fig. 2, Table 2) .

Past lineage distribution based on MIROC suggests that phylogroup A distribution at LGM was roughly the same than its present distribution, but maybe confined to lower elevations at the Andes mountain range compared to present distribution. On the other hand, CCSM based distribution model clearly over-predicts the past distribution of phylogroup A in a disjunct population of southern Argentina, at the eastern side of the Andes, far away from the southern distributional limit of the species. In contrast, phylogroup B appears to have had a much broader distribution towards the lowlands and the Coastal Cordillera at LGM,

according to both, CCSM and MIROC based distribution models. Nevertheless, latitudinal boundaries appear to remain constant since LGM (Fig. 2, Table 2).

Rhinella arunco This is the only species for which we did not obtain phylogenetic structure (Fig. 1). According to the distribution model at present conditions, *R. arunc*o has suitable habitats between 31°S and 37°S. Both CCSM and MIROC based distribution models at LGM predict that *R. arunco's* distributional range could have been slightly contracted (32°S to 36°S) in its latitudinal boundaries compared to its distribution at present (31°S to 37°S) (Fig. 2, Table 2) .

Alternative Biogeographic scenarios

To develop a biogeographic hypothesis to explain current distribution of vertebrate's cryptic diversity in the central Chile hotspot, we have made a comparison of present and past distribution at LGM, among lineages and across species. Our first conclusion was that, despite the highly similar intraspecific phylogenetic topology among species (except for *R. arunco* for which we did not recover intraspecific structure), there exists a general lack of phylogeographic congruence (i.e lineages among species are not codistributed, nor at present or during LGM). Nevertheless, we have identified some generalities in the distribution of those phylogroups with no internal subdivision ("B" phylogroups, Fig.1): all "B" phylogroups are currently distributed at, or were restricted to, approximately the same area between 32°S to 36°S (Fig 2) mainly at the valley and coastal cordillera but never in the Andes; that is to say this is an area of persistant distribution for vertebrate lineages during LGM, with the max degree of codistribution between 32°S – 35°S (Fig. 4). Therefore, our results consistently suggests that this geographic area appears to be a highly

persistent distribution center for vertebrate lineages in the central Chile hotspot, since the LGM to present times. Assuming that this hypothetically highly persistent distribution area could have behaved in a similar way across several glacial/interglacial cycles through the evolutionary history of those vertebrate species, we postulate that this area has harbored the most ancient haplotypes of the studied species. If this is true, we expect to see this phenomena reflected in the intraspecific phylogenetic trees: our hypothesis is that all phylogroups distributed independently of this high persistence area ("A1" and "A2" phylogroups, Fig. 1) must be more related to each other than any of them with "B" phylogroups from the persistence area, because "A" haplotypes must be derived from ancestral haplotypes which have been most likely preserved inside the area between 32°S and 35°S across glacial/ interglacial transitions; therefore "A" phylogroups could be a consequence of postglacial expansions from the area of persistent distribution.

As our intraspecific phylogenies were built from a single mitochondrial marker per species, we implemented a coalescent simulation approach (see Methods) to measure if our gene trees agreed with the hypothetical intraspecific trees expected according to our biogeographic hypothesis, or if those topologies could be expected by chance from a different biogeographic scenario. In order to perform the test, we defined the following hypothetical biogeographic scenarios:

H1) "Refuge" for ancestral haplotypes in the persistant distribution area across glacial/interglacial cycles explains current distribution of cryptic diversity: "A" phylogroups are expected to be more related to each other than any of them with "B" phylogroups in the intraspecific phylogeny , because "A" phylogroups are descendants from ancient phylogroups harbored inside the area between 32°S – 35°S (Figs. 3, 4).

H2) Ancient fragmentation: All phylogroups are expected to share an equally ancestral common ancestor in the intraspecific phylogeny, because current distribution of cryptic diversity is not related to the area between 32°S-35°S, but to fragmentation of a formerly continual distribution.

H3) and H4) "No refuge": Persistant distribution at the 32°S-35°S area does not explain current distribution of cryptic diversity because ancient haplotypes have no particular relationship with the area of persistant distribution across the last glacial/interglacial transition, and therefore any "A" phylogroup could be more related to "B" phylogroup than to the other "A" phylogroup in the intraspecific phylogeny.

Coalescent simulations were performed for each species (except *Rhinella arunco*) and each alternative hypothesis. Molecular evolution was constrained to observed parameters of length of DNA sequences, nucleotide frequencies, maximum observed long branches, expected topology, etc. In each iteration the number of deep coalescences was registered to build the expected null distribution of the nDC for each hypothesis (Fig. 3). Then we simply counted the observed nDC (blue arrows in Fig. 3) by contrasting the observed intraspecific phylogenies with the hypothetical tree used to constrain simulations. Results of this test were not conclusive because observed values for nDC were in the rejection zone for all the hypothesis with the exception of H1 in *Phyllotis darwini*. For the latter species we were able to confidently say that intraspecific genealogy agreeds with the null distribution of nDC expected for our biogeographic scenario H1. Even though we lack of statistical power to fully discern which hypothesis is closer to the real biogeographic scenario in the other two species (*L. monticola and T. elegans*), we can assure that the

smallest degree of discordance between the observed nDC and the expected null distribution of nDC, occurs also in the H1 biogeographic scenario.

Discussion

Phylogenetic structure of the endemic species studied is interesting in several ways. First, when we compared the intraspecific structure among four species belonging to three different classes of vertebrates (and two infraclasses of mammals), the general phylogenetic topology is remarkably similar among the species: with the exception of the amphibian, all the species with phylogenetic structure have two major intraspecific lineages and one of them is subdivided in two other phylogroups. Second, this topological pattern must not be interpreted as phylogeographic congruence, because the distribution of phylogroups at present is far from congruent despite many of the intraspecific lineages are largely sympatric (it is impossible to find coincidence in latitudinal or altitudinal boundaries between lineages among species). And third, we know from previous work that nodes which define major phylogroups have very different ages among species. For example, the major split in *P. darwini* may have occurred about 0.47 – 2.9 MYa for lineage B, and between 0.057 – 1.99 Mya for lineage A (Gutiérrez-Tapia & Palma, in prep.); in *L. monticola* confidence intervals for ages are: 0.41 – 0.94 Mya. for lineage A and 0.5 – 1.7 Mya. For lineage B (Gonzalez, 2013). However, for *T. elegans* the subdivision into lineages A and B it was hypothesized to have occurred long before 3.0 – 6.0 Mya (Boric-Bargetto, in prep.). Therefore, these apparently similar topologies are not necessarily the result of a synchronized intraspecific diversification triggered by some historical events; it

could be the outcome of chance or another historical process at the region without the need of synchronic diversification.

Once we established the occurrence of cryptic intraspecific lineages whit pseudo-congruent distributional pattern, the next step was to asses if species' distributional responses after LGM were homogenous or if lineages displayed independent distributional responses, and therefore species would not behave as distributional units after glacial retreat. Without considering intraspecific structure, we have found that those vertebrate species displayed two kinds of distributional responses after LGM until present: the ectothermic species *Rhinella arunco* and *Liolaemus monticola* seem to have a stable distribution through the last glacial/interglacial transition with only small latitudinal and altitudinal expansions after glacial retreat; the only notorious contraction occured in the southern portion of *L. monticola*'s distributional range. Whereas endothermic species *Thylamys elegans* and *Phyllotis darwini* exhibited the most dynamic distributional responses when its past distribution is compared to present distribution, with remarkable postglacial expansions towards the north and up to the Andean mountain range.

If we consider intraspecific lineage's distributional responses, the pattern is not dichotomic: within ectothermic species, *Rhinella arunco* (that with no intraspecific structure) displayed a small and homogenous expansion after glacial retreat (northward, southward and up to Andean mountain range). On the other hand, *Liolaemus monticola*'s lineages shared very different responses: even though latitudinal distribution has not change since LGM, lineage A has moderately expanded towards higher altitudes at Andean mountain ranges, whereas lineage B has experienced a more notorious contraction from lowlands and retreated to coastal and andean mountain ranges. Among ectothermic species, *Thylamys elegans*'s

lineages behave independently after glacial retreat: meanwhile lineage B has remained stable, lineage A appears to have expanded northward and upward into the andean mountain range. The other endothermic (*Phyllotis darwini*) displayed the most dynamic distributional responses after glacial retreat in both lineages: lineage B has notoriously expanded its latitudinal range but remains restricted mainly to the lowlands; meanwhile lineage A has largely expanded northwards but appears to be almost exclusively restricted to mountain ranges above 1500 m. Altogether those results indicated that complex distributional responses to climate change after LGM appeared to be a general phenomenon for vertebrate's species in the central Chile hotspot.

Despite largely independent distributional responses of vertebrate's lineages, we have identified an area around 32°S and 35°S with high persistence in situ and codistribution during LGM (Figs. 2, 4). Two of the three species with intraspecific phylogenetic structure has one lineage persistently distributed in that area since the LGM until present times (*Liolaemus* B and *Thylamys* B), and lineages from the third species were largely associated to this area during LGM (*Phyllotis* A and B). Besides the species with no phylogenetic structure, *Rhinella arunco*, was restricted to the same area during LGM and displayed a moderate expansion at present. For the above reasons, we hypothesized that the 32°S-35°S area could have behaved as a high distributional persistance area for vertebrates across previous glacial/interglacial transitions, and if this is truth, ancient haplotypes must be harbored here and the species trees should reflect this pattern (hypothetical scenario H1). This assumption is largely coincident with some conclusions reported in a review of phylogeographic patterns in Patagonia (Sercic *et al.*, 2011) which reports i) a phylogeographical break for terrestrial vertebrates in Chile between 33°S and 35°S, ii) a

high persistence area for terrestrial vertebrates north from 36°S in Chile, and iii) a high diversity spot for plants and terrestrial vertebrates combined, approximately at 35°S approximately.

Hypothesis testing for alternative biogeographical hypothesis rejected scenarios H2, H3 and H4 for the three examined species. Observed nDc value was inside the expected null distribution for scenario H1 only in *Phyllotis darwini* , but observed nDc in *Thylamys elegans* and *Liolaemus monticola* were always closer for expected distribution of nDc in that same scenario. Therefore, statistical performance was poor, but this approach is known to have a limited statistical power (Knowles & Maddison, 2002). Nevertheless, considering the observations of Sercic *et al*. for the area around 32° and 35°S along with the fact that scenario H1 is accepted for *P. darwini* and shares the lowest disagreement between observed and expected nDc null distribution in *Liolaemus monticola* and *Thylamys elegans*, we concluded that evidence is sufficient to suggest that current distribution of cryptic intraspecific diversity in vertebrates of the central Chile hotspot is explained by both, complex distributional responses in species with phylogenetic structure and high persistance of lineages in a common area through glacial/interglacial transitions.

Vertebrate's distribution dynamics and conservation priorities in the central Chile hotspot

Our main intention here is to raise the argument about the importance of detecting cryptic diversity as independent ESUs, and propose specific predictions of distributional dynamics for conservation issues, particularly in endangered areas with high endemism as biodiversity hotspots. Not just for practical reasons, but also because real distributional

dynamics of species have been (and probably will be) strongly determinate by phylogenetic structure at the intraspecific level.

The way in which vertebrate's cryptic diversity distribution has behaved across a major climatic transition allows us to asseverate that in current interglacial conditions, mountain ranges in central Chile are important biological corridors which may be considered as a conservation priority, while Coastal cordillera around 36°S is an important cryptic biodiversity reservoir through major climatic oscillations. We encourage considering this information in a long term conservation strategy in this biodiversity hotspot.

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Table legends.

Table 1. AUC average values for each distribution model.

Table 2. General description of hypothesized distribution with latitudinal extension and orographic characteristics in suitable areas. Rows are intraspecific lineages and files represent climatic models for current conditions (current) and LGM conditions (CCSM and MIROC). The last row indicates if a lineage has an stable distribution since LGM until present day, or its distribution range has changed.

Figure captions

Figure 1 - Molecular intraspecific phylogenies reconstructed in MultiBayes; numbers above the nodes are posterior probability values. Blue bars depict main intraspecific lineages (A and B) inside each species. A) *Liolaemus monticola* B) *Phyllotis darwini* C) *Thylamys elegans* D) *Rhinella arunco*

Figure 2 - The figure shows the species distribution models for present and two LGM climatic models (rows). Models were built independently for each intraspecific lineage (columns). Yellow represents suitability areas for lineages according to the maximum training sensitivity plus specificity logistic threshold; red areas represent suitability areas according to an arbitrary restrictive threshold, defined as the 50% highest value observed between the maximum training sensitivity plus specificity logistic threshold, and the maximum logistic probability value for each model.

Figure 3 – Number of deep coalescens (nDc) distribution. In columns are the simulated distribution for each species with phylogenetic structure. Rows are alternative biogeographical scenarios H1, H2, H3 and H4 (described in results section). Curves are the registered nDC value for each iteration by species and hypothesis; therefore it represents the expected null distribution of nDc under a particular hypothesis. Blue arrows are the observed nDc value for each reconstructed molecular phylogeny when compared to a particular genealogical hypothesis for each alternative biogeographic scenario.

Figure 4 – Summary of codistributed lineages during LGM. Colours represent the number of lineages that were codistributed in the same area during LGM.

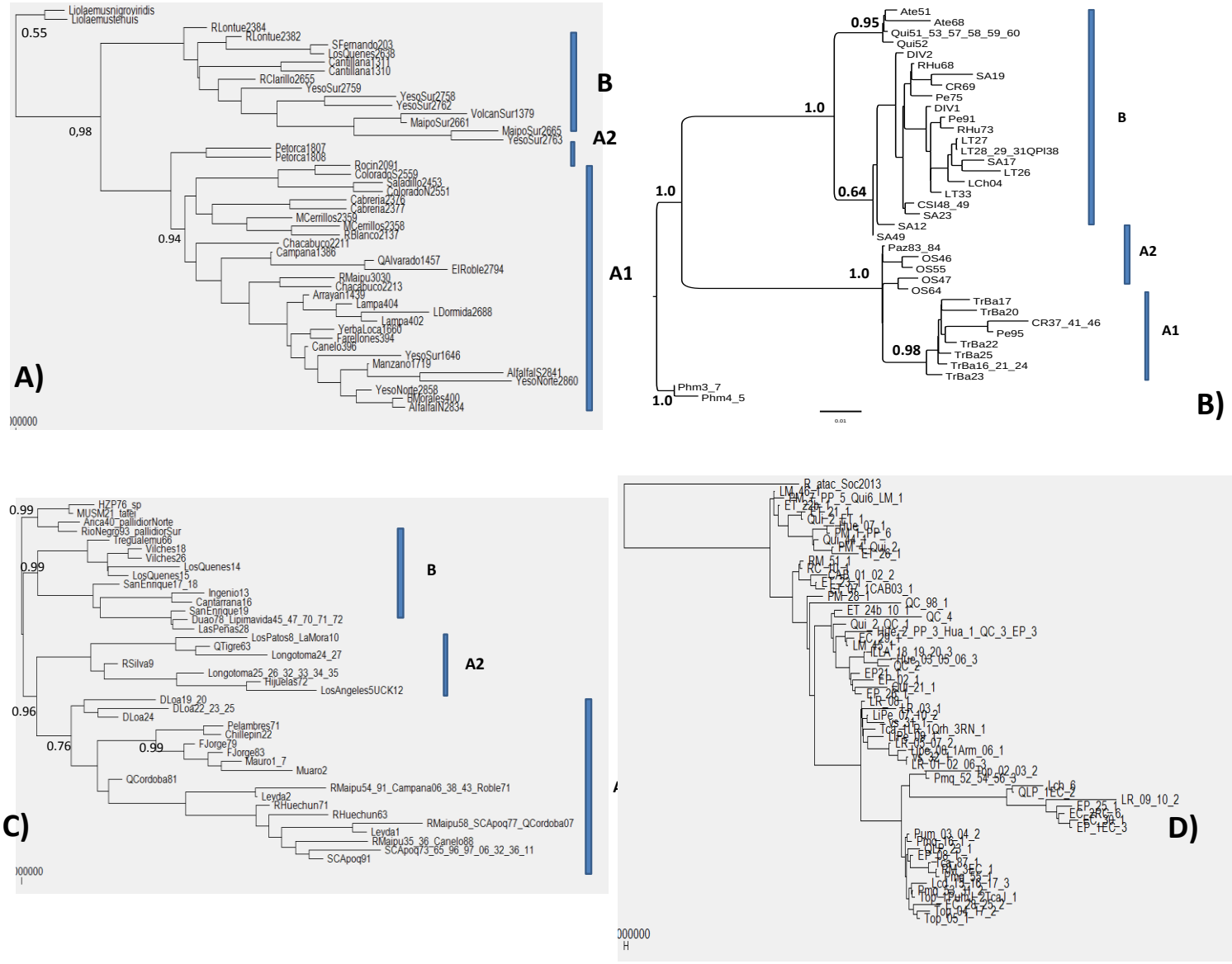
Table 1

MaxEnt Model	AUC Average	AUC stdv.
Phyllotis darwini whole specie´s range	0.971	0.022
Phyllotis darwini A	0.973	0.025
Phyllotis darwini B	0.835	0.157
*Thylamys elegan*s whole specie´s range	0.965	0.023
Thylamys elegans A	0.978	0.043
Thylamys elegans B	0.984	0.012
Liolaemus monticola whole specie´s range	0.992	0.006
Liolaemus monticola A	0.986	0.029
Liolaemus monticola B	0.996	0.002
Rhinella arunco whole specie´s range	0.981	0.01

Table 2.

	Liolaemus A	Liolaemus B	Thylamys A	Thylamys B	Phyllotis A	Phyllotis B	Rhinella
Present	32°S-34°S (Andes and coastal mountain ranges)	**33°S-36°S** (Andes and some populations in coastal mountain range)	30°S to 34°S (Valley and Coast)	**33°S-36°S** (Coast,Valley and Andes)	25°S-36°S (Highest logistic probability in Andes)	25°S-37°S (Mostly Valley and Coast)	**31°-37°S** (Valley, Andean and coastal mountain ranges)
CCSM	32°S-34°S (Andes and coastal mountain ranges; over-prediction in southern argentina)	**33°S-36°S** (Andes, valley and coast)	31°S - 33°S (Coast,Valley and Andes))	**33°S-36°S** (Coast,Valley and Andes)	25°S-35°S (Valley and Coast, disjunction?)	25°S-28°S//31°-35°S (Valley and Coast)	**32°-36°S** (Valley and coastal mountain range)
MIROC	32°S-34°S (Andes and coastal mountain ranges)	**33°S-36°S** (Andes, valley and coast)	31°S - 33°S (Coast,Valley and Andes)	**33°S-36°S** (Coast,Valley and Andes)	**31°S-35°S** (Valley, Coast and Andes)	**31°S-36°S** (Valley and Coast, 33°-34°: Max. logistic value)	**32°-36°S** (Valley and coastal mountain range)
	Range Stability	Range Stability	Range Dynamics	Range Stability	Range Dynamics	Range Dynamics	Range Stability

Fig.1



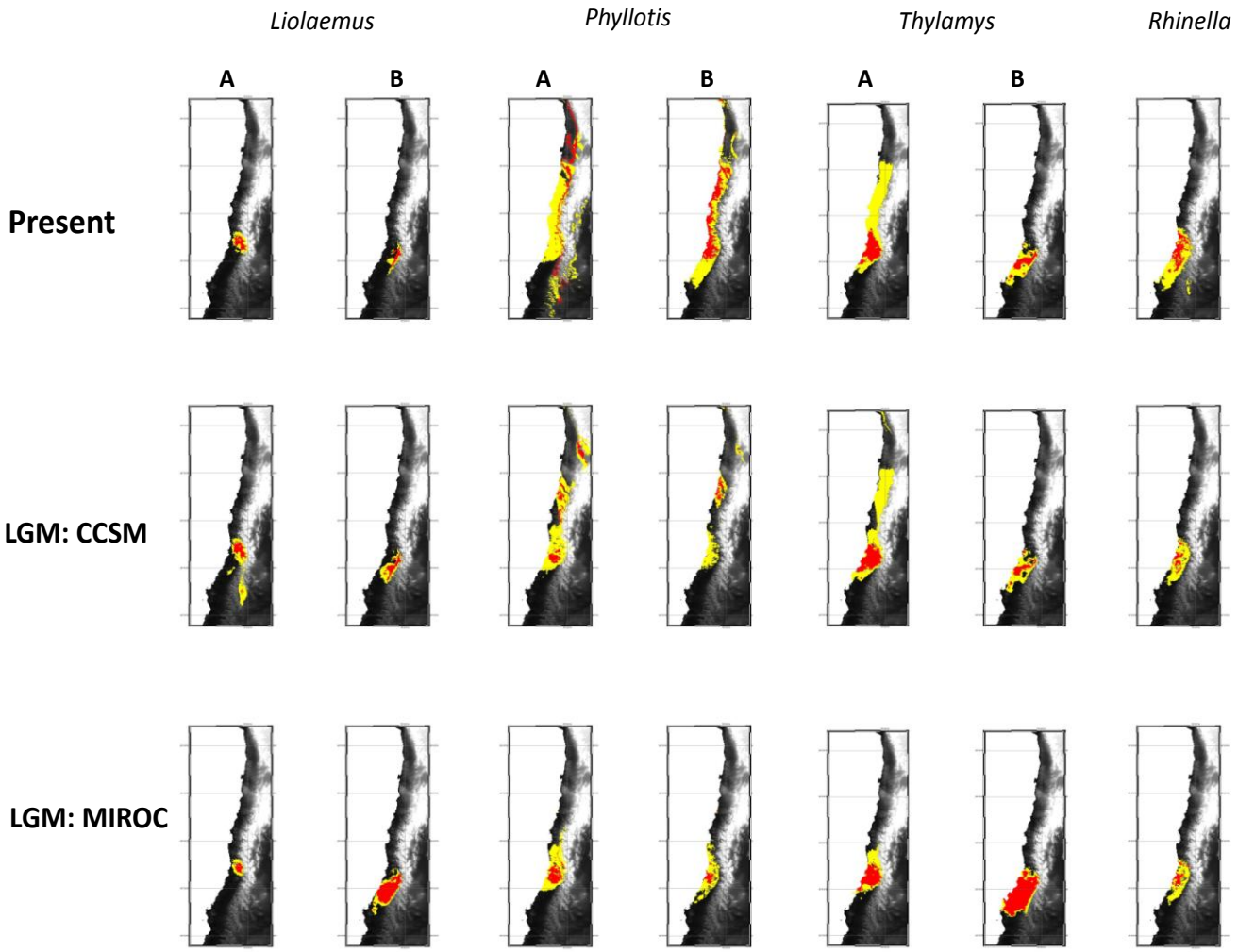

Liolaemus
Phyllotis
Thylamys
Rhinella
A
B
A
B
A
B
Present
LGM: CCSM
LGM: MIROC

Fig.2

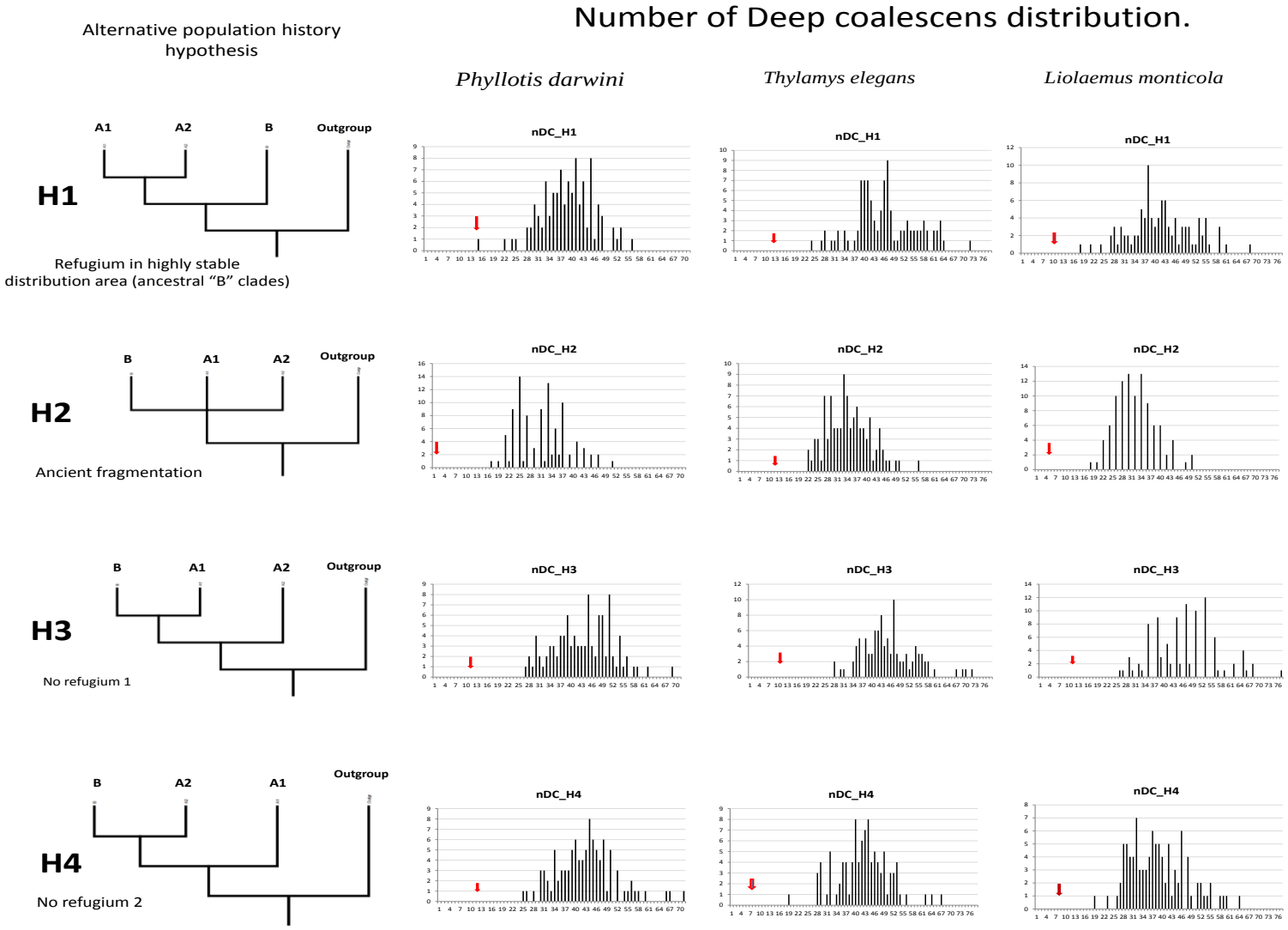
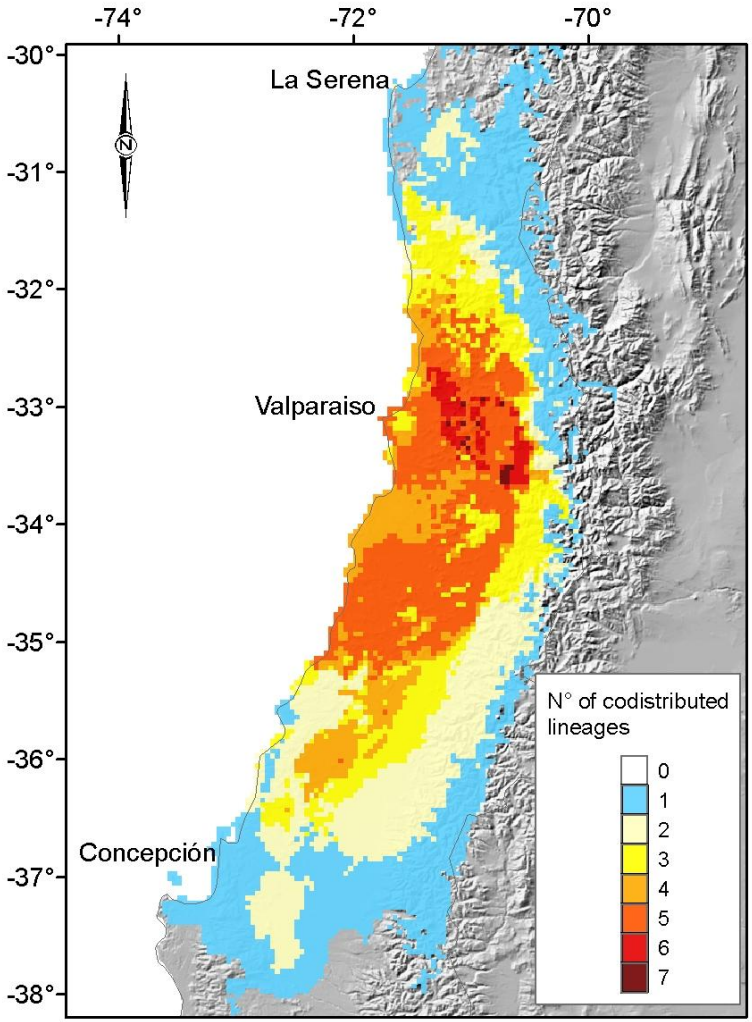

Alternative population history hypothesis
Number of Deep coalescens distribution.
Phyllotis darwini
Thylamys elegans
Liolaemus monticola
A1
A2
B
Outgroup
H1
Refugium in highly stable distribution area (ancestral "B" clades)
nDC_H1
H2
Ancient fragmentation
nDC_H2
H3
No refugium 1
nDC_H3
H4
No refugium 2
nDC_H4

Fig. 3

Fig. 4


-74°
-72°
-70°
-30°
-31°
-32°
-33°
-34°
-35°
-36°
-37°
-38°
N
La Serena
Valparaiso
Concepción
N° of codistributed lineages
0
1
2
3
4
5
6
7

Conclusiones Generales

Uno de los patrones más llamativos reportados en este trabajo es la particular distribución de los linajes intraespecíficos en el roedor *Phyllotis darwini*; el rasgo más característico de dicho patrón es la presencia de segregación altitudinal estricta entre ambos linajes, con uno de ellos distribuido en poblaciones disyuntas a lo largo de los Andes y Cordillera de la Costa siempre sobre los 1500 m, con la excepción de una localidad costera en la porción más septentrional de su rango. Considerando solamente la genealogía e información de la distribución actual de la especie este patrón resulta muy difícil de explicar, pero al modelar la distribución pasada de los linajes fue posible observar que frente a la configuración climática del LGM, la distribución montañosa del linaje de altura no era posible, y ambos linajes se encontraban aparentemente codistribuídos en el sur de la distribución presente, y principalmente en el valle y Cordillera de la Costa. Esta observación permitió hipotetizar que la distribución de los linajes en el presente, altitudinalmente segregada a través de toda la extensión latitudinal de la especie, es consecuencia de un mecanismo de recolonización post-glacial diferencial, con un linaje que expande su rango de distribución a través de ambos cordones montañosos, mientras que el segundo se distribuye predominantemente en el valle. En consecuencia, bajo el supuesto de conservación de nicho desde el LGM hasta el presente, debemos asumir que el nicho realizado del linaje distribuído sobre 1500 m en el presente, no le permitía alcanzar esa altitud en condiciones de temperaturas más bajas y precipitación incrementada, y que en la configuración climática presente no solo es capaz de estar distribuido en los cordones montañosos, sino que no se distribuye en casi ninguna localidad bajo este límite altitudinal; esta segregación distribucional entre ambos linajes aparece solo cuando el umbral de temperatura mínima se relaja, sugiriendo fuertemente que la divergencia intraespecífica de nicho ejerce algún efecto sobre la posibilidad de coexistencia de los linajes en diferentes configuraciones climáticas. En el segundo capítulo se exploró estas conclusiones preliminares sobre las dinámicas de rango de distribución de vertebrados en la última transición glacial/interglacial, esta vez con mayor resolución geográfica y añadiendo un segundo taxón (*Abrothrix olivaceus*). La principal conclusión de este capítulo es que el patrón de mezcla de haplotipos de ambos cordones cordilleranos al interior de los linajes de mamíferos es consistente con la hipótesis de desplazamientos de

rango de distribución de vertebrados análogos a los bien conocidos movimientos de cinturones vegetacionales desde los Andes hacia la costa durante el LGM. La única excepción observada a este patrón es un único linaje estrictamente andino en *A. olivaceus,* que está restringido a los Andes en el presente y es además el único de los linajes de mamíferos analizados que, de acuerdo al modelo de distribución en el LGM, no habría sido capaz de colonizar el valle durante ese evento, permaneciendo restringido a los faldeos cordilleranos. En conjunto, la evidencia sugiere fuertemente que el patrón de mezcla de haplotipos en la mayoría de los linajes (de ambos cordones montañosos) se debe a repetidas expansiones y contracciones del rango de distribución (del tipo de la descrita en el capítulo 1), a través de varias oscilaciones glacial/interglacial; es posible hipotetizar en consecuencia que la repetición de este fenómeno durante el Pleistoceno podría explicar en parte la diferenciación intraespecífica y la presente distribución geográfica de dichos linajes.

El tercer capítulo es un intento de encontrar un mecanismo biogeográfico más general para los vertebrados de la región, utilizando una aproximación comparada y multi-taxa. Sobre la base de información genealógica y modelos de distribución pasada y presente se formuló varios escenarios biogeográficos alternativos, en donde la hipótesis de interés es que los haplotipos ancestrales están asociados a un área de distribución persistente para todos los linajes de vertebrados analizados; esta zona se detectó entre los 26°S-36°S. La idea detrás de esta hipótesis es que, dado que se identificó una zona de distribución persistente para todos los linajes de vertebrados analizados, es razonable pensar que si el área de codistribución fue similar a través de las repetidas transiciones glacial/interglacial, entonces los haplotipos mas ancestrales de cada especie tendrán una alta probabilidad de permanecer distribuídos en dicha área hasta el presente; al mismo tiempo aquellas poblaciones que expandieron su distribución durante los períodos interglaciales debiesen haber sido más susceptibles a diversificación que aquellas asociadas aún a la zona de distribución persistente. La consecuencia de este proceso hipotético sería que todas las especies de vertebrados endémicos de Chile mediterráneo debiesen reflejar en algún grado esta dinámica en su estructura filogenética intraespecífica. Dicho de otro modo, existiría una topología general, en donde los filogrupos más ancestrales son aquellos asociados a la zona de persistencia, tanto en períodos glaciales como interglaciales, mientras que los filogrupos

con variaciones drásticas de rango de distribución y mayor grado de diferenciación, estarían siempre más relacionados entre sí que cualquiera de ellos con los filogrupos asociados a la zona de persistencia, simplemente porque los primeros descienden de los últimos. Si bien la simulación coalescente permitió sustentar dicha hipótesis solo para una especie, el menor grado de discordancia entre el estadígrafo observado y la distribución simulada bajo cada uno de los escenarios alternativos ocurrió siempre en el escenario que representa la hipótesis de interés. En conjunto, la evidencia presentada en esta tesis sugiere fuertemente que la persistencia distribucional de los linajes en dicha zona, a través de varias oscilaciones glaciares/interglaciares, es un componente importante en la diversificación de los vertebrados de Chile central al nivel intraespecífico. En el contexto de la hipótesis de Fuentes y Jaksic para la diversificación de vertebrados en Chile central, esta conclusión sustenta la idea de que existe una serie de maquinaria de diversificación para vertebrados compuesta por dos engranajes principales: Orografía de la región y ciclos glaciales. Sin embargo, el modelo de diversificación postulado en esta tesis no distingue entre dos modos de especiación asincrónicos e independientes para el valle (glacial) y montaña (interglacial), pues la evidencia apunta fuertemente a que el mecanismo es general para distintos grupos de vertebrados, y depende del área de distribución persistente durante el LGM sin excepción. Un corolario de este modelo es que la diversificación intraespecífica ocurriría principalmente en fases interglaciales, que además de ser las de mayor extensión temporal, posibilitarían drásticas expansiones del rango de distribución en interacción con una heterogeneidad del paisaje mucho mayor que la que es posible en condiciones glaciales en el área comprendida entre los 26°S y 36°S, principalmente en valle y codillera de la costa.

Desde el punto de vista metodológico, es destacable que este complicado mecanismo habría pasado desapercibido si no se hubiese considerado la distribución presente y pasada en conjunto con la estructura filogenética intraespecífica. Es decir, la inclusión de la información genealógica no solo mejora la resolución geográfica de los modelos, sino que revela información distribucional real y relevante que es simplemente omitida toda vez que una especie es considerada como una unidad distribucional simple sin investigar su estructura filogenética.

Los vertebrados terrestres de Chile mediterráneo son un grupo que alberga una cantidad importante de diversidad críptica intraespecífica. La identificación de estos linajes y su historia biogeográfica no es un mero ejercicio teórico sino una necesidad real en el contexto de la planificación de la conservación biológica en Chile. Por ejemplo, con los resultados precedentes, es posible afirmar que los cordones montañosos del hotspot de Chile central son importantes corredores biológicos en condiciones interglaciales. Una estrategia razonada de conservación debiese por lo tanto priorizar la creación de numerosas reservas de pequeña área pero alta conectividad a lo largo de los Andes y en los puntos en donde el valle es más estrecho y la separación entre ambas cordilleras es mínima. Por otro lado, existe suficiente evidencia que señala que el área entre los 33°S-36°S constituye un importante centro de distribución persistente de linajes de vertebrados a través de oscilaciones climáticas de gran escala, al menos desde el LGM hasta el presente, y muy probablemente a través de varias transiciones glacial/interglacial durante el Pleistoceno. Se debiese por tanto priorizar la presencia de áreas protegidas de gran extensión a lo largo de la Cordillera de la Costa en dicho segmento latitudinal.

Finalmente, la evidencia expuesta permite proponer la discusión sobre la importancia de la estructura filogenética en el contexto de las dinámicas de rango de distribución de las especies; en consecuencia se propone la siguiente tesis:

Las especies con estructura filogenética intraespecífica a menudo presentan un mosaico de respuestas distribucionales independientes, en donde cada linaje genealógico posee dinámicas de distribución que son independientes de las variaciones en la distribución de otros linajes de la misma especie, y frente a los mismos forzamientos climáticos.

Apéndices

Capítulo I

APPENDIX I.- List of individuals by catalog number, species, locality, initials, geographic coordinates and GeneBank accession number.

Catalogue Number	Species	Locality	Initials	Latitude (S)	Longitude (W)	Genebank accesion number
NK 106069	*Phyllotis darwini*	Cerro el Roble, Til-Til	CR69	32°59´24´´	71°1´12´´	JN226723
NK 106137	*Phyllotis darwini*	Cerro el Roble, Til-Til	CR37	32°59´24´´	71°1´12´´	JN226724
NK 106141	*Phyllotis darwini*	Cerro el Roble, Til-Til	CR41	32°59´24´´	71°1´12´´	JN226725
NK 106146	*Phyllotis darwini*	Cerro el Roble, Til-Til	CR46	32°59´24´´	71°1´12´´	JN226726
NK 105341	*Phyllotis darwini*	Cerro Santa Inés, Los Vilos	CSI41	32°9´0´´	71°28´48´´	JN226718
NK 105348	*Phyllotis darwini*	Cerro Santa Inés, Los Vilos	CSI48	32°9´0´´	71°28´48´´	JN226719
NK 105349	*Phyllotis darwini*	Cerro Santa Inés, Los Vilos	CSI49	32°9´0´´	71°28´48´´	JN226720
NK 95618	*Phyllotis darwini*	Chillepín	CH18	31°52’48´´	70°47´24´´	JN226690
NK 95620	*Phyllotis darwini*	Chillepín	CH20	31°52’48´´	70°47´24´´	JN226691
NK 95624	*Phyllotis darwini*	Chillepín	CH24	31°52’48´´	70°47´24´´	JN226692
NK 120051	*Phyllotis darwini*	Concepción, Tomé Agua Tendida	Ate51	36°38´24´´	72°47´24´´	JN226727
NK 120068	*Phyllotis darwini*	Concepción, Tomé Agua Tendida	Ate68	36°38´24´´	72°47´24´´	JN226728
ER3	*Phyllotis magister*	Desembocadura Río Loa	Phm3	21°25´33´´	70°33´36´´	JN226732

ER4	*Phyllotis magister*	Desembocadura Río Loa	Phm4	21°25´33´´	70°33´36´´	JN226733
ER5	*Phyllotis magister*	Desembocadura Río Loa	Phm5	21°25´33´´	70°33´36´´	JN226734
ER7	*Phyllotis magister*	Desembocadura Río Loa	Phm7	21°25´33´´	70°33´36´´	JN226735
SSUC-Ma-00451	*Phyllotis darwini*	El Guanaco, Quirihue, Ñuble	QUI51	36°24´0´´	72°37´30´´	JN226683
SSUC-Ma-00452	*Phyllotis darwini*	El Guanaco, Quirihue, Ñuble	QUI52	36°24´0´´	72°37´30´´	JN226684
SSUC-Ma-00453	*Phyllotis darwini*	El Guanaco, Quirihue, Ñuble	QUI53	36°24´0´´	72°37´30´´	JN226685
SSUC-Ma-00457	*Phyllotis darwini*	El Guanaco, Quirihue, Ñuble	QUI57	36°24´0´´	72°37´30´´	JN226686
SSUC-Ma-00458	*Phyllotis darwini*	El Guanaco, Quirihue, Ñuble	QUI58	36°24´0´´	72°37´30´´	JN226687
SSUC-Ma-00459	*Phyllotis darwini*	El Guanaco, Quirihue, Ñuble	QUI59	36°24´0´´	72°37´30´´	JN226688
SSUC-Ma-00460	*Phyllotis darwini*	El Guanaco, Quirihue, Ñuble	QUI60	36°24´0´´	72°37´30´´	JN226689
NK 96868	*Phyllotis darwini*	Fray Jorge	FJ68	30°40´12´´	71°37´48´´	JN226713
NK 96869	*Phyllotis darwini*	Fray Jorge	FJ69	30°40´12´´	71°37´48´´	JN226714
NK 96871	*Phyllotis darwini*	Fray Jorge	FJ71	30°40´12´´	71°37´48´´	JN226715
NK 96874	*Phyllotis darwini*	Fray Jorge	FJ74	30°40´12´´	71°37´48´´	JN226716
NK 105515	*Phyllotis darwini*	Fray Jorge	FJ15	30°40´12´´	71°37´48´´	JN226722
SSUC-Ma-00426	*Phyllotis darwini*	Las Tacas	LT26	30°0´0´´	71°22´26´´	JN226672
SSUC-Ma-00427	*Phyllotis darwini*	Las Tacas	LT27	30°0´0´´	71°22´26´´	JN226673
SSUC-Ma-	*Phyllotis*	Las Tacas	LT28	30°0´0´´	71°22´26´´	JN226674

00428	*darwini*					
SSUC-Ma-00429	*Phyllotis darwini*	Las Tacas	LT29	30°0´0´´	71°22´26´´	JN226675
SSUC-Ma-00431	*Phyllotis darwini*	Las Tacas	LT31	30°0´0´´	71°22´26´´	JN226676
SSUC-Ma-00433	*Phyllotis darwini*	Las Tacas	LT33	30°0´0´´	71°22´26´´	JN226677
NK 160004	*Phyllotis darwini*	Llanos del Challe	LCH04	28°4´48´´	71°8´24´´	JN226731
SSUC-Ma-00442	*Phyllotis darwini*	Los Molles	LMo42	30°14´24´	71°30´0´´	JN226681
NK 96846	*Phyllotis darwini*	Observatorio La Silla	OS46	29°15´0´´	70°43´48´´	JN226708
NK 96847	*Phyllotis darwini*	Observatorio La Silla	OS47	29°15´0´´	70°43´48´´	JN226709
NK 96852	*Phyllotis darwini*	Observatorio La Silla	OS52	29°15´0´´	70°43´48´´	JN226710
NK 96855	*Phyllotis darwini*	Observatorio La Silla	OS55	29°15´0´´	70°43´48´´	JN226711
NK 96864	*Phyllotis darwini*	Observatorio La Silla	OS64	29°15´0´´	70°43´48´´	JN226712
NK 142983	*Phyllotis darwini*	Pan de Azucar	Paz83	26°8´24´´	70°39´36´´	JN226729
NK 142984	*Phyllotis darwini*	Pan de Azucar	Paz84	26°8´24´´	70°39´36´´	JN226730
NK 96575	*Phyllotis darwini*	Pelambres	PE75	31°49´12´	70°34´48´´	JN226700
NK 96586	*Phyllotis darwini*	Pelambres	PE86	31°49´12´	70°34´48´´	JN226701
NK 96591	*Phyllotis darwini*	Pelambres	PE91	31°49´12´	70°34´48´´	JN226702
NK 96595	*Phyllotis darwini*	Pelambres	PE95	31°49´12´	70°34´48´´	JN226703
NK 96596	*Phyllotis darwini*	Pelambres	PE96	31°49´12´	70°34´48´´	JN226704

NK 105381	*Phyllotis darwini*	Llanos del Challe	LCH81	28°4´48´´	71°8´24´´	JN226721
SSUC-Ma-00435	*Phyllotis darwini*	Punta de Choros	PCh35	29°15´0´´	71°27´36´´	JN226678
SSUC-Ma-00437	*Phyllotis darwini*	Punta de Choros	PCh37	29°15´0´´	71°27´36´´	JN226679
SSUC-Ma-00445	*Phyllotis darwini*	Punta de Choros	PCh45	29°15´0´´	71°27´36´´	JN226682
SSUC-Ma-00438	*Phyllotis darwini*	Quebrada de la Plata	PCh38	29°15´0´´	71°27´36´´	JN226680
NK 96738	*Phyllotis darwini*	Quebrada del Tigre	QT38	32°33´36´´	71°25´48´´	JN226705
NK 96754	*Phyllotis darwini*	Quebrada del Tigre	QT54	32°33´36´´	71°25´48´´	JN226706
NK 95968	*Phyllotis darwini*	Rinconada Huechún	RHU68	33°0´36´´	70°48´36´´	JN226693
NK 95973	*Phyllotis darwini*	Rinconada Huechún	RHU73	33°0´36´´	70°48´36´´	JN226694
NK 96312	*Phyllotis darwini*	San Carlos Apoquindo	SA12	33°24´0´´	70°28´48´´	JN226695
NK 96319	*Phyllotis darwini*	San Carlos Apoquindo	SA19	33°24´0´´	70°28´48´´	JN226696
NK 96323	*Phyllotis darwini*	San Carlos Apoquindo	SA23	33°24´0´´	70°28´48´´	JN226697
NK 96349	*Phyllotis darwini*	San Carlos Apoquindo	SA49	33°24´0´´	70°28´48´´	JN226698
NK 96359	*Phyllotis darwini*	San Carlos Apoquindo	SA59	33°24´0´´	70°28´48´´	JN226699
NK 96817	*Phyllotis darwini*	San Carlos Apoquindo	SA17	33°24´0´´	70°28´48´´	JN226707
NK 105329	*Phyllotis darwini*	San Carlos Apoquindo	SA29	33°24´0´´	70°28´48´´	JN226717
SSUC-Ma-00416	*Phyllotis darwini*	Tranque de relaves Barahona	TrBa16	34°4´51´´	70°31´12´´	JN226664
SSUC-Ma-	*Phyllotis*	Tranque de relaves Barahona	TrBa1	34°4´51´´	70°31´12´´	JN226665

00417	*darwini*		7			
SSUC-Ma-	*Phyllotis*	Tranque de relaves Barahona	TrBa2	34°4´51´´	70°31´12´´	JN226666
00420	*darwini*		0			
SSUC-Ma-	*Phyllotis*	Tranque de relaves Barahona	TrBa2	34°4´51´´	70°31´12´´	JN226667
00421	*darwini*		1			
SSUC-Ma-	*Phyllotis*	Tranque de relaves Barahona	TrBa2	34°4´51´´	70°31´12´´	JN226668
00422	*darwini*		2			
SSUC-Ma-	*Phyllotis*	Tranque de relaves Barahona	TrBa2	34°4´51´´	70°31´12´´	JN226669
00423	*darwini*		3			
SSUC-Ma-	*Phyllotis*	Tranque de relaves Barahona	TrBa2	34°4´51´´	70°31´12´´	JN226670
00424	*darwini*		4			
SSUC-Ma-	*Phyllotis*	Tranque de relaves Barahona	TrBa2	34°4´51´´	70°31´12´´	JN226671
00425	*darwini*		5			

APPENDIX II .- List of haplotypes with the number of individuals by locality that shares each haplotype. The proportion of haplotypes by locality, the sample haplotype frequency and the proportional weighted frequency are listed.

	Localities										
Haplotype	**Agua Tendida**	**Cerro el Roble**	**Cerro Santa Inés**	**Chillepín**	**Fray Jorge**	**Las Tacas**	**Llanos de Challe**	**Los Molles**	**Obs. La Silla**	**PN Pan de Azúcar**	**Pelambres**
TrBa16_21_24	0	0	0	0	0	0	0	0	0	0	0
TrBa17	0	0	0	0	0	0	0	0	0	0	0
TrBa20	0	0	0	0	0	0	0	0	0	0	0
TrBa22	0	0	0	0	0	0	0	0	0	0	0
TrBa23	0	0	0	0	0	0	0	0	0	0	0
TrBa25	0	0	0	0	0	0	0	0	0	0	0
LT26	0	0	0	0	0	1	0	0	0	0	0
LT27	0	0	0	0	0	1	0	0	0	0	0
LT28_29_31QPl38	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**
LT33	0	0	0	0	0	1	0	0	0	0	0
DIV1	**0**	**0**	**0**	**2**	**4**	**0**	**1**	**1**	**1**	**0**	**2**
Qui51_53_57_58_59_60	0	0	0	0	0	0	0	0	0	0	0
Qui52	0	0	0	0	0	0	0	0	0	0	0
DIV2	**0**	**0**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
RHu68	0	0	0	0	0	0	0	0	0	0	0
RHu73	0	0	0	0	0	0	0	0	0	0	0
SA12	0	0	0	0	0	0	0	0	0	0	0

SA19	0	0	0	0	0	0	0	0	0	0	0
SA23	0	0	0	0	0	0	0	0	0	0	0
SA49	0	0	0	0	0	0	0	0	0	0	0
PE75	0	0	0	0	0	0	0	0	0	0	1
PE91	0	0	0	0	0	0	0	0	0	0	1
PE95	0	0	0	0	0	0	0	0	0	0	1
SA17	0	0	0	0	0	0	0	0	0	0	0
OS46	0	0	0	0	0	0	0	0	1	0	0
OS47	0	0	0	0	0	0	0	0	1	0	0
OS55	0	0	0	0	0	0	0	0	1	0	0
OS64	0	0	0	0	0	0	0	0	1	0	0
CSI48_49	0	0	2	0	0	0	0	0	0	0	0
CR69	0	1	0	0	0	0	0	0	0	0	0
CR37_41_46	0	3	0	0	0	0	0	0	0	0	0
Ate51	1	0	0	0	0	0	0	0	0	0	0
Ate68	1	0	0	0	0	0	0	0	0	0	0
Paz83_84	0	0	0	0	0	0	0	0	0	2	0
LCh04	0	0	0	0	0	0	1	0	0	0	0
TOTAL	**2**	**4**	**3**	**3**	**5**	**6**	**2**	**1**	**5**	**2**	**5**
Proportion from the total of haplotypes by locality	0.0294118	0.0588235	0.0441176	0.044117 6	0.0735294	0.0882 33	0.0294118	0.014705 9	0.073529 4	0.0294118	0.073529 4

APPENDIX II (CONTINUE).

Haplotype	Punta de Choros	Quebrada de la plata	Quebada del Tigre	Localities Quirihue	Rinconada Huechún	San Carlos de Apoquindo	Tranque de relaves Barahona	Total individuals by haplotype	Frequency	Weigthened frequency
TrBa16_21_24	0	0	0	0	0	0	3	3	0.044176	0.0051903
TrBa17	0	0	0	0	0	0	1	1	0.014759	0.0017301
TrBa20	0	0	0	0	0	0	1	1	0.014759	0.0017301
TrBa22	0	0	0	0	0	0	1	1	0.014759	0.0017301
TrBa23	0	0	0	0	0	0	1	1	0.014759	0.0017301
TrBa25	0	0	0	0	0	0	1	1	0.014759	0.0017301
LT26	0	0	0	0	0	0	0	1	0.014759	0.0012976
LT27	0	0	0	0	0	0	0	1	0.014759	0.0012976
LT28_29_31QPl38	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**4**	**0.058823**	**0.0060554**
LT33	0	0	0	0	0	0	0	1	0.0147	0.0012976

									059	
									0.2352	
DIV1	**3**	**0**	**2**	**0**	**0**	**0**	**0**	**16**	**941**	**0.0934256**
									0.0882	
Qui51_53_57_58_59_60	0	0	0	6	0	0	0	6	353	0.009083
									0.0147	
Qui52	0	0	0	1	0	0	0	1	059	0.0015138
									0.0735	
DIV2	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**5**	**294**	**0.0194637**
									0.0147	
RHu68	0	0	0	0	1	0	0	1	059	0.0004325
									0.0147	
RHu73	0	0	0	0	1	0	0	1	059	0.0004325
									0.0147	
SA12	0	0	0	0	0	1	0	1	059	0.0015138
									0.0147	
SA19	0	0	0	0	0	1	0	1	059	0.0015138
									0.0147	
SA23	0	0	0	0	0	1	0	1	059	0.0015138
									0.0147	
SA49	0	0	0	0	0	1	0	1	059	0.0015138
									0.0147	
PE75	0	0	0	0	0	0	0	1	059	0.0010813
PE91	0	0	0	0	0	0	0	1	0.0147	0.0010813

									059	
									0.0147	
PE95	0	0	0	0	0	0	0	1	059	0.0010813
									0.0147	
SA17	0	0	0	0	0	1	0	1	059	0.0015138
									0.0147	
OS46	0	0	0	0	0	0	0	1	059	0.0010813
									0.0147	
OS47	0	0	0	0	0	0	0	1	059	0.0010813
									0.0147	
OS55	0	0	0	0	0	0	0	1	059	0.0010813
									0.0147	
OS64	0	0	0	0	0	0	0	1	059	0.0010813
									0.0294	
CSI48_49	0	0	0	0	0	0	0	2	118	0.0012976
									0.0147	
CR69	0	0	0	0	0	0	0	1	059	0.0008651
									0.0441	
CR37_41_46	0	0	0	0	0	0	0	3	176	0.0025952
									0.0147	
Ate51	0	0	0	0	0	0	0	1	059	0.0004325
									0.0147	
Ate68	0	0	0	0	0	0	0	1	059	0.0004325
Paz83_84	0	0	0	0	0	0	0	2	0.0294	0.0008651

LCh04	0	0	0	0	0	0	0	1	118 0.0147 059	0.0004325
TOTAL	**3**	**1**	**2**	**7**	**2**	**7**	**8**	**68**	**1**	
Proportion from the total of haplotypes by locality	0.0441176	0.0147059	0.0294118	0.1029412	0.0294118	0.1029412	0.1176471			

Capítulo II

APPENDIX I.- List of individuals by catalog number, species, locality, initials, geographic coordinates and GeneBank accession number.

Species	Catalogue Number	Locality	Initials	Latitude (S)	Longitude (W)	Genebank accesion numbe
Abrothrix olivaceus	NK95341	San Carlos de Apoquindo	NK95341SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Abrothrix olivaceus	NK95549	San Carlos de Apoquindo	NK95549SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Abrothrix olivaceus	NK95671	San Carlos de Apoquindo	NK95671SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Abrothrix olivaceus	NK95672	San Carlos de Apoquindo	NK95672SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Abrothrix olivaceus	NK95720	San Carlos de Apoquindo	NK95720SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Abrothrix olivaceus	NK95812	San Carlos de Apoquindo	NK95812SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Abrothrix olivaceus	NK96309	San Carlos de Apoquindo	NK96309SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Abrothrix olivaceus	NK96348	San Carlos de Apoquindo	NK96348SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Abrothrix olivaceus	NK104631	San Carlos de Apoquindo	NK104631SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Abrothrix olivaceus	NK105427	San Carlos de Apoquindo	NK105427SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Abrothrix olivaceus	NK106136	El Roble	NK106136ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	NK106138	El Roble	NK106138ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	NK106142	El Roble	NK106142ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	NK106143	El Roble	NK106143ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	NK106144	El Roble	NK106144ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	NK106145	El Roble	NK106145ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	NK106147	El Roble	NK106147ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	NK106148	El Roble	NK106148ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	NK106149	El Roble	NK106149ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	EP492	El Roble	EP492ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	EP554	El Roble	EP554ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending

Abrothrix olivaceus	EP557	El Roble	EP557ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	EP562	El Roble	EP562ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Abrothrix olivaceus	NK96786	Rinconada de Maipu	NK96786RMaipu	33° 29' 40.92"	70° 53' 34.50"	Pending
Abrothrix olivaceus	NK96787	Rinconada de Maipu	NK96787RMaipu	33° 29' 40.92"	70° 53' 34.50"	Pending
Abrothrix olivaceus	NK96788	Rinconada de Maipu	NK96788RMaipu	33° 29' 40.92"	70° 53' 34.50"	Pending
Abrothrix olivaceus	EP580	Farellones	EP580Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP581	Farellones	EP581Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP582	Farellones	EP582Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP583	Farellones	EP583Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP585	Farellones	EP585Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP586	Farellones	EP586Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP617	Farellones	EP617Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP619	Farellones	EP619Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP622	Farellones	EP622Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP623	Farellones	EP623Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP624	Farellones	EP624Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP628	Farellones	EP628Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	EP629	Farellones	EP629Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Abrothrix olivaceus	NK105804	Rabuco	NK105804Rabuco	32° 52' 11.30"	71° 7' 20.70"	Pending
Abrothrix olivaceus	NK105808	Rabuco	NK105808Rabuco	32° 52' 11.30"	71° 7' 20.70"	Pending
Abrothrix olivaceus	NK106153	Melipilla	NK106153Melipilla	33° 44' 5.49"	71° 13' 2.38"	Pending
Abrothrix olivaceus	NK106154	Melipilla	NK106154Melipilla	33° 44' 5.49"	71° 13' 2.38"	Pending
Abrothrix olivaceus	NK106155	Melipilla	NK106155Melipilla	33° 44' 5.49"	71° 13' 2.38"	Pending
Abrothrix olivaceus	NK106156	Melipilla	NK106156Melipilla	33° 44' 5.49"	71° 13' 2.38"	Pending
Abrothrix olivaceus	NK106157	Melipilla	NK106157Melipilla	33° 44' 5.49"	71° 13' 2.38"	Pending
Abrothrix olivaceus	NK106158	Melipilla	NK106158Melipilla	33° 44' 5.49"	71° 13' 2.38"	Pending
Abrothrix olivaceus	NK106159	Melipilla	NK106159Melipilla	33° 44' 5.49"	71° 13' 2.38"	Pending
Abrothrix olivaceus	NK106162	Melipilla	NK106162Melipilla	33° 44' 5.49"	71° 13' 2.38"	Pending

Abrothrix olivaceus	NK106163	Melipilla	NK106163Melipilla	33° 44' 5.49"	71° 13' 2.38"	Pending
Abrothrix olivaceus	NK106164	Melipilla	NK106164Melipilla	33° 44' 5.49"	71° 13' 2.38"	Pending
Abrothrix olivaceus	NK105908	Villa Alemana	NK105908VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK105909	Villa Alemana	NK105909VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK105910	Villa Alemana	NK105910VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK105915	Villa Alemana	NK105915VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK105917	Villa Alemana	NK105917VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK105919	Villa Alemana	NK105919VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK105920	Villa Alemana	NK105920VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK105921	Villa Alemana	NK105921VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK105922	Villa Alemana	NK105922VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK105923	Villa Alemana	NK105923VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK105924	Villa Alemana	NK105924VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK105925	Villa Alemana	NK105925VAlemana	33° 4' 22.29"	71° 21' 16.70"	Pending
Abrothrix olivaceus	NK108712	Campo Ahumada	NK108712CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Abrothrix olivaceus	NK108717	Campo Ahumada	NK108717CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Abrothrix olivaceus	NK108719	Campo Ahumada	NK108719CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Abrothrix olivaceus	NK108720	Campo Ahumada	NK108720CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Abrothrix olivaceus	NK129190	La Florida	NK129190LaFlorida	33° 33' 48.96"	70° 31' 54.48"	Pending
Abrothrix olivaceus	NK129191	La Florida	NK129191LaFlorida	33° 33' 48.96"	70° 31' 54.48"	Pending
Abrothrix olivaceus	NK129192	La Florida	NK129192LaFlorida	33° 33' 48.96"	70° 31' 54.48"	Pending
Abrothrix olivaceus	NK129194	La Florida	NK129194LaFlorida	33° 33' 48.96"	70° 31' 54.48"	Pending
Abrothrix olivaceus	NK129195	La Florida	NK129195LaFlorida	33° 33' 48.96"	70° 31' 54.48"	Pending
Abrothrix olivaceus	NK129196	La Florida	NK129196LaFlorida	33° 33' 48.96"	70° 31' 54.48"	Pending
Abrothrix olivaceus	NK129198	La Florida	NK129198LaFlorida	33° 33' 48.96"	70° 31' 54.48"	Pending
Abrothrix olivaceus	NK129199	La Florida	NK129199LaFlorida	33° 33' 48.96"	70° 31' 54.48"	Pending
Abrothrix olivaceus	NK129200	La Florida	NK129200LaFlorida	33° 33' 48.96"	70° 31' 54.48"	Pending
Abrothrix olivaceus	NK120005	Paine	NK120005Paine	33° 52' 5.29"	70° 50' 12.09"	Pending

Abrothrix olivaceus	NK120007	Paine	NK120007Paine	33° 52' 5.29"	70° 50' 12.09"	Pending
Abrothrix olivaceus	NK120008	Paine	NK120008Paine	33° 52' 5.29"	70° 50' 12.09"	Pending
Abrothrix olivaceus	EP410	Quebrada de Tarapacá	EP410QTarapaca	Outgroup		Pending
Abrothrix olivaceus	EP413	Quebrada de Tarapacá	EP413QTarapaca	Outgroup		Pending
Abrothrix olivaceus	EP435	Quebrada de Camarones	EP435QCamarones	Outgroup		Pending
Abrothrix olivaceus	EP438	Quebrada de Camarones	EP438QCamarones	Outgroup		Pending
Phyllotis darwini	NK95305	San Carlos de Apoquindo	NK95305SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Phyllotis darwini	NK95336	San Carlos de Apoquindo	NK95336SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Phyllotis darwini	NK96318	San Carlos de Apoquindo	NK96318SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Phyllotis darwini	NK96359	San Carlos de Apoquindo	NK96359SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Phyllotis darwini	NK95531	San Carlos de Apoquindo	NK95531SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Phyllotis darwini	NK95544	San Carlos de Apoquindo	NK95544SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Phyllotis darwini	NK120403	San Carlos de Apoquindo	NK120403SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Phyllotis darwini	NK160855	San Carlos de Apoquindo	NK160855SCApoq	33˚ 24' 13"	70˚ 29' 01"	Pending
Phyllotis darwini	EP493	El Roble	EP493ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP544	El Roble	EP544ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP546	El Roble	EP546ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP548	El Roble	EP548ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP553	El Roble	EP553ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP558	El Roble	EP558ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP560	El Roble	EP560ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP566	El Roble	EP566ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP567	El Roble	EP567ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP569	El Roble	EP569ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP571	El Roble	EP571ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP575	El Roble	EP575ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	NK106069	El Roble	NK106069ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	NK106137	El Roble	NK106137ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending

Phyllotis darwini	NK106141	El Roble	NK106141ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	NK106146	El Roble	NK106146ElRoble	32˚ 58' 34"	71˚ 00' 50"	Pending
Phyllotis darwini	EP563	Farellones	EP563Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Phyllotis darwini	EP587	Farellones	EP587Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Phyllotis darwini	EP588	Farellones	EP588Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Phyllotis darwini	EP606	Farellones	EP606Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Phyllotis darwini	EP614	Farellones	EP614Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Phyllotis darwini	EP621	Farellones	EP621Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Phyllotis darwini	EP625	Farellones	EP625Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Phyllotis darwini	EP626	Farellones	EP626Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Phyllotis darwini	EP630	Farellones	EP630Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Phyllotis darwini	EP633	Farellones	EP633Farellones	33˚ 21' 36"	70˚ 17' 28"	Pending
Phyllotis darwini	NK108713	Campo Ahumada	NK108713CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Phyllotis darwini	NK108714	Campo Ahumada	NK108714CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Phyllotis darwini	NK108715	Campo Ahumada	NK108715CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Phyllotis darwini	NK108716	Campo Ahumada	NK108716CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Phyllotis darwini	NK108718	Campo Ahumada	NK108718CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Phyllotis darwini	NK108721	Campo Ahumada	NK108721CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Phyllotis darwini	NK108722	Campo Ahumada	NK108722CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Phyllotis darwini	NK108723	Campo Ahumada	NK108723CAhumada	32° 40' 27.11"	70° 31' 58.07"	Pending
Phyllotis darwini	EP539	La Campana	EP539LaCampana	32˚ 57' 42"	71˚ 07' 37"	Pending
Phyllotis darwini	EP540	La Campana	EP540LaCampana	32˚ 57' 42"	71˚ 07' 37"	Pending
Phyllotis darwini	EP542	La Campana	EP542LaCampana	32˚ 57' 42"	71˚ 07' 37"	Pending
Phyllotis darwini	UCK156	Altos de Cantillana	UCK156Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending
Phyllotis darwini	UCK157	Altos de Cantillana	UCK157Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending
Phyllotis darwini	UCK158	Altos de Cantillana	UCK158Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending
Phyllotis darwini	UCK159	Altos de Cantillana	UCK159Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending
Phyllotis darwini	UCK160	Altos de Cantillana	UCK160Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending

Phyllotis darwini	UCK162	Altos de Cantillana	UCK162Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending
Phyllotis darwini	UCK163	Altos de Cantillana	UCK163Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending
Phyllotis darwini	UCK164	Altos de Cantillana	UCK164Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending
Phyllotis darwini	UCK165	Altos de Cantillana	UCK165Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending
Phyllotis darwini	UCK166	Altos de Cantillana	UCK166Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending
Phyllotis darwini	UCK167	Altos de Cantillana	UCK167Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending
Phyllotis darwini	UCK168	Altos de Cantillana	UCK168Cantillana	33˚ 55' 40.95"	70˚ 57' 49.9"	Pending
Phyllotis darwini	UCK177	Altos de Chicauma	UCK177Chicauma	33˚ 16' 59"	70˚ 58' 14"	Pending
Phyllotis darwini	UCK178	Atos de Chicauma	UCK178Chicauma	33˚ 16' 59"	70˚ 58' 14"	Pending
Phyllotis darwini	UCK181	Altos de Chicauma	UCK181Chicauma	33˚ 16' 59"	70˚ 58' 14"	Pending
Phyllotis darwini	UCK183	Altos de Chicauma	UCK183Chicauma	33˚ 16' 59"	70˚ 58' 14"	Pending
Phyllotis darwini	NK108791	El Canelo	NK108791Elcanelo	33° 33' 21.89"	70° 27' 15.99"	Pending
Phyllotis magister	ER4	Desembocadura Rio Loa	4Pmagister	Outgroup		JN226733
Phyllotis magister	ER5	Desembocadura Rio Loa	5Pmagister	Outgroup		JN226734

Capítulo III

APPENDIX I.- List of individuals by catalog number, species, locality, initials, geographic coordinates and GeneBank accession number.

Species	Catalogue Number	Locality	Initials	Latitude (S)	Longitude (W)	Genebank accesion number
Phyllotis darwini	NK 106069	Cerro el Roble, Til-Til	CR69	32°59´24´´	71°1´12´´	JN226723
Phyllotis darwini	NK 106137	Cerro el Roble, Til-Til	CR37	32°59´24´´	71°1´12´´	JN226724
Phyllotis darwini	NK 106141	Cerro el Roble, Til-Til	CR41	32°59´24´´	71°1´12´´	JN226725
Phyllotis darwini	NK 106146	Cerro el Roble, Til-Til	CR46	32°59´24´´	71°1´12´´	JN226726
Phyllotis darwini	NK 105341	Cerro Santa Inés, Los Vilos	CSI41	32°9´0´´	71°28´48´´	JN226718
Phyllotis darwini	NK 105348	Cerro Santa Inés, Los Vilos	CSI48	32°9´0´´	71°28´48´´	JN226719
Phyllotis darwini	NK 105349	Cerro Santa Inés, Los Vilos	CSI49	32°9´0´´	71°28´48´´	JN226720
Phyllotis darwini	NK 95618	Chillepín	CH18	31°52’48´´	70°47´24´´	JN226690
Phyllotis darwini	NK 95620	Chillepín	CH20	31°52’48´´	70°47´24´´	JN226691
Phyllotis darwini	NK 95624	Chillepín	CH24	31°52’48´´	70°47´24´´	JN226692
Phyllotis darwini	NK 120051	Concepción, Tomé Agua Tendida	Ate51	36°38´24´´	72°47´24´´	JN226727
Phyllotis darwini	NK 120068	Concepción, Tomé Agua Tendida	Ate68	36°38´24´´	72°47´24´´	JN226728
Phyllotis magister	ER3	Desembocadura Río Loa	Phm3	21°25´33´´	70°33´36´´	JN226732
Phyllotis magister	ER4	Desembocadura Río Loa	Phm4	21°25´33´´	70°33´36´´	JN226733
Phyllotis magister	ER5	Desembocadura Río Loa	Phm5	21°25´33´´	70°33´36´´	JN226734
Phyllotis magister	ER7	Desembocadura Río Loa	Phm7	21°25´33´´	70°33´36´´	JN226735
Phyllotis darwini	SSUC-Ma-00451	El Guanaco, Quirihue, Ñuble	QUI51	36°24´0´´	72°37´30´´	JN226683
Phyllotis darwini	SSUC-Ma-00452	El Guanaco, Quirihue, Ñuble	QUI52	36°24´0´´	72°37´30´´	JN226684
Phyllotis darwini	SSUC-Ma-00453	El Guanaco, Quirihue, Ñuble	QUI53	36°24´0´´	72°37´30´´	JN226685
Phyllotis darwini	SSUC-Ma-00457	El Guanaco, Quirihue, Ñuble	QUI57	36°24´0´´	72°37´30´´	JN226686
Phyllotis darwini	SSUC-Ma-00458	El Guanaco, Quirihue, Ñuble	QUI58	36°24´0´´	72°37´30´´	JN226687

Phyllotis darwini	SSUC-Ma-00459	El Guanaco, Quirihue, Ñuble	QUI59	36°24´0´´	72°37´30´´	JN226688
Phyllotis darwini	SSUC-Ma-00460	El Guanaco, Quirihue, Ñuble	QUI60	36°24´0´´	72°37´30´´	JN226689
Phyllotis darwini	NK 96868	Fray Jorge	FJ68	30°40´12´´	71°37´48´´	JN226713
Phyllotis darwini	NK 96869	Fray Jorge	FJ69	30°40´12´´	71°37´48´´	JN226714
Phyllotis darwini	NK 96871	Fray Jorge	FJ71	30°40´12´´	71°37´48´´	JN226715
Phyllotis darwini	NK 96874	Fray Jorge	FJ74	30°40´12´´	71°37´48´´	JN226716
Phyllotis darwini	NK 105515	Fray Jorge	FJ15	30°40´12´´	71°37´48´´	JN226722
Phyllotis darwini	SSUC-Ma-00426	Las Tacas	LT26	30°0´0´´	71°22´26´´	JN226672
Phyllotis darwini	SSUC-Ma-00427	Las Tacas	LT27	30°0´0´´	71°22´26´´	JN226673
Phyllotis darwini	SSUC-Ma-00428	Las Tacas	LT28	30°0´0´´	71°22´26´´	JN226674
Phyllotis darwini	SSUC-Ma-00429	Las Tacas	LT29	30°0´0´´	71°22´26´´	JN226675
Phyllotis darwini	SSUC-Ma-00431	Las Tacas	LT31	30°0´0´´	71°22´26´´	JN226676
Phyllotis darwini	SSUC-Ma-00433	Las Tacas	LT33	30°0´0´´	71°22´26´´	JN226677
Phyllotis darwini	NK 160004	Llanos del Challe	LCH04	28°4´48´´	71°8´24´´	JN226731
Phyllotis darwini	SSUC-Ma-00442	Los Molles	LMo42	30°14´24´´	71°30´0´´	JN226681
Phyllotis darwini	NK 96846	Observatorio La Silla	OS46	29°15´0´´	70°43´48´´	JN226708
Phyllotis darwini	NK 96847	Observatorio La Silla	OS47	29°15´0´´	70°43´48´´	JN226709
Phyllotis darwini	NK 96852	Observatorio La Silla	OS52	29°15´0´´	70°43´48´´	JN226710
Phyllotis darwini	NK 96855	Observatorio La Silla	OS55	29°15´0´´	70°43´48´´	JN226711
Phyllotis darwini	NK 96864	Observatorio La Silla	OS64	29°15´0´´	70°43´48´´	JN226712
Phyllotis darwini	NK 142983	Pan de Azucar	Paz83	26°8´24´´	70°39´36´´	JN226729
Phyllotis darwini	NK 142984	Pan de Azucar	Paz84	26°8´24´´	70°39´36´´	JN226730
Phyllotis darwini	NK 96575	Pelambres	PE75	31°49´12´´	70°34´48´´	JN226700
Phyllotis darwini	NK 96586	Pelambres	PE86	31°49´12´´	70°34´48´´	JN226701
Phyllotis darwini	NK 96591	Pelambres	PE91	31°49´12´´	70°34´48´´	JN226702
Phyllotis darwini	NK 96595	Pelambres	PE95	31°49´12´´	70°34´48´´	JN226703
Phyllotis darwini	NK 96596	Pelambres	PE96	31°49´12´´	70°34´48´´	JN226704
Phyllotis darwini	NK 105381	Llanos del Challe	LCH81	28°4´48´´	71°8´24´´	JN226721

Phyllotis darwini	SSUC-Ma-00435	Punta de Choros	PCh35	29°15´0´´	71°27´36´´	JN226678
Phyllotis darwini	SSUC-Ma-00437	Punta de Choros	PCh37	29°15´0´´	71°27´36´´	JN226679
Phyllotis darwini	SSUC-Ma-00445	Punta de Choros	PCh45	29°15´0´´	71°27´36´´	JN226682
Phyllotis darwini	SSUC-Ma-00438	Quebrada de la Plata	PCh38	29°15´0´´	71°27´36´´	JN226680
Phyllotis darwini	NK 96738	Quebrada del Tigre	QT38	32°33´36´´	71°25´48´´	JN226705
Phyllotis darwini	NK 96754	Quebrada del Tigre	QT54	32°33´36´´	71°25´48´´	JN226706
Phyllotis darwini	NK 95968	Rinconada Huechún	RHU68	33°0´36´´	70°48´36´´	JN226693
Phyllotis darwini	NK 95973	Rinconada Huechún	RHU73	33°0´36´´	70°48´36´´	JN226694
Phyllotis darwini	NK 96312	San Carlos Apoquindo	SA12	33°24´0´´	70°28´48´´	JN226695
Phyllotis darwini	NK 96319	San Carlos Apoquindo	SA19	33°24´0´´	70°28´48´´	JN226696
Phyllotis darwini	NK 96323	San Carlos Apoquindo	SA23	33°24´0´´	70°28´48´´	JN226697
Phyllotis darwini	NK 96349	San Carlos Apoquindo	SA49	33°24´0´´	70°28´48´´	JN226698
Phyllotis darwini	NK 96359	San Carlos Apoquindo	SA59	33°24´0´´	70°28´48´´	JN226699
Phyllotis darwini	NK 96817	San Carlos Apoquindo	SA17	33°24´0´´	70°28´48´´	JN226707
Phyllotis darwini	NK 105329	San Carlos Apoquindo	SA29	33°24´0´´	70°28´48´´	JN226717
Phyllotis darwini	SSUC-Ma-00416	Tranque de relaves Barahona	TrBa16	34°4´51´´	70°31´12´´	JN226664
Phyllotis darwini	SSUC-Ma-00417	Tranque de relaves Barahona	TrBa17	34°4´51´´	70°31´12´´	JN226665
Phyllotis darwini	SSUC-Ma-00420	Tranque de relaves Barahona	TrBa20	34°4´51´´	70°31´12´´	JN226666
Phyllotis darwini	SSUC-Ma-00421	Tranque de relaves Barahona	TrBa21	34°4´51´´	70°31´12´´	JN226667
Phyllotis darwini	SSUC-Ma-00422	Tranque de relaves Barahona	TrBa22	34°4´51´´	70°31´12´´	JN226668
Phyllotis darwini	SSUC-Ma-00423	Tranque de relaves Barahona	TrBa23	34°4´51´´	70°31´12´´	JN226669
Phyllotis darwini	SSUC-Ma-00424	Tranque de relaves Barahona	TrBa24	34°4´51´´	70°31´12´´	JN226670
Phyllotis darwini	SSUC-Ma-00425	Tranque de relaves Barahona	TrBa25	34°4´51´´	70°31´12´´	JN226671
Thylamys elegans	NK95358	Rinconada de Maipú	RMaipu58	33°29'40,92"	70°53'34,5"	Pending
Thylamys elegans	NK95677	San Carlos de Apoquindo	SCApoq77	33°28'8,28"	70°29'18,54"	KF164529
Thylamys elegans	NK105407	Quebrada de Córdoba	QCordoba07	33°26'18,06"	71°39'14,76"	Pending
Thylamys elegans	NK95435	Rinconada de Maipú	RMaipu35	33°29'40,92"	70°53'34,5"	Pending
Thylamys elegans	NK95436	Rinconada de Maipú	RMaipu36	33°29'40,92"	70°53'34,5"	KF164526

Thylamys elegans	NK108788	El Canelo	Canelo88	33°33'21,9"	70°27'16"	Pending
Thylamys elegans	NK95673	San Carlos de Apoquindo	SCApoq73	33°28'8,28"	70°29'18,54"	Pending
Thylamys elegans	NK96065	San Carlos de Apoquindo	SCApoq65	33°28'8,28"	70°29'18,54"	Pending
Thylamys elegans	NK96096	San Carlos de Apoquindo	SCApoq96	33°28'8,28"	70°29'18,54"	Pending
Thylamys elegans	NK96097	San Carlos de Apoquindo	SCApoq97	33°28'8,28"	70°29'18,54"	Pending
Thylamys elegans	NK96306	San Carlos de Apoquindo	SCApoq06	33°28'8,28"	70°29'18,54"	Pending
Thylamys elegans	NK105332	San Carlos de Apoquindo	SCApoq32	33°28'8,28"	70°29'18,54"	Pending
Thylamys elegans	NK105336	San Carlos de Apoquindo	SCApoq36	33°28'8,28"	70°29'18,54"	Pending
Thylamys elegans	NK95111	San Carlos de Apoquindo	SCApoq11	33°28'8,28"	70°29'18,54"	HM583378
Thylamys elegans	SSUC-Ma-00519	Desembocadura Río Loa	DLoa19	21°25'43,75"	70°03'30,35"	KF164532
Thylamys elegans	SSUC-Ma-00520	Desembocadura Río Loa	DLoa20	21°25'43,75"	70°03'30,35"	KF164533
Thylamys elegans	NK96879	Fray Jorge	FJorge79	30°38'18,276"	71°39'16,596"	KF164531
Thylamys elegans	NK27583	Fray Jorge	FJorge83	30°38'18,276"	71°39'16,596"	HM583376
Thylamys elegans	NK95354	Rinconada de Maipú	RMaipu54	33°29'40,92"	70°53'34,5"	KF164527
Thylamys elegans	NK96791	Rinconada de Maipú	RMaipu91	33°29'40,92"	70°53'34,5"	KF164530
Thylamys elegans	NK27606	La Campana	Campana06	32°57'42"	71°07'37"	HM583377
Thylamys elegans	EP538	La Campana	Campana38	32°57'42"	71°07'37"	Pending
Thylamys elegans	EP543	La Campana	Campana43	32°57'42"	71°07'37"	Pending
Thylamys elegans	NK108071	Cerro el Roble, Til-Til	Roble71	33°0'37,44"	71°0'46,08"	Pending
Thylamys elegans	NK95971	Rinconada Huechún	RHuechun71	33°1'3,9"	70°49'1,31"	KF164530
Thylamys elegans	NK95691	San Carlos de Apoquindo	SCApoq91	33°28'8,28"	70°29'18,54"	KF164528
Thylamys elegans	NK160518	Vilches	Vilches18	35°35'4,7"	71°5'28"	KF164535
Thylamys elegans	NK160526	Vilches	Vilches26	35°35'4,7"	71°5'28"	KF164534
Thylamys elegans	NK160466	Tregualemu	Tregualemu66	35°56'59,6"	72°44'38,4"	KF164536
Thylamys elegans	NK106178	Duao	Duao78	34°52'55,56"	72°09'15,08"	KF164537
Thylamys elegans	NK160945	Lipimávida	Lipimavida45	34°52'12,2"	72°08'50,6"	KF164538
Thylamys elegans	NK60947	Lipimávida	Lipimavida47	34°52'12,2"	72°08'50,6"	Pending
Thylamys elegans	NK160970	Lipimávida	Lipimavida70	34°52'12,2"	72°08'50,6"	Pending

Thylamys elegans	NK160971	Lipimávida	Lipimavida71	34°52'12,2"	72°08'50,6"	Pending
Thylamys elegans	NK160972	Lipimávida	Lipimavida72	34°52'12,2"	72°08'50,6"	Pending
Thylamys elegans	NK105928	Las Peñas	LasPenas28	34°46'	70°46'	KF164540
Thylamys elegans	NK95963	Rinconada Huechún	RHuechun63	33°1'3,9"	70°49'1,31"	Pending
Thylamys elegans	NK96571	Pelambres	Pelambres71	31°49'13,7"	70°34'56,1"	Pending
Thylamys elegans	NK95622	Chillepín	Chillepin22	31°53'12,12"	70°47'47,04"	Pending
Thylamys elegans	NK96763	Quebrada del Tigre	QTigre63	32°33'36,396"	71°26'18,672"	HM583379
Thylamys elegans	SSUC-Ma-00522	Desembocadura Río Loa	DLoa22	21°25'43,75"	70°03'30,35"	Pending
Thylamys elegans	SSUC-Ma-00523	Desembocadura Río Loa	DLoa23	21°25'43,75"	70°03'30,35"	Pending
Thylamys elegans	SSUC-Ma-00525	Desembocadura Río Loa	DLoa25	21°25'43,75"	70°03'30,35"	Pending
Thylamys elegans	SSUC-Ma-00524	Desembocadura Río Loa	DLoa24	21°25'43,75"	70°03'30,35"	Pending
Thylamys pallidior	EP440	Quebrada de Camarones	Arica40_pallidiorNorte	19°11'25,3"	70°16'07"	Pending
Thylamys pallidior	UP793	Establecimiento San Nicolás	RioNegro93_pallidiorSur	41º43'50"	67º09'49"	Pending
Thylamys sp	HZP3576	Acho, Ayo, Valle de los Volcanes, Castilla	HZP76_sp	15º39’47,67”	72º18’16,02’’	KF164541
Thylamys tatei	MUSM23121	Pallasca, Pampas, 10 km to Pallasca	MUSM21_tatei	8°13’45,984’’	77°54’18,684’’	KF164555
Thylamys elegans	NK106072	Hijuelas	Hijuelas72	32°48'44,64"	71°5'20,76"	Pending
Thylamys elegans	NK106113	El Ingenio	Ingenio13	33°47'9,96"	70°14'51"	Pending
Thylamys elegans	NK108881	Quebrada de Cordoba	QCordoba81	33°26'18,06"	71°39'14,76"	Pending
Thylamys elegans	NK160924	Longotoma	Longotoma24	32°16'38,9"	71°14'13,3"	Pending
Thylamys elegans	NK160927	Longotoma	Longotoma27	32°16'38,9"	71°14'13,3"	Pending
Thylamys elegans	NK160925	Longotoma	Longotoma25	32°16'38,9"	71°14'13,3"	Pending
Thylamys elegans	NK160926	Longotoma	Longotoma26	32°16'38,9"	71°14'13,3"	Pending
Thylamys elegans	NK160932	Longotoma	Longotoma32	32°16'38,9"	71°14'13,3"	Pending
Thylamys elegans	NK160933	Longotoma	Longotoma33	32°16'38,9"	71°14'13,3"	Pending
Thylamys elegans	NK160934	Longotoma	Longotoma34	32°16'38,9"	71°14'13,3"	Pending
Thylamys elegans	NK160935	Longotoma	Longotoma35	32°16'38,9"	71°14'13,3"	Pending
Thylamys elegans	UCK807	Leyda	Leyda1	33°34'08,1"	71°22'22,3"	Pending
Thylamys elegans	UCK808	Leyda	Leyda2	33°34'08,1"	71°22'22,3"	Pending

Thylamys elegans	UCK809	El Mauro	Mauro1	31°58'24,01"	71°0'15,80"	Pending
Thylamys elegans	UCK816	El Mauro	Mauro7	31°58'24,01"	71°0'15,80"	Pending
Thylamys elegans	UCK810	El Mauro	Mauro2	31°58'24,01"	71°0'15,80"	Pending
Thylamys elegans	UCK8	Los Patos	LosPatos8	32°31'59,3"	70°39'27,1"	Pending
Thylamys elegans	UCK10	La Mora	LaMora10	32°29'45,8"	70°55'40,2"	Pending
Thylamys elegans	RdeSilvaUCK9	Rinconada de Silva	RSilva9	32°38'38,8"	70°41'39,2"	Pending
Thylamys elegans	UCK12	Puente Angeles	LosAngeles5UCK12	32°30'51,5"	70°48'06,8"	Pending
Thylamys elegans	UCK14	Los Queñes	LosQuenes14	35°00'14,8"	70°49'54,9"	Pending
Thylamys elegans	UCK15	Los Queñes	LosQuenes15	35°00'14,8"	70°49'54,9"	Pending
Thylamys elegans	UCK16	Cantarrana	Cantarrana16	34°23'22,7"	71°03'16,9"	Pending
Thylamys elegans	UCK17	San Enrique	SanEnrique17	33°53'38,6"	71°44'47,7"	Pending
Thylamys elegans	UCK18	San Enrique	SanEnrique18	33°53'38,6"	71°44'47,7"	Pending
Thylamys elegans	UCK19	San Enrique	SanEnrique19	33°53'38,6"	71°44'47,7"	Pending
Liolaemus monticola	CUCH 1808	Petorca	Petorca1808	32°17’	71°02’	AY851704
Liolaemus monticola	CUCH 1807	Petorca	Petorca1807	32°17’	71°02’	AY851704
Liolaemus monticola	CUCH 2358	Mina Cerrillos	MCerrillos2358	32°42’	70°55’	AY851706
Liolaemus monticola	CUCH 2359	Mina Cerrillos	MCerrillos2359	32°42’	70°55’	AY851707
Liolaemus monticola	CUCH 2376	Cabrería	Cabreria2376	32°49’	71°05’	AY851708
Liolaemus monticola	CUCH 2377	Cabrería	Cabreria2377	32°49’	71°05’	AY851709
Liolaemus monticola	CUCH 2091	Rocín	Rocin2091	32°28’	70°27’	AY851710
Liolaemus monticola	CUCH 2453	Saladillo	Saladillo2453	32°55’	70°10’	AY851711
Liolaemus monticola	CUCH 2137	Río Blanco	RBlanco2137	32°55’	70°16’	AY851712
Liolaemus monticola	CUCH 2551	Colorado Norte	ColoradoN2551	32°50’	70°24’	AY851713
Liolaemus monticola	CUCH 2559	Colorado Sur	ColoradoS2559	32°51’	70°23’	AY851714
Liolaemus monticola	CUCH 1386	La Campana	Campana1386	32°57’	71°07’	AY851715
Liolaemus monticola	CUCH 2794	El Roble	ElRoble2794	32°58’	71°00’	AY851716
Liolaemus monticola	CUCH 2688	La Dormida	LDormida2688	33°03’	71°02’	AY851717
Liolaemus monticola	CUCH 2213	Cuesta Chacabuco	Chacabuco2213	32°58’	70°42’	AY851718

Liolaemus monticola	CUCH 2211	Cuesta Chacabuco	Chacabuco2211	32°58’	70°42’	AY851719
Liolaemus monticola	MZUC 28604	Lampa	Lampa406	33°16’	70°53’	AY851720
Liolaemus monticola	MZUC 28606	Lampa	Lampa404	33°16’	70°53’	AY851721
Liolaemus monticola	CUCH 2860	Yeso Norte	YesoNorte2860	33°47’	70°12’	AY850614
Liolaemus monticola	CUCH 2858	Yeso Norte	YesoNorte2858	33°47’	70°12’	AY851722
Liolaemus monticola	CUCH 2841	Alfalfal Sur	AlfalfalS2841	33°32’	70°16’	AY850615
Liolaemus monticola	CUCH 2834	Alfalfal Norte	AlfalfalN2834	33°32’	70°16’	AY850616
Liolaemus monticola	CUCH 1719	El Manzano	Manzano1719	33°35’	70°24’	AY850617
Liolaemus monticola	MZUC 28609	El Canelo	Canelo396	33°33’	70°27’	AY851723
Liolaemus monticola	CUCH 3030	Rinconada de Maipu	RMaipu3030	33°29’	70°53’	AY851724
Liolaemus monticola	CUCH 1439	Arrayán	Arrayan1439	33°20’	70°28’	AY850618
Liolaemus monticola	CUCH 1660	Yerba Loca	YerbaLoca1660	33°19’	70°20’	AY850619
Liolaemus monticola	MZUC 28597	Farellones	Farellones394	33°20’	70°19’	AY851725
Liolaemus monticola	CUCH 1457	Quebrada de Alvarado	QAlvarado1457	33°07’	71°07’	AY851726
Liolaemus monticola	MZUC 28601	Baños Morales	BMorales400	33°49’	70°04’	AY851727
Liolaemus monticola	CUCH 1646	Yeso Sur	YesoSur1646	33°47’	70°13’	AY850620
Liolaemus monticola	CUCH 2763	Yeso Sur	YesoSur2763	33°47’	70°13’	AY851728
Liolaemus monticola	CUCH 2762	Yeso Sur	YesoSur2762	33°47’	70°13’	AY851729
Liolaemus monticola	CUCH 2758	Yeso Sur	YesoSur2758	33°47’	70°13’	AY851730
Liolaemus monticola	CUCH 2759	Yeso Sur	YesoSur2759	33°47’	70°13’	AY851731
Liolaemus monticola	CUCH 2661	Maipo Sur	MaipoSur2661	33°48’	70°09’	AY851732
Liolaemus monticola	CUCH 2665	Maipo Sur	MaipoSur2665	33°48’	70°09’	AY851733
Liolaemus monticola	CUCH 1379	Volcán Sur	VolcanSur1379	33°48’	70°09’	AY851734
Liolaemus monticola	CUCH 1310	Cantillana	Cantillana1310	33°57’	70°56’	AY851735
Liolaemus monticola	CUCH 1311	Cantillana	Cantillana1311	33°57’	70°56’	AY851736
Liolaemus monticola	CUCH 2655	Rio Clarillo	RClarillo2655	33°43’	70°30’	AY851737
Liolaemus monticola	MZUC 28603	San Fernando	SFernando203	34°45’	70°47’	AY851738
Liolaemus monticola	CUCH 2638	Los Queñes	LosQuenes2638	35°00’	70°58’	AY851739

Liolaemus monticola	CUCH 2384	Rio Lontué	RLontue2384	35°10’	71°10’	AY851740
Liolaemus monticola	CUCH 2382	Rio Lontué	RLontue2382	35°10’	71°10’	AY851741
Liolaemus tenuis	CUCH 2656	Outgroup				AY850633
Liolaemus nigroviridis	MZUC 26300	Outgroup				AY173795
Rhinella arunco	DBGUCH 1002003	Huentelauquén (río Choapa)	Hue2003	31°35'16,6"	71°31'30,1"	KC778247
Rhinella arunco	DBGUCH 1002004	Huentelauquén (río Choapa)	Hue2004	31°35'16,6"	71°31'30,1"	KC778248
Rhinella arunco	DBGUCH 1002005	Huentelauquén (río Choapa)	Hue2005	31°35'16,6"	71°31'30,1"	KC778249
Rhinella arunco	DBGUCH 1002006	Huentelauquén (río Choapa)	Hue2006	31°35'16,6"	71°31'30,1"	KC778250
Rhinella arunco	DBGUCH 1002007	Huentelauquén (río Choapa)	Hue2007	31°35'16,6"	71°31'30,1"	KC778251
Rhinella arunco	DBGUCH 1002008	Huentelauquén (río Choapa)	Hue2008	31°35'16,6"	71°31'30,1"	KC778252
Rhinella arunco	DBGUCH 0708002	Pupío Medio	PM8002	31°51'55,0"	71°18'45,5"	HQ132630
Rhinella arunco	DBGUCH 0708004	Pupío Medio	PM8004	31°51'55,0"	71°18'45,5"	HQ132631
Rhinella arunco	DBGUCH 0708006	Pupío Medio	PM8006	31°51'55,0"	71°18'45,5"	HQ132632
Rhinella arunco	DBGUCH 0708007	Pupío Medio	PM8007	31°51'55,0"	71°18'45,5"	HQ132633
Rhinella arunco	DBGUCH 0708008	Pupío Medio	PM8008	31°51'55,0"	71°18'45,5"	HQ132634
Rhinella arunco	DBGUCH 0708009	Pupío Medio	PM8009	31°51'55,0"	71°18'45,5"	HQ132635
Rhinella arunco	DBGUCH 0708010	Pupío Medio	PM8010	31°51'55,0"	71°18'45,5"	HQ132636
Rhinella arunco	DBGUCH 0708011	Pupío Medio	PM8011	31°51'55,0"	71°18'45,5"	HQ132637
Rhinella arunco	DBGUCH 0808027	Pupío Medio	PM8027	31°51'55,0"	71°18'45,5"	HQ132638
Rhinella arunco	DBGUCH 0808028	Pupío Medio	PM8028	31°51'55,0"	71°18'45,5"	HQ132639
Rhinella arunco	DBGUCH 0808031	Pupío Medio	PM8031	31°51'55,0"	71°18'45,5"	HQ132640
Rhinella arunco	DBGUCH 0808030	Pupío Medio	PM8030	31°51'55,0"	71°18'45,5"	HQ132641
Rhinella arunco	DBGUCH 0808029	Pupío Medio	PM8029	31°51'55,0"	71°18'45,5"	HQ132642
Rhinella arunco	DBGUCH 0701210	Puente Pupío	PP210	31°52'14,1"	71°23'55,2"	HQ132616
Rhinella arunco	DBGUCH 0701211	Puente Pupío	PP211	31°52'14,1"	71°23'55,2"	HQ132617
Rhinella arunco	DBGUCH 0701212	Puente Pupío	PP212	31°52'14,1"	71°23'55,2"	HQ132618
Rhinella arunco	DBGUCH 0701202	Puente Pupío	PP202	31°52'14,1"	71°23'55,2"	HQ132619
Rhinella arunco	DBGUCH 0705005	Puente Pupío	PP005	31°52'14,1"	71°23'55,2"	HQ132620

Rhinella arunco	DBGUCH 0701178	Puente Pupío	PP178	31°52'14,1"	71°23'55,2"	HQ132621
Rhinella arunco	DBGUCH 0701199	Puente Pupío	PP199	31°52'14,1"	71°23'55,2"	HQ132622
Rhinella arunco	DBGUCH 0808036	Puente Pupío	PP8036	31°52'14,1"	71°23'55,2"	HQ132623
Rhinella arunco	DBGUCH 0808037	Puente Pupío	PP8037	31°52'14,1"	71°23'55,2"	HQ132624
Rhinella arunco	DBGUCH 0908001	Puente Pupío	PP8001	31°52'14,1"	71°23'55,2"	HQ132625
Rhinella arunco	DBGUCH 0908002	Puente Pupío	PP8002	31°52'14,1"	71°23'55,2"	HQ132626
Rhinella arunco	DBGUCH 0908004	Puente Pupío	PP8004	31°52'14,1"	71°23'55,2"	HQ132627
Rhinella arunco	DBGUCH 0908005	Puente Pupío	PP8005	31°52'14,1"	71°23'55,2"	HQ132628
Rhinella arunco	DBGUCH 0908006	Puente Pupío	PP8006	31°52'14,1"	71°23'55,2"	HQ132629
Rhinella arunco	DBGUCH 0810012	Quilimarí Medio	QM0012	32°07'04,1"	71°19'25,3"	KC778253
Rhinella arunco	DBGUCH 0701227	Quilimarí	Qui227	32°07'12,6"	71°28'10,6"	HQ132643
Rhinella arunco	DBGUCH 0701228	Quilimarí	Qui228	32°07'12,6"	71°28'10,6"	HQ132644
Rhinella arunco	DBGUCH 0701246	Quilimarí	LP246	32°07'12,6"	71°28'10,6"	HQ132645
Rhinella arunco	DBGUCH 0701247	Quilimarí	LP247	32°07'12,6"	71°28'10,6"	HQ132646
Rhinella arunco	DBGUCH 0701243	Quilimarí	LP1243	32°07'12,6"	71°28'10,6"	HQ132647
Rhinella arunco	DBGUCH 0701244	Quilimarí	LP1244	32°07'12,6"	71°28'10,6"	HQ132648
Rhinella arunco	DBGUCH 0701245	Quilimarí	Qui1245	32°07'12,6"	71°28'10,6"	HQ132649
Rhinella arunco	DBGUCH 0907001	Quilimarí	Qui7001	32°07'12,6"	71°28'10,6"	HQ132650
Rhinella arunco	DBGUCH 0907002	Quilimarí	Qui7002	32°07'12,6"	71°28'10,6"	HQ132651
Rhinella arunco	DBGUCH 0701221	Quilimarí	Qui1221	32°07'12,6"	71°28'10,6"	HQ132652
Rhinella arunco	DBGUCH 0909011	Quilimarí	Qui9011	32°07'12,6"	71°28'10,6"	HQ132653
Rhinella arunco	DBGUCH 0909012	Quilimarí	Qui9012	32°07'12,6"	71°28'10,6"	HQ132654
Rhinella arunco	DBGUCH 0909013	Quilimarí	Qui9013	32°07'12,6"	71°28'10,6"	HQ132655
Rhinella arunco	DBGUCH 0909014	Quilimarí	Qui9014	32°07'12,6"	71°28'10,6"	HQ132656
Rhinella arunco	DBGUCH 0812047	Los Molles	LM2047	32°13'28,4"	71°29'58,7"	KC778264
Rhinella arunco	DBGUCH 0812045	Los Molles	LM2045	32°13'28,4"	71°29'58,7"	KC778265
Rhinella arunco	DBGUCH 0812046	Los Molles	LM2046	32°13'28,4"	71°29'58,7"	KC778266
Rhinella arunco	DBGUCH 0810003	El Sobrante	ES0003	32°13'44,7"	70°44'11,3"	KC778271

Rhinella arunco	DBGUCH 0701255	Palquico	Pal255	32°14'28,5"	71°08'05,2"	Pending
Rhinella arunco	DBGUCH 0711001	Petorca	PET001	32°15'05,6"	70°55'09,5"	KC778276
Rhinella arunco	DBGUCH 0711003	Petorca	PET003	32°15'05,6"	70°55'09,5"	KC778277
Rhinella arunco	DBGUCH 0711002	Petorca	Pet1002	32°15'05,6"	70°55'09,5"	KC778278
Rhinella arunco	DBGUCH 0701257	Santa Julia	SJ257	32°18'21,2"	71°03'16,6"	Pending
Rhinella arunco	DBGUCH 0902011	Huaquén	Hua2011	32°20'01,9"	71°25'01,2"	KC778267
Rhinella arunco	DBGUCH 0902012	Huaquén	Hua2012	32°20'01,9"	71°25'01,2"	KC778268
Rhinella arunco	DBGUCH 0902014	Huaquén	Hua2014	32°20'01,9"	71°25'01,2"	KC778270
Rhinella arunco	DBGUCH 0902021	El Trapiche	ET2021	32°18'45,2"	71°16'38,6"	HQ132657
Rhinella arunco	DBGUCH 0902023	El Trapiche	ET2023	32°18'45,2"	71°16'38,6"	HQ132658
Rhinella arunco	DBGUCH 0902025	El Trapiche	ET2025	32°18'45,2"	71°16'38,6"	HQ132659
Rhinella arunco	DBGUCH 0902026	El Trapiche	ET2026	32°18'45,2"	71°16'38,6"	HQ132660
Rhinella arunco	DBGUCH 0902022	El Trapiche	ET2022b	32°18'45,2"	71°16'38,6"	HQ132661
Rhinella arunco	DBGUCH 0902024	El Trapiche	ET2024b	32°18'45,2"	71°16'38,6"	HQ132662
Rhinella arunco	DBGUCH 0902007	El Trapiche	ET2007	32°18'45,2"	71°16'38,6"	KC778272
Rhinella arunco	DBGUCH 0902009	El Trapiche	ET2009	32°18'45,2"	71°16'38,6"	KC778273
Rhinella arunco	DBGUCH 0902010	El Trapiche	ET2010	32°18'45,2"	71°16'38,6"	KC778274
Rhinella arunco	DBGUCH 0902008	El Trapiche	ET2008	32°18'45,2"	71°16'38,6"	KC778275
Rhinella arunco	DBGUCH 0902018	Illalolén	Illa2018	32°26'23,5"	71°14'10,0"	KC778279
Rhinella arunco	DBGUCH 0902020	Illalolén	Illa2020	32°26'23,5"	71°14'10,0"	KC778280
Rhinella arunco	DBGUCH 0902019	Illalolén	Illa2019	32°26'23,5"	71°14'10,0"	KC778281
Rhinella arunco	DBGUCH 1102001	Campo Ahumada bajo	CAB2001	32°43'51,0"	70°34'01,5"	KC778282
Rhinella arunco	DBGUCH 1102002	Campo Ahumada bajo	CAB2002	32°43'51,0"	70°34'01,5"	KC778283
Rhinella arunco	DBGUCH 1102003	Campo Ahumada bajo	CAB2003	32°43'51,0"	70°34'01,5"	KC778284
Rhinella arunco	DBGUCH 0611002	Las Chilcas	LCL1	32°52'04,7"	70°50'35,1"	KC778285
Rhinella arunco	DBGUCH 0611004	Las Chilcas	LCh1004	32°52'04,7"	70°50'35,1"	KC778286
Rhinella arunco	DBGUCH 0909008	Las Chilcas	LCh9008	32°52'04,7"	70°50'35,1"	KC778287
Rhinella arunco	DBGUCH 0611003	Las Chilcas	LCh1003	32°52'04,7"	70°50'35,1"	KC778288

Rhinella arunco	DBGUCH 1002001	Las Chilcas	LCh2001	32°52'04,7"	70°50'35,1"	KC778289
Rhinella arunco	DBGUCH 1002002	Las Chilcas	LCh2002	32°52'04,7"	70°50'35,1"	KC778290
Rhinella arunco	DBGUCH 0909009	Las Chilcas	LCh9009	32°52'04,7"	70°50'35,1"	Pending
Rhinella arunco	DBGUCH 1011021	Estero Puangue	EP1021	33°15'26,7"	71°09'03,3"	KC778300
Rhinella arunco	DBGUCH 1011022	Estero Puangue	EP1022	33°15'26,7"	71°09'03,3"	KC778301
Rhinella arunco	DBGUCH 1011023	Estero Puangue	EP1023	33°15'26,7"	71°09'03,3"	KC778302
Rhinella arunco	DBGUCH 1011024	Estero Puangue	EP1024	33°15'26,7"	71°09'03,3"	KC778303
Rhinella arunco	DBGUCH 1011025	Estero Puangue	EP1025	33°15'26,7"	71°09'03,3"	KC778304
Rhinella arunco	DBGUCH 1011026	Estero Puangue	EP1026	33°15'26,7"	71°09'03,3"	KC778305
Rhinella arunco	DBGUCH 1011027	Estero Puangue	EP1027	33°15'26,7"	71°09'03,3"	KC778306
Rhinella arunco	DBGUCH 1102050	Río Molina	RM2050	33°22'24,1"	70°23'47,3"	KC778307
Rhinella arunco	DBGUCH 1102051	Río Molina	RM2051	33°22'24,1"	70°23'47,3"	KC778308
Rhinella arunco	DBGUCH 1102049	Río Molina	RM2049	33°22'24,1"	70°23'47,3"	KC778309
Rhinella arunco	DBGUCH 1102048	Río Molina	RM2048	33°22'24,1"	70°23'47,3"	KC778310
Rhinella arunco	DBGUCH 2096	Curacaví	Bc2096Curacavi	33°24'42,7"	71°09'15,8"	Pending
Rhinella arunco	DBGUCH 2154	Curacaví	Bchi45Curacavi2154	33°24'42,7"	71°09'15,8"	Pending
Rhinella arunco	DBGUCH 0611005	Quebrada de la Plata	QP005	33°29'20,3"	70°53'38,2"	KC778321
Rhinella arunco	DBGUCH 2398	Quebrada de Cordoba	Bc2398QuebradadeCord	33°26'27,6"	71°39'38,0"	HQ132663
Rhinella arunco	DBGUCH 2400	Quebrada de Córdova	Bc2400QuebradadeCord	33°26'27,6"	71°39'38,0"	HQ132664
Rhinella arunco	DBGUCH 0610063	Quebrada de Córdova	QC0063	33°26'27,6"	71°39'38,0"	KC778311
Rhinella arunco	DBGUCH 0610064	Quebrada de Córdova	QC0064	33°26'27,6"	71°39'38,0"	KC778312
Rhinella arunco	DBGUCH 0611010	Camino a El Yeso	SEY010	33°47'14,9"	70°11'03,1"	KC778334
Rhinella arunco	DBGUCH 0901001	Topocalma	Top1001	34°06'54,1"	71°55'40,1"	HQ132665
Rhinella arunco	DBGUCH 0901002	Topocalma	Top1002	34°06'54,1"	71°55'40,1"	HQ132666
Rhinella arunco	DBGUCH 0901003	Topocalma	Top1003	34°06'54,1"	71°55'40,1"	HQ132667
Rhinella arunco	DBGUCH 0901004	Topocalma	Top1004	34°06'54,1"	71°55'40,1"	KC778335
Rhinella arunco	DBGUCH 0910016	Los Cardos	LCd0016	34°41'15,8"	71°26'29,8"	KC778345
Rhinella arunco	DBGUCH 0910015	Los Cardos	LCd0015	34°41'15,8"	71°26'29,8"	KC778346

Rhinella arunco	DBGUCH 0910017	Los Cardos	LCd0017	34°41'15,8"	71°26'29,8"	KC778347
Rhinella arunco	DBGUCH 3203	Pumaitén	Bc3203Pumaiten	34°58'00,3"	71°07'29,3"	KC778348
Rhinella arunco	DBGUCH RaJ3	Pumaitén	BcJ3Pumaiten	34°58'00,3"	71°07'29,3"	KC778349
Rhinella arunco	DBGUCH 2387	Talca	Bc2387Talca	35°26'00,8"	71°41'59,4"	Pending
Rhinella arunco	DBGUCH 2427	Talca	Bc2427Talca	35°26'00,8"	71°41'59,4"	Pending
Rhinella arunco	DBGUCH TalJ1	Talca	Bchi71TalcaJuvenil1	35°26'00,8"	71°41'59,4"	Pending
Rhinella arunco	DBGUCH 0802007	Linares de Perales	LiPe2007	35°28'09,2"	71°51'54,0"	HQ132668
Rhinella arunco	DBGUCH 0802008	Linares de Perales	LiPe2008	35°28'09,2"	71°51'54,0"	HQ132669
Rhinella arunco	DBGUCH 0802009	Linares de Perales	LiPe2009	35°28'09,2"	71°51'54,0"	HQ132670
Rhinella arunco	DBGUCH 0802010	Linares de Perales	LiPe2010	35°28'09,2"	71°51'54,0"	KC778352
Rhinella arunco	DBGUCH 0612001	Manzanares	BcQ7	36°21'35,8"	72°30'56,3"	KC817175
Rhinella atacamensis	DBGUCH 1002013	Outgroup	R_atacSoc_2013	30°44'04,5"	71°29'36,0"	HQ132571