American Genetic Association

Journal of Heredity, 2015, 546–559
doi:10.1093/jhered/esv039
Symposium Article

OXFORD

Symposium Article

Evolution and Conservation on Top of the World: Phylogeography of the Marbled Water Frog (*Telmatobius marmoratus* Species Complex; Anura, Telmatobiidae) in Protected Areas of Chile

Pedro F. Victoriano, Carla Muñoz-Mendoza, Paola A. Sáez, Hugo F. Salinas, Carlos Muñoz-Ramírez, Michel Sallaberry, Pablo Fibla, and Marco A. Méndez

From the Depto. de Zoología, Fac. de Cs. Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile (Victoriano and Muñoz-Mendoza); Laboratorio de Genética y Evolución, Facultad de Ciencias, Universidad de Chile, Santiago, Chile (Sáez, Salinas, Sallaberry, Fibla, and Méndez); Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA (Muñoz-Ramírez).

Address correspondence to Pedro F. Victoriano at the address above, or e-mail: pvictori@gmail.com

Data deposited at Dryad: http://dx.doi.org/doi:10.5061/dryad.kv1hd

Received September 2, 2014; First decision October 20, 2014; Accepted May 19, 2015.

Corresponding editor: Dr Antonio Solé-Cava

Abstract

The Andean Altiplano has served as a complex setting throughout its history, driving dynamic processes of diversification in several taxa. We investigated phylogeographic processes in the *Telmatobius marmoratus* species complex occurring in this region by studying the geographic patterns of genetic variability, genealogies, and historical migration, using the cytochrome b (cyt-b) gene as a marker. DNA sequences from *Telmatobius gigas* and *Telmatobius culeus*, Bolivian species with an uncertain taxonomic status, were also included. Additionally, we evaluated the phylogenetic diversity (PD) represented within Chilean protected areas and the complementary contribution from unprotected populations. Phylogenetic reconstructions from 148 cyt-b sequences revealed 4 main clades, one of which corresponded to *T. culeus*. *T. gigas* was part of *T. marmoratus* clade indicating paraphyletic relationships. Haplotypes from Chilean and Bolivian sites were not reciprocally monophyletic. Geographic distribution of lineages, spatial Bayesian analysis, and migration patterns indicated that *T. marmoratus* displays a weaker geographic structure than expected based on habitat distribution and physiological requirements. Demographic and statistical phylogeography analyses pointed out to a scenario of recent population expansion and high connectivity events of a more recent age than the post Last Glacial Maximum, probably associated to more humid events in Altiplano. PD of *T. marmoratus* populations within protected areas represents 55.6% of the total estimated PD. The unprotected populations that would contribute the most to PD are Caquena and Quebe (21%). Recent evolutionary processes and paleoclimatic changes, potentially driving shifts in habitat connectivity levels and population sizes, could explain the phylogeographic patterns recovered herein.

Resumen

El altiplano andino ha funcionado como un complejo escenario a través de su historia, el cual ha involucrado procesos dinámicos de diversificación en varios taxa. Nosotros investigamos los procesos filogeográficos en el complejo de especies *Telmatobius marmoratus* que existen en esta zona, mediante el estudio de patrones geográficos de variabilidad genética, genealogías y migración histórica, mediante el uso del gen mitocondrial citocromo-b (cit-b). También incluimos secuencias de ADN de *Telmatobius gigas* y *T. culeus*, especies bolivianas con un estatus taxonómico incierto. Adicionalmente, evaluamos la diversidad filogenética (DF) representada dentro de las áreas protegidas de Chile y la contribución por complementariedad de parte de áreas no protegidas. Las reconstrucciones filogenéticas de 148 secuencias de cit-b recuperaron cuatro clados principales, uno de los cuales correspondió a *Telmatobius culeus*. La especie *T. gigas* fue parte del clado *T. marmoratus*, sugiriendo una relación parafilética. Los haplotipos de localidades chilenas y bolivianas no conformaron grupos recíprocamente monofiléticos. La distribución geográfica de linajes, análisis bayesianos geográfiamente explícitos y los patrones de migración, sugirieron para *T. marmoratus* una menor estructura genética que la esperada en función de su distribución de habitat y requerimientos ecológicos. Análisis demográficos y de filogeografía estadística reconstruyeron un escenario de expansión poblacional reciente y eventos de alta conectividad de edad menor que el término del Último Máximo Glacial, probablemente asociados a períodos más lluviosos en el altiplano. La DF de las poblaciones de *T. marmoratus* dentro de las áreas protegidas representan un 55,6% del total estimado para Chile. Las poblaciones no protegidas que contribuirían con mayor DF son Caquena y Quebe (21%). Cambios paleoclimáticos recientes habrían modulado variaciones en la conectividad del hábitat y en los tamaños poblacionales, lo que podría explicar el patrón filogeográfico recuperado.

Subject areas: Conservation genetics and biodiversity; Population structure and phylogeography
Key words: Andean Altiplano, Chile, Phylogenetic Diversity, *Telmatobius*

Geological history and paleoclimatic fluctuations can be determinants of the current patterns of genetic diversity and the geographic distribution of lineages within a species (Sérsic et al. 2011). Tectonic uplift events that result in the formation of mountain ranges generally lead to the occurrence of complex reliefs that could subsequently act as barriers. Nevertheless, climatic fluctuations during the Quaternary have been recognized as the main historic processes influencing spatial patterns of genetic diversity (Hewitt 1996, 2004). According to Gregory-Wodzicki (2000), the Altiplano Plateau extends from 15°S to 24°S at over 3700 m.a.s.l. and about 250 km wide (Figure 1). The Andean Altiplano is the second highest plateau on Earth and, while it originated during the Cretaceous period, it did not reach its current height until the Plio-Pleistocene (Gregory-Wodzicki 2000; Moon 2008). Therefore, it has long existed as an element that, combined with contrasting and cyclic climates (Ochsenius 1986), would have acted as a dynamic generator of species diversity. Evidence for the latter is the high degree of species richness within the fish genera *Orestias* and *Trichomycterus* (Vila et al. 2011, 2013) and in aquatic gastropods (Collado et al. 2013). Although high species diversity has been noted, the evolutionary mechanisms related to the origin of this diversity remain poorly understood. Previous studies suggest allopatric speciation associated to vicariant events and historical fragmentation of previously continuous aquatic environments as the main drivers of this diversification (e.g., Vila et al. 2011; Collado et al. 2013). Unfortunately, few studies have explored the biodiversity and diversification processes that occur in the Andean Altiplano system, despite its interesting geological history and its peculiarities. Recent studies have uncovered the occurrence of cryptic diversity in mollusks within this region (Collado et al. 2013), suggesting that hidden diversity could also be present in other biological groups in this area.

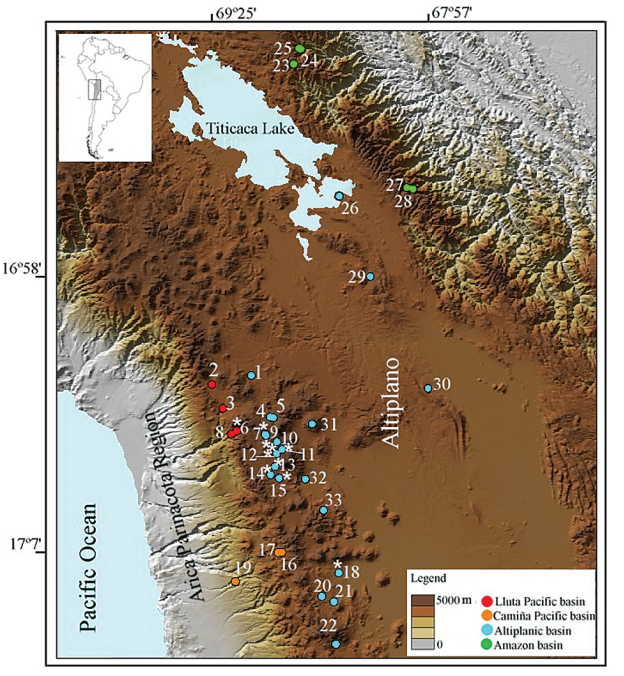

Figure 1. Sampled locations for the *Telmatobius marmoratus* species complex considered in this study. The numbers correspond to the site numbers detailed in Table 1. Color codes correspond to basin systems, which are detailed in Table 1. *Locations within protected areas as detailed in Table 1.

Table 1. Sites of sampled mtDNA cyt-b haplotypes for the *Telmatobius marmoratus* species complex, grouped by country, region/district, site name and geographical coordinates, and basin category

Site	Country	Región/district	Locality	Latitude	Longitude	Basin	*N*	Cluster	Haplotype
1	Chile	Arica y Parinacota	Umaqui	17°44'	69°23'	Altiplanic basin	3	2	**H29**, H31
2	Chile	Arica y Parinacota	Surapalca	18°43'	69°25'	Pacific basin	4	2	H7
3	Chile	Arica y Parinacota	Allane	17°59'	69°37'	Pacific basin	10	2	H5
4	Chile	Arica y Parinacota	Colpa	18°03'	69°13'	Altiplanic basin	2	2	**H1**
5	Chile	Arica y Parinacota	Caquena	18°03'	69°12'	Altiplanic basin	6	2	H14
6	Chile	Arica y Parinacota	Pacollo[a]	18°10'	69°30'	Pacific basin	5	2	**H18**
7	Chile	Arica y Parinacota	Lauca[a]	18°32'	69°09'	Altiplanic basin	4	2	**H3, H18**
8	Chile	Arica y Parinacota	Putre	18°11'	69°33'	Pacific basin	4	2	**H3**
9	Chile	Arica y Parinacota	Parinacota[a]	18°12'	69°16'	Altiplanic basin	12	2	**H3**
10	Chile	Arica y Parinacota	Malpaso[a]	18°15'	69°10'	Altiplanic basin	1	2	**H3**
11	Chile	Arica y Parinacota	Chungará[a]	18°18'	69°8'	Altiplanic basin	9	2	**H3**
12	Chile	Arica y Parinacota	Chiriguaya[a]	18°20'	69°10'	Altiplanic basin	10	2	**H12**, **H18**, H21, H30
13	Chile	Arica y Parinacota	Ancuta[a]	18°26'	69°11'	Altiplanic basin	5	2	**H3**, **H12**, H18, H29
14	Chile	Arica y Parinacota	Lauca Vichuta[a]	18°30'	69°13'	Altiplanic basin	1	2	H11
15	Chile	Arica y Parinacota	Lauca Sur[a]	18°32'	69°09'	Altiplanic basin	2	2	H25
16	Chile	Tarapacá	Pumiri	19°6'	69°8'	Pacific basin	5	4	**H2,H8**
17	Chile	Tarapacá	Toculla	19°7'	69°10'	Pacific basin	3	4	**H2,H8**
18	Chile	Tarapacá	Isluga[a]	19°15'	68°42'	Altiplanic basin	20	3	**H1**,H10,**H12**,H13,H22
19	Chile	Tarapacá	Quebrada Tana	19°22'	69°32'	Pacific basin	4	4	**H2,H8**
20	Chile	Tarapacá	Quebe	19°27'	68°48'	Altiplanic basin	16	4	H4,H24
21	Chile	Tarapacá	Toroni	19°30'	69°42'	Altiplanic basin	2	4	**H1**,H9
22	Chile	Tarapacá	Cancosa	19°57'	68°41'	Altiplanic basin	8	4	H6,**H17**
23	Bolivia	La Paz	Rio Wasawayqo	15°10'	68°59'	Amazon basin	1	1	**H16**
24	Bolivia	La Paz	Charazani	15°10'	69°0'	Amazon basin	1	1	**H17**, **H16**
25	Bolivia	La Paz	Charazani Escoma	15°13'	69°2'	Amazon basin	2	1	H20
26	Bolivia	La Paz	Lago Titicaca	16°15'	68°44'	Altiplanic basin	1	1	H15
27	Bolivia	La Paz	Zongo	16°16'	68°6'	Amazon basin	1	1	**H1**
28	Bolivia	La Paz	KkotaPata	16°16'	68°4'	Amazon basin	1	1	H28
29	Bolivia	La Paz	Comanche	16°58'	68°25'	Altiplanic basin	1	1	H19
30	Bolivia	Oruro	Huayllamarca	17°50'	67°57'	Altiplanic basin	1	1	H27
31	Bolivia	Oruro	Sajama	18°6'	68°59'	Altiplanic basin	1	1	H23
32	Bolivia	Oruro	Lago Macaya	18°33'	68°56'	Altiplanic basin	1	2	H26
33	Bolivia	Oruro	Rio Packohaua	18°45'	68°41'	Altiplanic basin	1	3	**H18**
							148		

Bold haplotypes are present in more than one site.
Cluster, genetic group assigned by Geneland; *N*, sample size.
[a]Locations within protected areas as indicated in Figure 1.

Genetic variation is widely recognized as one of several currencies for the evaluation of diversity (Ehrlich & Wilson 1991; Humphries et al. 1995), and the protection of diversity is incorporated into many national and international conventions (Moritz & Faith, 1998). For single species conservation, it is important to identify the evolutionary relationship between populations in order to retain the maximum genetic diversity and to incorporate information on historical population processes (Avise 1989, Moritz 1994, 1995). The use of molecular data in phylogeographic studies has led to the discovery of many cryptic phylogeographic lineages (Daniels et al. 2003), usually not reflected in morphological variation. By combining the resulting gene trees with the geographic location at which each individual was sampled, one can elucidate the geographical distributions of major gene lineages that comprise a gene tree (Arbogast and Kenagy 2008). This is a key issue for the delimitation of evolutionary significant units and for defining intraspecific biodiversity units for conservation purposes. Moritz (1999) suggested that in order to define conservation units one should identify and protect groups of historically isolated evolutionarily significant units (ESUs) in the first place, and that groups that would maximize the potential for evolutionary adaptation are protected for each ESU (Fraser and Bernatchez 2001). The phylogenetic diversity (PD) (Faith 1992a) is a quantitative measure of phylogenetic diversity. Larger PD values are expected to correspond to greater feature diversity (Faith 1992b; 1994). This index allows a priority system to be established that reflects the value of the taxonomic diversity, which is very important when resources are limited or when the goal of conservation is to maintain the most hierarchical variation (Faith 1992a); PD can also be used to measure the complementary diversity of a taxon (or taxa) that is not covered by a reference set of taxa (Faith 1992b). For instance, for a set of populations distributed outside protected areas, it is possible to determine which of them would provide greater phylogenetic diversity complementary to those already protected and prioritize their conservation.

The genus *Telmatobius* Wiegmann 1834 constitutes a very diverse genus that is associated with Andean landscapes, and distributed from Ecuador, throughout the Bolivian and Peruvian highlands, reaching southwards into Argentina and Chile (Aguilar and Valencia 2009). These amphibians have aquatic and semi-aquatic habits, occurring in

lakes, river systems, and shallow wetlands between 1000 and 5200 m.a.s.l. (De la Riva 2005). About 10 *Telmatobius* species are known to occur in northern Chile, from which 8 are endemic to Chile. According to recent studies (Sáez et al. 2014), the species group that occurs in Chile is composed of 3 clades that does not form a reciprocal monophyletic unit relative to the *Telmatobius* species from neighboring Andean regions. The latter makes the *Telmatobius* species an ideal model for the inference of diversification processes and for the reconstruction of evolutionary relationships. One of the northern Chilean clades within this genus includes *T. marmoratus* (Duméril and Bibron 1841), a species with a wide distribution range in the highlands that extends to the Bolivian provinces of Oruro and La Paz. Its taxonomic history, characterized by a long list of synonyms, indicates that this is a taxon whose delimitation has been challenging and it is currently under development. Systematic uncertainties have led researchers to include within this clade several putative species that show a low degree of divergence from *T. marmoratus*, such as *T. culeus* (Garman 1875) and *T. gigas* Vellard 1969 from Bolivia (Benavides et al. 2002; De la Riva et al. 2010). As a result, it becomes compulsory to consider *T. marmoratus* as a species complex. According to recent results (Sáez et al. 2014), some Chilean populations whose evolutionary relationships were uncertain, belong to the *T. marmoratus* clade, *T. gigas* is apparently part of *T. marmoratus*, and *T. culeus* could be the sister species of *T. marmoratus*. As such, investigating the degree of genetic differentiation between *T. marmoratus sensu stricto* and weakly differentiated species like *T. culeus* and *T. gigas*, as well as the genealogical relationships within *T. marmoratus sensu lato*, becomes a very interesting undertaking.

From a phylogeographic viewpoint, it would be interesting to characterize patterns of diversity, both inter- and intrapopulations belonging to this species complex and associate these with geological and climatic historical processes that have occurred in the Andean Altiplano. As *Telmatobius* species are aquatic, their distribution are strongly associated with water bodies. The habitat range occupied by the *T. marmoratus* complex in the Altiplano is highly fragmented and shows low levels of connectivity. In general, amphibians display low levels of vagility relative to other vertebrates, and many species are philopatric (Correa et al. 2010), frequently generating high levels of structure even in areas separated by narrow or moderate distances. Additionally, this habitat can be assumed to be historically unstable due to the dynamic paleoclimatic history of these highlands that were subjected to recurrent glacial events and to cyclical dry and wet periods. Unstable environments are often associated with low levels of local genetic variability due to recurrent decreases in effective population size (Carnaval et al. 2009). Therefore, based on the factors described above, we predict low levels of local genetic variability and high levels of structure coupled with low levels of historical gene flow among populations of the *T. marmoratus* complex.

Regarding its conservation status, *T. marmoratus* is listed as vulnerable as its population is expected to decline by more than 30% between 2010 and 2020 (Icochea et al. 2010). One of the major threats for this species is chytridiomycosis, an infectious disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* that is believed to be responsible for the decline of some Peruvian populations (Seimon et al. 2007). Another relevant threat for the Chilean populations of *T. marmoratus* is habitat alteration due to increased mining, groundwater withdrawals, and intensive grazing by domestic livestock. The IUCN Red List provides scientific decision-making guidelines to assign species into categories of threat based on threshold values of population parameters, such as distribution range and population decline (Mace and Lande 1991). However, these criteria do not ensure the preservation of intraspecific biodiversity. For example, many species with a broad distribution and high number of records are not listed as threatened. But in these cases, this system of classification does not consider the geographic distribution of their genetic variability, or the evolutionary uniqueness of their populations. Many species differ substantially in the amount of unique genetic information they embody (Crozier 1997), which is not commonly considered as a criterion in conservation measures.

The major goal of this study is to investigate from a phylogeographic standpoint, the spatial patterns of genetic diversity and lineage distribution within the *T. marmoratus* species complex, and the historical levels of connectivity among the populations assignable to this species based on variants of a mitochondrial gene. Additionally, we evaluate to which extent the patterns of PD are represented inside the National System of Protected Areas of Chile (Sistema Nacional de Áreas Protegidas de Chile, SNASPE), and which unprotected populations would contribute the most in increasing the amount of protected PD if their range were to be included in the SNASPE.

Materials and Methods

Sample Collection

We analyzed a total of 148 sequences from specimens of the *Telmatobius marmoratus* complex distributed in Chile and Bolivia. From these, 136 corresponded to individuals collected in 22 sites between the Arica and Parinacota Region and the Tarapacá Region. DNA samples were obtained from buccal swabs, interdigital membranes, or both. In some cases, muscular tissue was extracted and preserved in absolute ethanol. All samples were catalogued with the geographic coordinates of the sampling site recorded by GPS (Table 1; Figure 1). For analyses, sites were grouped in 3 basin categories: Pacific, Altiplano, and Amazona basins (Table 1; Figure 1). The Bolivian samples of *T. marmoratus* studied here are the same that De la Riva et al. (2010) included in their work with Bolivian *Telmatobius*. (GenBank accession codes GU060589 – GU060612). In this work, we complemented the sampling coverage performed in Sáez et al. 2014 by taking samples from sites outside of the Lauca and Isluga National Parks and including samples from the Las Vicuñas National Reserve, which results in a relatively continuous sampling of *T. marmoratus* specimens along the high Andean zone of the Arica y Parinacota and Tarapacá regions. It is important to point out that this work extends the actual known distribution of *T. marmoratus* in Chile (from 11 to 22 localities; see Sáez et al. 2014).

Data Archiving

All new sequences were deposited in GenBank (accession codes KT156848--KT156960). We have deposited the primary data underlying these analyses in Dryad following data archiving guidelines (Baker 2013).

Laboratory Protocols

Genomic DNA was extracted using the commercial Kit Wizard SV Genomic (Promega). Subsequently, extraction was verified by electrophoresis in a 2% agarose gel using 3 microlitres of extraction products, followed by staining of the gel with SYBR Safe®. A fragment of the mitochondrial gene (mtDNA) cytochrome b (cyt-b) was amplified using the primers MVZ15 (GAA CTA ATG GCC CAC ACW WTA CGN AA) (Moritz et al. 1992) and CYTBAR-h (TAW AAG GGT CTT CTA CTG GTT G) (Goebel et al. 1999). DNA

amplification was performed by polymerase chain reaction (PCR) in a final volume of 30 µL containing: 1.5 U of Taq polymerase (Invitrogen), 3 mM of $MgCl_2$, 0.12 mM of each dNTP, 0.1 µM of each primer, and 10–50 ng of genomic DNA. The PCR reactions were performed using the following conditions: initial denaturation at 94 °C for 2 min, followed by 40 cycles (45 s at 94 °C, 1 min at 53 °C, and 1.3 min a 72 °C), and a final elongation at 72 °C for 10 min.

PCR products were visualized using a 2% agarose gel stained with SYBR Safe® and purified using a MultiScreen $PCR_{\mu 96}$ Filter plate (Millipore) according to the protocol provided by the manufacturer. PCR products were sequenced on both directions through automatic sequencing using the equipment ABI3730XL of Macrogen (Korea). Sequences were aligned and edited using Codon Code Aligner v. 3.0.3 (Codon Code Corporation 2007), and later translated into amino acids in order to corroborate the absence of stop codons.

Saturation levels of the matrix were evaluated following the protocols described in DAMBE v. 5.0.11 (Xia and Xie 2001). Tests were performed with 100 iterations using the proportion of invariant sites informed by the software jModeltest v. 0.1.1 (Posada 2008).

Phylogenetic Analyses

Phylogenetic relationships were inferred using the Bayesian approach implemented in MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003). Four Markov chains were run for 5 million iterations and sampled at intervals of 1000 generations. The first 1250 trees (25%) were discarded as burn-in. Posterior probabilities were obtained from 50% majority-rule consensus tree. The best-fit model of sequence evolution for each gene was selected with JModeltest 0.1.1 (Posada 2008). The species *Telmatobius bolivianus* was selected as the outgroup (accession number of GeneBank GU060588).

The haplotype network was inferred using statistical parsimony in TCS (Clement et al. 2000). Ambiguities within the network were solved according to the criteria of Crandall and Templeton (1993): 1) Frequency criterion: haplotypes are more likely to be connected to high-frequency haplotypes than to other sequences with lower frequencies; 2) Topological criterion: haplotypes are more likely to be connected to interior haplotypes than to tip haplotypes; and 3) Geographic criterion: haplotypes are more likely to be connected to other sequences belonging to the same population or region, than to haplotypes occurring in distant populations.

Neutrality Test, Population Genetic, and Historical Demographic Analyses

To asses if cyt-b gene sequences behave neutrally (Ballard and Whitlock 2004), the McDonald and Kreitman (1991) neutrality test was performed using the program DnaSP v. 5 (Librado and Rozas 2009). In order to detect deviations from a constant population size under a neutral model, we applied Tajima's D test (Tajima 1989) implemented in the program DnaSP, and Fu's Fs (Fu 1997) using the Arlequin program (Excoffier et al. 2005). Negative and statistically significant values for these statistics suggest an excess of low-frequency mutations, relative to what is expected under a neutral model (e.g., strict selective neutrality of variants and constant population size). Additionally, analyses of pairwise haplotype comparisons ("mismatch distribution") were performed using Arlequin. To test if the data deviate from what is expected under an expansion model, the Raggedness index was calculated. In order to determine the most likely number of populations (cluster), we used a Bayesian approach implemented in the program Geneland 4.0.3 (Guillot et al. 2005a, Guillot et al. 2005b, Guillot 2008, Guedj and Guillot 2011, Guillot et al. 2012). This program provides a frequency distribution graphic that indicates the most probable number of populations and their approximate geographic limits. We performed a preliminary run where *K* was allowed to vary from 1 to 33 (number of localities collected) to determine the modal number of clusters. All runs were conducted using the spatial Dirichlet model for the priors in allele frequency and 5 runs with fixed *K* were performed for the spatially explicit model, and for each run, the posterior probability of subpopulation membership was computed for each pixel of the spatial domain (100 × 100 pixels). The Markov chain Monte Carlo (MCMC) repetitions were set at 500,000, thinning was set at 100, and the burn-in period was set at 200 iterations. Because use of Geneland with nonrecombining DNA sequence data could incur a considerable loss of information, it should not be viewed as a substitute for methods that model the genealogy of genes (Guillot et al. 2012). For this reason we also use coalescent methods to estimate the historical population connectivity. Four possible models of gene flow were tested using Migrate-n v. 3.6 (Beerli 2006) (see Supplementary Table A online). The marginal likelihood of the model was estimated followed by a ranking of the Bayes factor of each one (Beerli and Palczewski 2010). The starting genealogy was taken from a UPGMA tree and initial theta and *M* values were derived from the FST calculation. Static heating was applied to 4 independent chains using temperature settings of 1.0, 1.5, 3.0, and 1 000 000.0. A total of 500 000 steps were run, recorded every 100 generations, of which 12 500 were discarded as the burn-in. Stationarity was assessed by examining the effective sample size and distribution of each parameter in Tracer v 1.5 (Drummond and Rambaut 2007).

Genetic distance between and within clusters was calculated using MEGA 5 (Tamura et al. 2011), while haplotype diversity (Hd; Nei 1987), nucleotide diversity (π; Nei 1987) and the average number of nucleotide differences (*k*; Tajima 1989) were estimated using the program DnaSP.

Based on the results from the phylogenetic and gene flow analyses, a hypothesis of postglacial gene flow was proposed and tested against the alternative hypothesis of ancestral polymorphism retained from an earlier divergence using a model-based phylogeographic approach. These hypotheses were tested by performing coalescent simulations in the program Mesquite ver. 3.01 (Maddison and Maddison 2008) to produce thousands of expected genealogies under 2 models representing the 2 hypotheses being tested. For each simulated genealogy, the s statistic (Slatkin and Maddison 1989) was calculated and its frequency distribution from each of the 2 models was then compared with the s-value obtained from the empirical dataset. The s statistic is used here as a measure of departure from reciprocal monophyly. The higher the value, the higher the lack of monophyly. Support for a given model is assessed based on where the observed *s*-value falls relative to the distribution of the simulated *s* values, as the different models may produce different expectations regarding the degree of reciprocal monophyly. Simulations were initially performed for 2 models differing in the time since divergence (14 000 ybp vs. 7000 ybp) (Figure 2). However, based on the preliminary results, additional divergence times were also simulated (1000 and 400 ybp). For simplicity, we test our models with populations from cluster C2, which we divided in 2 subpopulations based on the basin in which they were collected (west: Lluta River basin or east: Altiplano basin). Simulations were performed assuming a population size of 10 000 individuals for each model. Models were

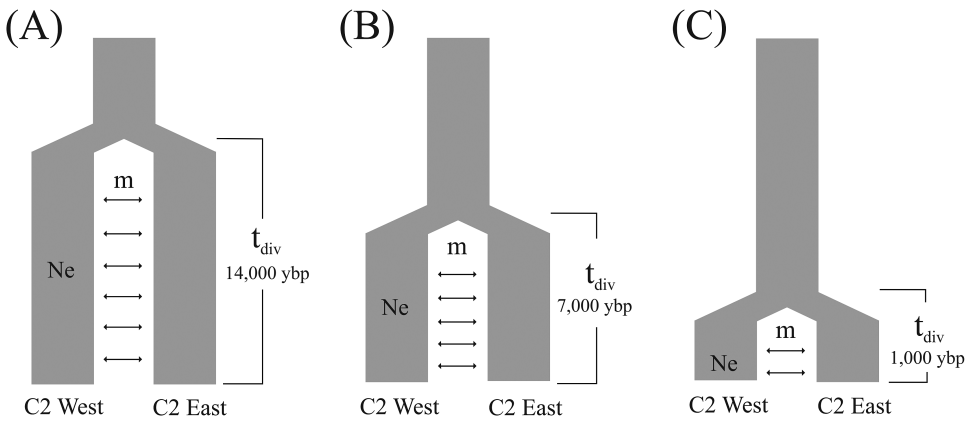

Figure 2. Models of coalescent simulations performed in the program Mesquite ver. 3.01. Ne, effective sample size; tdiv, time since divergence; m, migration rate between basins; C2 West, localities from cluster C2 collected in Lluta Pacific basin (west basin); C2 East, localities from cluster C2 collected in Altiplano basin.

also run assuming 2 scenarios of migration, a without-migration scenario (m = 0) and a with-migration scenario (m = 0.001; percent of migrants per generation). These values of m are close to those obtained by Migrate, and we selected a slightly larger m value for the with-migration scenario to account for the larger uncertainty in the estimation of this parameter when estimated from mtDNA only.

Phylogenetic Diversity and Its Representation in Protected Areas in Chile

In order to evaluate how much of the *T. marmoratus* diversity is sheltered by protected areas in Chile, we calculated the PD index (Faith 1992b) for all populations within the SNASPE, and compared it to the PD of populations outside protected areas. Protected and unprotected sites are showed in Figure 1. Additionally, we determined which of the currently unprotected populations, if included in the protected areas system, would contribute the most to raise the total phylogenetic diversity contained in the SNASPE. This approach enables the prioritization of those populations that could contribute the most toward the PD while minimizing the number of populations to protect. These analyses were conducted using the Picante package for R (Phylocom Integration, Community Analyses, Null-models, Traits and Evolution in R) (Kembel et al. 2010) and the R function "Phylorare" (Nipperes and Matsen 2013). The "Phylorare" function calculates mean rooted phylogenetic diversity and can be used to standardize a set of samples to a particular level of sampling effort. This allows comparing PD between different sets (protected and unprotected) with unequal sample size.

Results

The final alignment consisted of 148 sequences representing an 815 bp fragment of the gene coding for cyt-b, from 33 sampling sites. All sequences (including the outgroup) showed low levels of saturation according to Xia's test. None of the cyt-b sequences presented gaps or stop codons, which indicates they represent functional copies of mtDNA. Neutrality test indicated the sequences evolve neutrally instead of evolving as a response to selective processes. A total of 60 segregating sites were found among 31 haplotypes.

Phylogenetic Analyses

The phylogenetic tree obtained from Bayesian analysis is shown in Figure 3. Four major clades can be delimited (A, B, C, and D), but the relationship among them are not resolved, represented by polytomies and low geographical structure. Clade A shows a strong support (0.99) and it includes 10 haplotypes, from both one of the 2 Pacific basins and from part of the Altiplano basin. The specimen from Huayllamarca, Oruro, which was putatively assigned to *T. gigas* in De la Riva et al. (2010), is nested within this group. Clade B is represented by only one haplotype and corresponds to specimens from Allane in the Lluta Pacific basin, whereas clade C is constituted only by the specimen from Titicaca Lake, which was designated as *T. culeus* prior to these analyses. Clade D was weakly supported (PP = 0.57) and includes sequences from specimens collected in most of the sampling sites in both Chile and Bolivia, from Lluta Pacific basin, Altiplano, and Amazon basin. Within this group, clade E includes haplotypes from Arica-Parinacota (Pacific basin), and samples from part of the Altiplano basin. Clade F is also strongly supported (PP = 0.98) and comprises haplotypes from a wide geographic area, in which the haplotypes from the Amazon basin are also included. In summary, the Bayesian genealogy shows a lack of genetic structure and no reciprocal monophily according to each river system.

The haplotype network showed 3 main haplogroups (Figure 4), with low geographical structure. The haplogroups were designated using the same codes as the Bayesian phylogenetic tree (Figure 3). The haplotype from clade B (from the Lluta Pacific basin) is closely associated with the haplogroup F that is distributed both in the Altiplano and Amazon basins. Haplogroup E shows a clear star-like shape with an internal high-frequency haplotype broadly distributed in the Lluta Pacific basin and Altiplano. Both clades A and F present a wider geographic distribution with no star-like shape, and without a clear dominant haplotype. Within clade F, one haplotype (H1) presented the widest geographic distribution and was also the most frequent. Overall, the network shows connections with few mutational steps with the exception of the unique haplotype forming clade C (preliminarily assigned to *T. culeus*), which is separated from the nearest haplogroup (F) by 10 mutational steps.

Neutrality Test, Population Genetics, and Historical Demographic Analyses

Analyses of population genetic structure (Geneland) indicated that the most probable number of population units is K = 4, which were largely, but not completely concordant with the basins limits (Figure 5). Cluster 1 (C1 in Figure 5) represented all sites from La Paz. This unit shows a wide north to south extension, suggesting that in La Paz there is a low genetic differentiation among

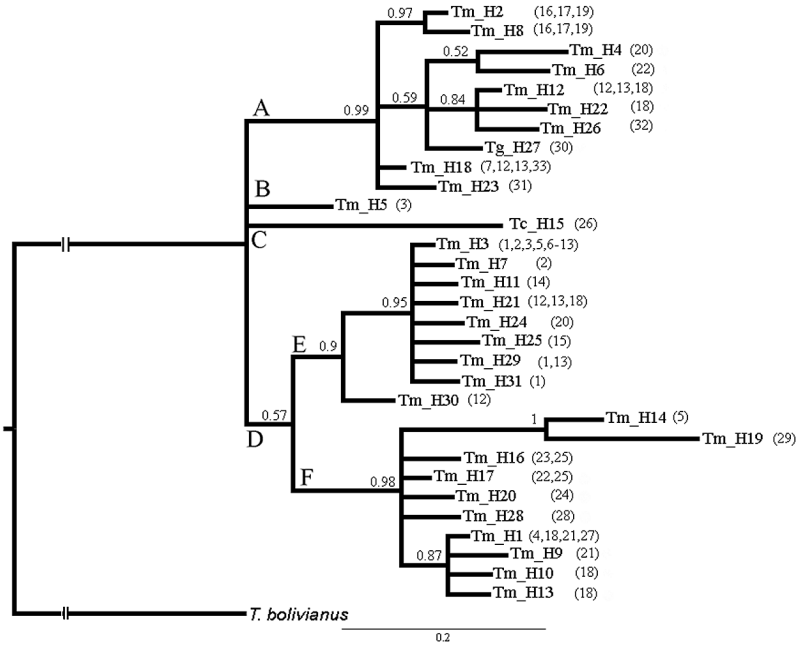

Figure 3. Bayesian majority-rule consensus tree depicting relationships of the *Telmatobius marmoratus* species complex based on sequences of the cyt-b gene. Numbers above nodes represent Bayesian posterior probability values, and numbers between parentheses indicate the corresponding site number according to Table 1. Tm = *Telmatobius marmoratus*. Tg = *Telmatobius gigas*, according to De la Riva 2010. Tc = *Telmatobius culeus*, according De la Riva 2010. H = haplotype codes according to Table 1.

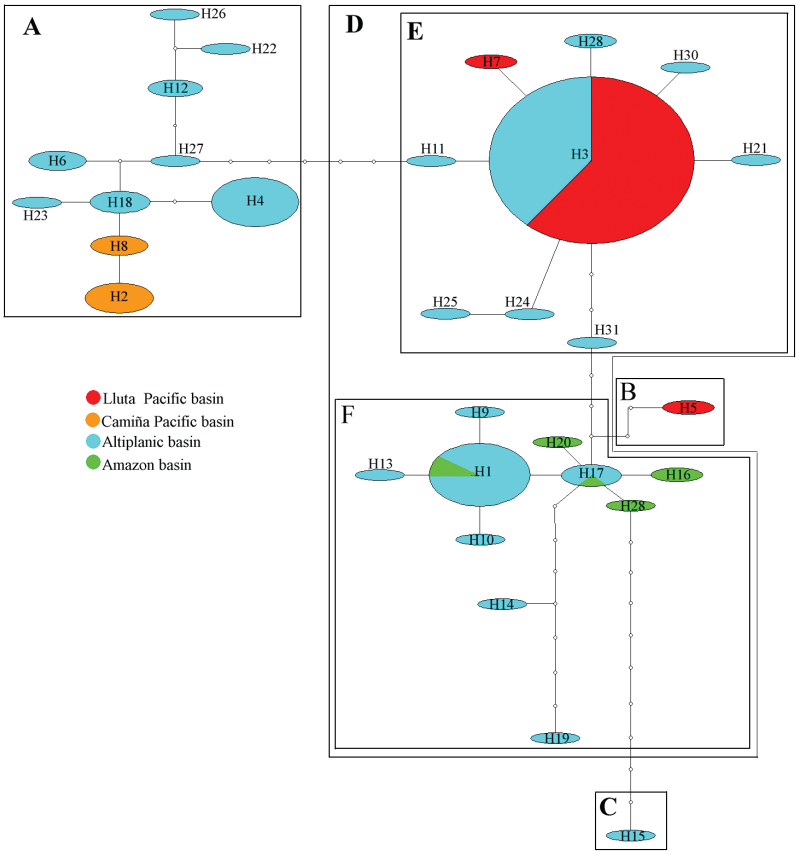

Figure 4. Haplotype network inferred using cyt-b gene sequences by statistical parsimony (TCS), for the *Telmatobius marmoratus* species complex. Color codes correspond to basin systems as shown in Figure 1. White circles indicate mutational steps, for details of haplotype codes see Table 1.

local populations. Cluster C2 included localities mainly from Arica-Parinacota, with the exception of a bordering site adjacent to Chile, in the Oruro province (Site 32, Macaya Lake). The C3 group included only 2 sites, one from Tarapacá, near the Bolivian border (site 18, Isluga), and the Bolivian site of Río Packohaua (site 33). Cluster C4 included only sites in Tarapacá, Chile.

Haplotype (Hd) and nucleotide diversity (π) for all cyt-b sequences was Hd = 0.8148 and π = 0.0076, respectively (Table 2). Diversity estimators were also calculated separately for each population inferred by Geneland. Higher haplotype and nucleotide diversity was observed for the group C1 which is distributed in La Paz with a wide geographic range, and the lowest estimated value corresponded to cluster C3, composed by only 2 border localities between Chile and Bolivia. Mostly, the above results were congruent with distance values within and between clusters (Table 3); generally, such genetic distance values were low (between 0.005 and 0.013). Pairwise

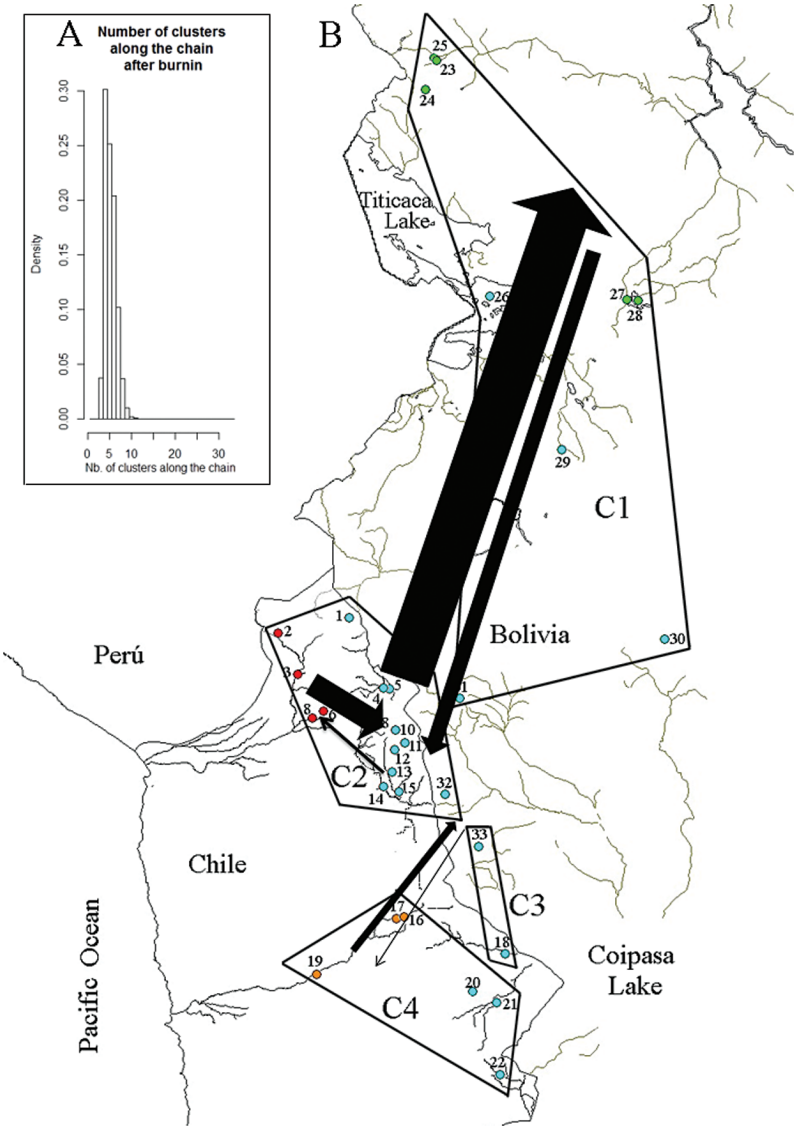

Figure 5. (**a**) Probability density for the number of populations simulated and obtained with Geneland. (**b**) Spatial distribution of each cluster (C1 to C4), defined by Geneland at $K = 4$. Cluster codes and basins included in each cluster are detailed in Table 1. Arrows show the Migrate gene flows between basins (see details in Table 4).

Table 2. Nucleotide diversity statistics, tests for neutrality, and demographic expansion of mtDNA cyt-b sequences for the clusters retrieved by Geneland in the *Telmatobius marmoratus* species complex

	N	*S*	*H*	*k*	Ragg	Hd	π	Fs Fu	D Tajima
Cluster 1	9	26	8	6.611111	0.15509259	0.9722	0.009391	−1.98453	−1.53992
Cluster 2	80	34	15	2.910759	0.20216452	0.4734	0.003058	−2.77868	**−1.82096**
Cluster 3	21	15	6	2.1	0.28272109	0.4286	0.002211	−0.04573	**−1.80962**
Cluster 4	38	23	9	6.042674	**0.16540371**	0.7895	0.006347	2.77071	0.3518
Total	148	60	32	7.291046	**0.04313333**	0.8148	0.007659	−3.55073	−0.99927

Basins included in each cluster are detailed in Table 1.

H, number of haplotypes; Hd, haplotype diversity; *k*, average number of nucleotide differences; *N*, number of sequences; π, nucleotide diversity; Ragg, Raggedness index; *S*, variable sites.

Bold values represent significance at $P < 0.05$.

distance average within each group was lower than between groups, except when comparing group C1 to group C3.

According to Fu's Fs values and Tajima's D, only groups C2 ($D = -1.82096$ from Arica-Parinacota) and C3 ($D = -1.80962$) show signals of recent bottlenecks and are currently experiencing a demographic expansion, as suggested by the significant negative values of one or both indicators (Table 2). As suggested by mismatch graphics, although some populations showed a tendency toward a multimodal distribution, a significant deviation from the expected curve under a demographic expansion model was only observed for one of the 4 haplogroups (see Raggedness values in Table 2). The mismatch graphics showing pairwise distances mostly in low values (Figure 6) correspond to clusters C2 and C3. The former is in agreement with the significant Fu's Fs value.

Migration values between clusters are shown in Table 4 and represented in Figure 5. Comparison of migration models revealed that the Bisected stepping stone model had the highest support from the data, with a log Bayes Factor difference > 10^5 units relative to the other migration model (where a difference of > 10 units provide very strong support for one model over another; Kass and Raferty 1995). According to these results, the historic migration scenario for the analyzed populations shows a prevalence of asymmetric migration rates. Higher migration magnitudes correspond to gene flow from C3 to C1, from C4 to C1, and from C2 to C1. Therefore, the cluster that historically acts as a sink is cluster C1, distributed in the La Paz highlands. Cluster C3 (formed by 2 sites, one in Oruro and the other in Tarapacá) could have acted primarily as a source. Cluster C4 distributed in Tarapacá, Chile, appears to have functioned more as a source than as a sink, although at a lower scale when compared to cluster C3.

Table 3. Pairwise sequence divergence for cyt-b haplotypes between clusters of the *Telmatobius marmoratus* complex, retrieved by Geneland

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster 1	**0.01 (0.002)**	0.011	0.007	0.013
Cluster 2	(0.003)	**0.003 (0.001)**	0.009	0.012
Cluster 3	(0.002)	(0.003)	**0.004 (0.001)**	0.013
Cluster 4	(0.003)	(0.003)	(0.003)	**0.008 (0.002)**

Basins included in each cluster are detailed in Table 1.

The values on the diagonal (in bold) represent mean differences within clusters, while those above the diagonal represent mean differences between clusters. Standard deviation values are shown in parentheses.

Because patterns of historical migration can be commonly confounded with the process of incomplete lineage sorting, we performed coalescent simulations to test how likely the later scenario could be for a number of models with different population splitting times. These analyses indicated that the low genetic structure found in *Telmatobius marmoratus* was likely not due to incomplete lineage sorting, but rather to migration. Simulations at 3 different times of population splitting and no migration between the basins (Figure 7, upper row) showed that all simulated gene genealogies produced *s* values lower than the observed *s* value. Additional simulations showed that only after 400 years it was possible to obtain *s* values equal or higher than the observed *s* value in at least 5% of the cases (data not shown). On the other hand, simulations that incorporated the effect of migration produced a range of *s* values that were not significantly different from the observed *s* value ($P > 0.5$), regardless of the time of population splitting (1000, 7000, and 14 000 ybp; Figure 7, lower row). All together, these results indicate that only the models incorporating migration account for the lack of reciprocal monophyly observed across populations.

Phylogenetic Diversity and Its Representation in Protected Areas in Chile

Faith's Phylogenetic Diversity Index (1992b) was estimated with the "phylorare" function in R and the PICANTE package for R, including all *T. marmoratus* populations in Chile. The Phylorare analysis with $m = 110$ showed that the PD for all Chilean populations was 1.87. When considering only populations sampled from protected areas, the index value decreases considerably to 1.04, representing 55.6% of Chile's phylogenetic diversity for *T. marmoratus*. We obtained the

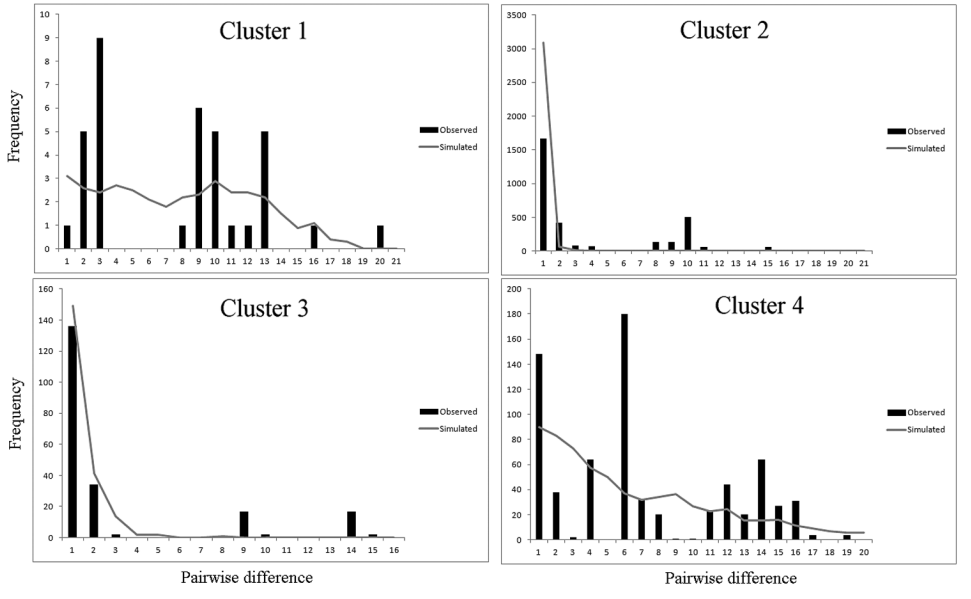

Figure 6. Mismatch distribution of haplotype lineages for each cluster (C1 to C4) in the *Telmatobius marmoratus* species complex. Raggedness index values are detailed in Table 2.

Table 4. Long-term female effective migration rates (ΘM) among basins, estimated by MIGRATE-N

	Donor basin			
Receiver basin	Amazon	Altiplanic	Lluta Pacific	Camiña Pacific
Amazon	*0.20 (0.04–0.20)*	3.21 (0–35.1)	—	—
Altiplanic	1.88 (0–337)	*0.12 (0.06–0.19)*	2.27 (0–28.1)	0.89 (0–5.38)
Lluta Pacific	—	0.38 (0–13.8)	*00.02 (0–0.09)*	—
Camiña Pacific	—	0.05 (0–1.83)	—	*0.01 (0–0.07)*

The 95% confidence interval is constructed from minimum and maximum estimates of 0.025 and 0.975 percentiles.
Theta values (Θ) for each basin is given on the identity diagonal in italics.

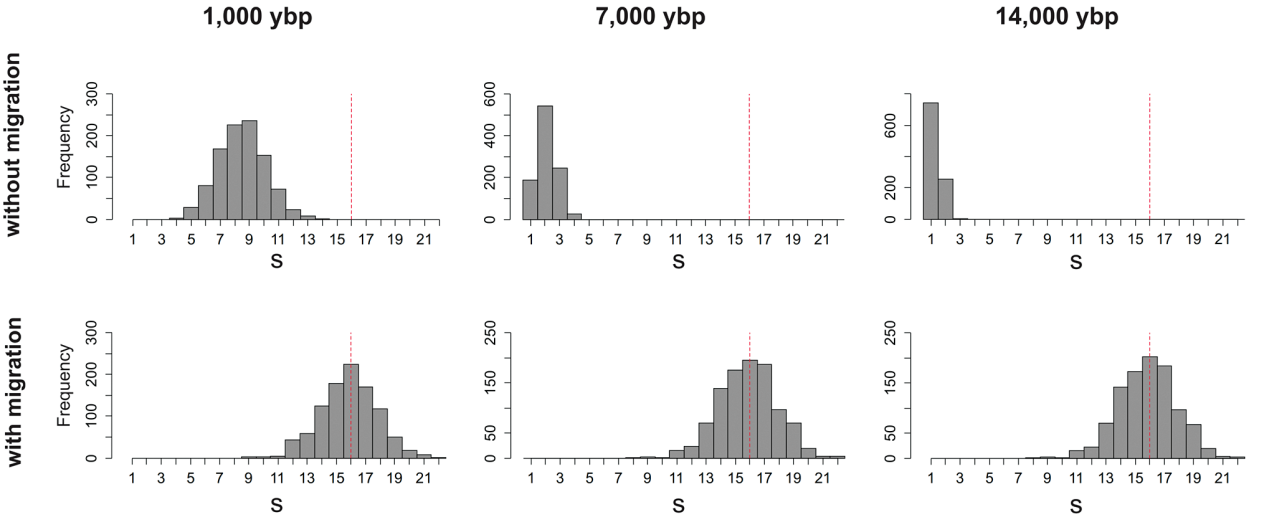

Figure 7. Comparison of the *s* value from the reconstructed gene tree (observed for groups explained in Figure 2) to the expected distributions of s from gene trees simulated by neutral coalescence with (lower row) and without (upper row) migration ($m = 0.001$ and $m = 0$, respectively) and Ne = 10 000, over a range of different times of population splitting (measured in generations). Vertical dashed line indicates the observed *s* value.

same results with the PICANTE package for R. The population from Caquena, currently unprotected, was retrieved as the most likely to complement at the highest degree the phylogenetic diversity included in protected areas (see Supplementary Table B online). This population is represented by one haplotype (H14) that, together with H19, would form a sister-clade relationship with haplotypes from the locality of Isluga, 150 km away (H1, H10, and H13). If this population was to be included, the phylogenetic diversity of protected areas would increase from 55.6% to 66.3%. Another population that would also contribute to phylogenetic diversity is that located in Quebe. This locality alone has a high phylogenetic diversity (PD = 0.53), potentially due to the presence of 2 haplotypes belonging to well-differentiated clades (H4 and H24). Providing protected status to the populations in Caquena and Quebe would raise the phylogenetic diversity in protected areas to 76%.

Discussion

Although not all clades from our inferred phylogenetic tree display high support values, it is interesting to analyze the position of *T. culeus* and *T. gigas* in the tree topology and in the haplotype network. From both species, only *T. culeus*, from the Titicaca Lake, appears as a well-differentiated clade relative to the rest. On the other hand, the specimen assigned to *T. gigas* prior to analyses shows a relationship to the *T. marmoratus* clade that includes specimens from the localities of Oruro, Arica-Parinacota, and Tarapacá. The strong evolutionary relationship between *T. marmoratus* and *T. gigas* has been noted in previous studies (e.g., Benavides 2005). De la Riva et al. (2010), when analyzing Bolivian *Telmatobius* species, could not find a relationship of reciprocal monophyly between samples of *T. marmoratus* and *T. culeus* from Titicaca. These authors also found evidence of relatively recent divergence between *T. marmoratus* and *T. gigas* (no more than 300 000 to 600 000 ybp), which could explain the absence of complete lineage sorting in both taxa. In conclusion, relative to taxonomy, the cyt-b gene suggests that *T. gigas* could be cospecific with *T. marmoratus*, while *T. culeus* is clearly a different species. However, the genetic variability analyzed in this study relied on only one gene of uniparental inheritance. In order to produce more robust results, a broader sampling in Bolivia, and an integrative approach is needed in order to establish the definite characterization of *T. gigas* as a separate species, including the use of nonlinked genomic markers (nuclear genes) and other characteristics such as morphological markers (Sites and Marshall 2004).

Contrary to our expectations of high structured populations, we found patterns of low genetic structure that suggest the occurrence of a complex scenario in terms of lineage distribution, genetic variability, and historic migrations within the *T. marmoratus* species complex. Based on our results, we were not able to statistically corroborate our prediction of highly structured populations within *T. marmoratus*. Overall, *T. marmoratus* presented moderate levels of population structure and low levels of genetic variation. We have recovered considerable levels of historic gene flow among some of

its populations. Nonetheless, Bayesian analyses using Geneland suggest the existence of 4 populations units or clusters, which although presenting low support values, serves as an indicator of the existence of moderately differentiated geographic groups. According to the star-like shape of the cluster C2 from Arica-Parinacota, and the significant negative values for the Fu's Fs and Tajima's D statistic, this population unit is expected to have undergone a recent population size reduction, and it would currently be at a phase of demographic expansion. Although not all groups showed significant values for expansion or for bottleneck indicators, 2 clusters exhibited significant negative Tajima's D values, both distributed in the Arica-Parinacota region and border areas between Chile and Bolivia. In this regard, only the localities included in the C4 cluster, which are mainly distributed in Tarapacá, Chile, showed a significant Raggedness value relative to the expected distribution under an expansion model. Below we discuss the plausible mechanisms that could explain the lack of population structure found.

The Andean highlands are believed to have first appeared around the Late Cretaceous, while the Western Cordillera and the Eastern Cordillera arose later, during the Miocene. Subsequently, during the Plio-Pleistocene, the Andean mountain range rose to nearly its current height, at approximately 4000 m (Moon 2008). Therefore, the Andean highlands have existed as an ecosystem for a long enough time to have generated dynamic evolutionary processes that promoted diversification in several biological groups, probably associated to vicariant events and fragmentation of aquatic environments (e.g., Collado et al. (2013)). At an intraspecific level, Vila et al. (2013) conducted a phylogeographic analysis for *Orestias ascotanensis*, a fish species that now inhabits only the Ascotan saltmarsh, but with isolated populations in nonconnected watersheds. The authors found highly differentiated populations when comparing those from distant watersheds, but less differentiation and more diversity between populations from watersheds closer together. Although this suggests a history of low connectivity for some isolated aquatic habitats, this also suggest that, in the past, water levels may have connected some other geographically closer populations that are currently isolated. Evidence for low genetic structure in amphibians from the same area considered in this study was also found for *Rhinella spinulosa*, suggesting high connectivity in the recent past. Correa et al. (2010) found a unique widely distributed haplotype for this frog. De la Riva and collaborators (2010) suggest that the current distribution of the *T. marmoratus* species complex could have arisen from refugial populations from lowlands, during cold and dry periods. Individuals could have dispersed from these refugia and could have quickly colonized territories at higher elevations during wetter periods, resulting in present-day populations. According to our results, most of the historical migrations (see Migrate results), would have been directional and strongly asymmetric originating from the high western Altiplano in Chile, towards the east in Bolivia. The haplogroup from Arica-Parinacota (C2) occurs in basins clearly topographically separated from adjacent basins in Chile, where the Tarapacá populations (C4) exist. This could be explained because some of these basins flow into Bolivian territory, allowing a greater connectivity among Chilean and Bolivian populations than between both Chilean groups. The occurrence of the some broadly distributed haplotypes, as in the case of the haplotypes from the Arica-Parinacota region, could be taken as evidence for recent dispersal events. One explanation for the genetic variation and haplotype distribution recovered for the *T. marmoratus* species complex is the probable influence of climate shifts and recent glacial events. The wide distribution exhibited by some haplotypes and the low structure found could be explained by events that resulted in increased habitat connectivity during postglacial melting. Glacial processes would have had an important impact on the biodiversity of the highlands, through a cycling of dry and wet periods (Ochsenius 1986). According to Maldonado and Rozas (2008), during the Last Glacial Maximum (LGM), temperatures dropped while rainfall increased in the north of Chile. A wet cycle would have occurred 14 000 to 11 000 years ago, whereas around 7000 years ago the climatic conditions would have been extremely arid. At the end of each glacial cycle, following the melting of the ice-sheet, lakes would have suffered an increase in both depth and extension. The former suggests that during these periods, aquatic environments could have been saturated with water, becoming the most common habitat, covering a larger area, and hence being closer to each other. According to Villagrán (1993), during the early Holocene (between 8000 and 4000 years), climatic conditions would have been more unstable with cycles of dry and wet periods taking place in different regions of South America. As a consequence, paleoenvironmental changes in the Andean highlands would have repeatedly caused reductions in the suitable habitat for *T. marmoratus* (Duellman 1982), especially during dry periods. This could have resulted in a reduction in population size, therefore leading to periods of fragmented populations and low connectivity which would have alternated with periods of glaciation, with lower temperatures and more rainfall (Maldonado and Rozas 2008). In turn, connectivity would have been reestablished during postglacial periods, when melting ice would have raised the water level of lakes, extending their coverage (Ochsenius 1986) and probably flooding or saturating wide areas that currently do not form water bodies or wetlands. This phenomenon could have increased connectivity levels for the *Telmatobius* populations among wide areas, leading to secondary contact of certain lineages and reducing genetic structure levels.

Although one of the reasons that may account for patterns of absence of genetic structure is the retention of ancestral polymorphism (Funk and Omland 2003), our coalescent simulations indicated that the low genetic structure found in *T. marmoratus* was unlikely to be due to incomplete lineage sorting. Our results indicate that only the models incorporating migration account for the observed patterns of low structure. While expected cycles of greater connectivity in the Altiplano after ice melting during postglacial phases (e.g., post Last Glacial Maximum) are a plausible explanation for the high historical gene flow in *T. marmoratus*, our coalescent simulations suggest that high connectivity events should have also occurred during more recent periods. Models suggest that high levels of gene flow in *T. marmoratus* might have occurred within the last 1000 years. Several paleoenvironmental evidence sources about the climate patterns in the Altiplano show that wet levels have been very unstable, with contrasting rainfall cycles, and with an important role of El Niño Southern Oscillation (ENSO), after the Puna Glaciation (Maldonado and Rozas 2008). Even during the Holocene, climatic fluctuations were common, and, for example, Lake Titicaca reached its current size only 3500 ybp (De La Riva et al. 2010). Bräuning (2009) provides an overview of what can be derived about changes of temperature and moisture conditions in the humid and arid parts of the Andes. He suggests that a progressive increase of humidity is evidenced during the late Pleistocene in humid Andes, since approximately 1000 ybp. Studies based on paleohydrological reconstructions in the central-southern Altiplano (18°–26 °S) show abrupt paleohydrological and paleoclimatic changes synchronous with the termination of the Little Ice Age from approximately 500 ybp (Valero-Garcés et al. 2003). Latorre et al. (2003), based on midden records from central Andes in arid prepuna (22–23°S), identify conditions wetter

than today during several periods including up 1.2 ka, and Rech et al. (2003), studying mid-Holocene deposits, suggest that local ground-water levels rise and wetland deposits aggrade in deep canyon systems, such as in the Río Puripica (approximately 23°S), which is connected to the west Altiplano. In their analysis they show evidence for many depositional environments, which indicate that it formed during a period of higher regional ground-water levels that were sustained by enhanced precipitation and recharge in the High Andes as recently as 500 ybp. According to the above, although we do not discard the role of increased connectivity during postglacial cycles, our results and previous paleoclimatic evidence suggest that population connectivity in *T. marmoratus* should have lasted until very recently (a few centuries ago), which is consistent with paleoclimatic evicence of increased humidity during the late Holocene.

Biodiversity, in simple terms, refers to all of the different life forms on our planet, and includes both species diversity and genetic diversity (Freeland 2005). Genetic erosion is assumed to decrease the mean fitness of populations (Reed and Frankham, 2003), and population genetic diversity (alpha and beta spatial scales) is believed to be essential to ensure species viability (Berthier et al. 2005). The most effective way of preserving biodiversity is by maintaining self–sustaining populations of native species in their natural ecosystems (Rodrigues and Gaston, 2002). This often requires the designation of nature reserves, areas where the conservation of biodiversity is a priority over other forms of land use (Pérez-Lozada and Crandall 2003). In this sense, biodiversity conservation strategies adopt a form of risk analysis that involves estimating patterns of variation, and then trying to conserve as much of that estimated variation as possible as a way to retain "options" (possible values) for the future (Faith and Baker 2006). The current system of protected areas in Chile does not adequately represent terrestrial vertebrate diversity. Chilean protected areas increase in frequency and size toward the south and have a strong bias towards temperate forest ecosystems (Tognelli et al. 2008). This partly justifies an urgent assessment of the geographic distribution of intraspecific conservation values based on phylogenetic diversity, mainly regarding the biodiversity components of central and northern Chile.

One difficulty we can foresee is that limited resources for conservation may impose practical limitations on the conservation of these units of diversity (the so-called 'resources' problem, McNeely et al. 1990). In order to optimize the prioritization of conservation units (areas), PD was proposed by Faith (1992b) as a measure of biodiversity option value. The evolutionary value of populations within a species should be one of the key components of any system that assigns conservation priorities. In reference to the proportion of PD of the *T. marmoratus* complex that is currently protected in Chile, our results show that all lineages are adequately represented within areas from the SNASPE. Of the unprotected areas (Figure 1), those that would contribute the most in raising the PD are the populations from the sites of Caquena and Quebe, from Arica-Parinacota and Tarapacá, Chile, respectively. The importance of protecting the Caquena population stems from its status as an independent evolutionary lineage relative to close populations, and that its closest sister-lineage within Chile is 150 km south, in the Isluga site. On the other hand, the Quebe population stands out because of its high phylogenetic diversity, as this population included haplotypes from different lineages, adding to a higher value of branch length.

Studies focusing on intraespecific diversity in these localities are scarce and the taxonomic and systematic knowledge in this area of the Altiplano is currently under development. Therefore, phylogenetic diversity for other taxa distributed in Caquena and Quebe had not been explored, so we do not know whether these sites also possess a high conservation value for multiple species. Correa et al (2010), in regards to populations of amphibian *Rhinella spinulosa*, detected a clear northern lineage associated to small endorheic drainage systems that included the Caquena and Quebe sites. Additionally, Collado et al (2011) working with snails from genus *Biomphalaria*, described 2 clustered monophyletic groups restricted to several aquatic systems within the Caquena and Lauca basins that may represent separate candidate species. More studies are needed in order to determinate priority areas in these systems, considering information from co-distributed taxa in those populations (including Caquena and Quebe). On the other hand, current legislation in Chile regarding protected areas is disperse, disarticulated, and incomplete, weakening strategies that could be potentially adopted in order to protect and conserve biodiversity. For this reason, the creation of a legislative bill that aims to create the Service of Biodiversity and Protected Areas is currently in process, whose objectives are to improve the representativeness of inland water ecosystems. Although the present criteria for prioritizing conservation areas are mainly based in neutrally evolving DNA, we are aware that this should be complemented with ecological and adaptive criteria (Crandall et al. 2000). However, our results are a good starting point for improving proposals for designing protected areas to preserve intraspecific variants of the Chilean biota.

The present study is the first that focuses on phylogeographic patterns for *Telmatobius* species occurring in Chile's highlands, as previous publications concentrated on relationships at the supraspecific level and were centered on species from Bolivia and Peru (Benavides et al. 2002; Benavides 2005; Aguilar et al. 2012). Additionally, this work constitutes the first approach in a *Telmatobius* species complex that quantifies evolutionary intraspecific diversity including protected areas in Chile. This type of approach has been used previously in vertebrates, but evaluating the protected PD at the multispecies level (Zupan et al. 2014). Our results highlight the importance of inferring historical processes that explain the current geographic distribution of lineages and considering this information in the optimization of conservation strategies at an intraspecific level.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

Funding

Fondo Nacional de Desarrollo Científico y Tecnológico, Chile: grant numbers 1140540 (M. M.) and 1120872 (P. V.). P. F. thank CONICYT-PCHA/Doctorado Nacional/2014-21140355.

Acknowledgments

Collection permits from Servicio Agrícola y Ganadero (Resolución SAG N° 3037), and Corporación Nacional Forestal (CONAF), Chile, are greatly appreciated. We thank Alejandra Panzera and Celia Fernandes de Lima for helping in the process this manuscript.

References

Arbogast B, Kenagy G. 2008. Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography*. 28:819–825.

Aguilar C, Catenazzi A, Venegas PJ, Siu-Ting K. 2012. Morphological variation of *Telmatobius atahualpai* (Anura: Telmatobiidae) with comments on its phylogenetic relationships and synapomorphies for the genus. *Phyllomedusa: Journal of Herpetology*. 11:37–49.

Aguilar C, Valencia N. 2009. Relaciones filogenéticas entre telmatobiinidos (Anura, Ceratophryidae, Telmatobiinae) de los Andes centrales basado en la morfología e los estados larval y adultos. *Revista Peruana de Biología*. 16:43–50.

Avise JC. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution*. 43:1192–1208.

Baker CS. 2013. Journal of Heredity adopts joint data archiving policy. *Journal of Heredity*. 104:1.

Ballard JW, Whitlock MC. 2004. The incomplete history of mitochondria. *Molecular Ecology*. 13:729–744.

Beerli P. 2006. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. *Bioinformatics*. 22:341–345.

Beerli P, Palczewski M. 2010. Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics*. 185:313–326.

Benavides E. 2005. The *Telmatobius* species complex in Lake Titicaca: applying phylogeographic and coalescent approaches to evolutionary studies of highly polymorphic Andean frogs. *Monografías de Herpetología*. 7:167–185.

Benavides E, Ortiz JC, Sites JW. 2002. Species boundaries among the *Telmatobius* (Anura, Leptodactylidae) of the Lake Titicaca basin: allozyme and morphological evidence. *Herpetologica*. 58:31–55.

Berthier K, Galan M, Foltete JC, Charbonnel N, Cosson JF. 2005. Genetic structure of the cyclic fossorial water vole (Arvicola terrestris): landscape and demographic influences. *Molecular Ecology*. 14:2861–2871.

Bräuning A. 2009. Climate variability of the tropical Andes since the late Pleistocene. *Advances in Geosciences*. 22:13–25.

Carnaval AC, Hickerson MJ, Haddad CF, Rodrigues MT, Moritz C. 2009. Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science*. 323:785–789.

Clement M, Posada D, Crandall K. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology*. 9:1657–1659.

Collado G, Valladares M, Méndez M. 2013. Hidden diversity in spring snails from the Andean Altiplano, the second highest plateau on Earth, and the Atacama Desert, the driest place in the World. *Zoological Studies*. 52:1–13.

Collado GA, Vila I, Mendez MA. 2011. Monophyly, candidate species and vicariance in Biomphalaria snails (Mollusca: Planorbidae) from the Southern Andean Altiplano. *Zoologica Scripta*. 40:613–662.

Correa C, Pastene L, Sallabery M, Veloso A, Méndez M. 2010. Phylogeography of *Rhinella spinulosa* (Anura: Bufonidae) in northern Chile. *Amphibia-Reptilia*. 31:85–96

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK. 2000. Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*. 15:290–295.

Crandall KA, Templeton AR. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics*. 134:959–969.

Crozier RH. 1997. Preserving the information content of species: Genetic diversity, phylogeny, and conservation worth. *Annual Review of Ecological Systematics*. 28:243–268.

Daniels SR, Gouws GB, Stewart A, Coke M. 2003. Molecular and morphometric data demonstrate the presence of cryptic lineages among freshwater crabs (Decapoda: Potamonautidae: Potamonautes) from the Drakensberg Mountains, South Africa. *Biological Journal of the Linnean Society*. 78:129–147.

De la Riva I, Aparicio J, Ríos N. 2005. New species of *Telmatobius* (Anura: Leptodactylidae) from humid paramo of Perú and Bolivia. *Journal of Herpetology*. 39:409–416.

De la Riva I, García-Paris M, Parra-Olea G. 2010. Systematics of Bolivian frogs of the genus *Telmatobius* (Anura, Ceratophryidae) based on mtDNA sequences. *Systematics and Biodiversity*. 8:49–61.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*. 7:214.

Duellman W. 1982. Compresión climática cuaternaria en Los Andes. Efectos sobre especiación. In: Salinas PJ, editor. *Proceedings of the VIII Latinoamerican Congress of Zoology*; 1980 Oct 5–11; Mérida, Venezuela. p. 184–201.

Ehrlich P, Wilson E. 1991 Biodiversity studies: Science and policy. *Science*. 253:758–762.

Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*. 1:47–50.

Faith DP. 1992a. Conservation evaluation and phylogenetic diversity. *Biological Conservation*. 61:1–10.

Faith DP. 1992b. Systematics and conservation: on predicting the feature diversity of subsets of taxa. *Cladistics*. 8:361–373.

Faith DP. 1994. Phylogenetic pattern and the quantification of organismal biodiversity. *Philosophical Transactions of the Royal Society B*. 345:45–58.

Faith DP and Baker MA. 2006. Phylogenetic diversity (PD) and biodiversity conservation: Some bioinformatics challenges. *Evolutionary Bioinformatics Online*. 2:70–77.

Fraser, DJ, Bernatchez, L. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology*. 10:2741–2752.

Freeland JR. 2005. *Molecular ecology*. Chichester, UK: John Wiley and Sons Ltd.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*. 147:915–925.

Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology Evolution and Systematics*. 34:397–423.

Goebel AM, Donnelly JM, Atz ME. 1999. PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome b in bufonids and others Frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. *Molecular Phylogenetics and Evolution*. 11:163–199.

Gregory-Wodzicki K. 2000. Uplift of the Central and Northern Andes: A review. *GSA Bulletin*. 112:1091–1105.

Guedj B and Guillot G. 2011. Estimating the location and shape of hybrid zones. *Molecular Ecology Resources*. 11:1119–1123.

Guillot G. 2008. Inference of structure in subdivided populations at low levels of genetic differentiation–the correlated allele frequencies model revisited. *Bioinformatics*. 24:2222–2228.

Guillot G, Estoup A, Mortier F, Cosson JF. 2005a. A spatial statistical model for landscape genetics. *Genetics*. 170:1261–1280.

Guillot G, Mortier F, and Estoup A. 2005b. Geneland: A computer package for landscape genetics. *Molecular Ecology Notes*. 5:708–711.

Guillot G, Renaud S, Ledevin R, Michaux J, and Claude J. 2012. A unifying model for the analysis of phenotypic, genetic and geographic data. *Systematic Biology*. 61:897–911.

Hewitt GM. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*. 58:247–276.

Hewitt GM. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*. 359:183–195.

Humphries CJ, Williams PH, Vane-Wright RI. 1995. Measuring biodiversity value for conservation. *Annual Review of Ecology and Systematics*. 26:93–111.

Icochea J, Arizabal W, Lehr E, De la Riva I, Veloso A, Núñez H, Lavilla E. 2010. *Telmatobius marmoratus*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.2. Available from: URL http://www.iucnredlist.org. Accessed July 25, 2014.

Kass RE, Raferty, AE. 1995. Bayes factors. *Journal of American Statistical Association*. 90:773–795.

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*. 26:1463–1464.

Latorre C, Betancourt JL, Rylander KA, Quade J, Matthei, O. 2003. A vegetation history from the arid prepuna of northern Chile (22–23°S) over the last 13 500 years. *Palaeogeography, Palaeoclimatology, Palaeoecology*. 194:223–246.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*. 25:1451–1452.

Mace GM, and Lande R. 1991. Assessing extinction threats toward a reevaluation of the IUCN threatened species categories. *Conservation Biology*. 5:148–157.

Maddison, WP, Maddison DR. 2008. Mesquite: A modular system for evolutionary analysis. *Evolution*. Available from: URL http://mesquiteproject.org.

Maldonado A, Rozas E. 2008. Clima y paleoambiente durante el Cuaternario tardío en la Región de Atacama. In: Squeo F, Arancio G, Gutierrez J, editors. *Libro Rojo de la Flora y Fauna Nativa para su Conservación: Región de Atacama*. La Serena: Ediciones Universidad de la Serena. p. 293–304.

McDonald JH, Kreitman M. 1991. Adaptive protein evolution at the Adh locus in *Drosophila*. *Nature*. 54:1218–1233.

McNeely JA, Miller KR, Reid WV, Mittermeier RA, Werner TB. 1990. *Conserving the WorM's biological diversity*. International Union for Conservation of Nature and Natural Resources/World Resources Institute/Conservation International/World Wildlife Fund/US. World Bank.

Moon MA. 2008. The geology and physiography of the altiplano of Perú and Bolivia. *Transactions of the Linnean Society of London*. 1:27–43.

Moritz C. 1994. Defining 'evolutionarily significant units' for conservation. *Trends in Ecology & Evolution*. 9:373–375.

Moritz C. 1995. Uses of molecular phylogenies for conservation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 349:113–118.

Moritz C. 1999. A molecular perspective of biodiversity. In: Kato S, editor. *The Biology of Biodiversity*. Tokyo: Springer Verlag.

Moritz C, Faith DP. 1998. Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology*. 7:419–429.

Moritz C, Schneider CJ, Wake DB. 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. *Systematic Biology*. 41:273–291.

Nei M. 1987. *Molecular Evolutionary Genetics*. NY: Columbia University Press.

Ochsenius C. 1986. La Glaciación Puna durante el Wisconsin, deglaciación y máximo lacustre en la transición Wisconsin-Holoceno y refugios de megafauna postglaciales en la Puna y Desierto de atacama. *Revista de Geografía Norte Grande*. 13:29–58.

Nipperes DA, Matsen FA. 2013. The mean and variance of phylogenetic diversity under rarefaction. *Methods in Ecology and Evolution*. 4:566–572.

Pérez-Lozada M, Crandall KA. 2003. Can taxonomic richness be used as a surrogate for phylogenetic distinctness indices for ranking areas for conservation?. *Animal Biodiversity and Conservation* 26.1:77–84.

Posada D. 2008. jModeltest: Phylogenetic model averaging. *Molecular Biology and Evolution*. 25:1253–1256.

Rech JA, Pigati JS, Jay Quade J, Betancourt JL. 2003. Palaeogeography, re-evaluation of mid-Holocene deposits at Quebrada Puripica, northern Chile. *Palaeogeography, Palaeoclimatology and Palaeoecology*. 194:207–222.

Reed DH, Frankham R. 2003. Correlation between fitness and genetic diversity. *Conservation Biology*. 17:230–237.

Rodrigues ASL, Gaston K J. 2002. Maximizing phylogenetic diversity in the selection of networks of conservation areas. *Biological Conservartion*. 105:103–111.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*. 19:1572–1574.

Sáez P, Fibla P, Correa P, Sallaberry M, Salinas H, Veloso A, Mella J, Iturra P, Méndez M. 2014. A new endemic lineage of the Andean frog genus *Telmatobius* (Anura, Telmatobiidae) from the western slopes of the central Andes. *Zoological Journal of the Linnean Society*. 171:769–782.

Seimon TA, Seimon A, Daszak P, Halloy SRP, Schloegel LM, Aguilar CA, Sowell P, Hyatt AD, Konecky B, Simmons JE. 2007. Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation. *Global Change Biology*. 13:288–299.

Sérsic A, Cosacov A, Cocucci AA, Johnson L, Pozner R, Avila LJ, Sites JW, Morando M. 2011. Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. *Biological Journal of the Linnean Society*. 103:475–494.

Sites JW, Marshall J. 2004. Operational criteria for delimiting species. *Annual Review of Ecology, Evolution and Systematics*. 35:199–227.

Slatkin M, Maddison WP. 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics*. 123:603–613.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*. 123:585–595.

Tamura K, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Molecular Biology and Evolution*. 28:2731–2739.

Tognelli MF, Ramirez de Arellano PI, Marquet PA. 2008. How well do the existing and proposed reserve networks represent vertebrate species in Chile?. *Diversity and Distribution*. 14:148–158

Valero-Garcés BL, Delgado-Huertas A, Navas A, Edwards L, Schwalb A, Ratto N. 2003. Patterns of regional hydrological variability in centralsouthern Altiplano (18°-26°S) lakes during the last 500 years. *Palaeogeography, Palaeoclimatology, Palaeoecology*. 194:319–338.

Vila I, Morales P, Scott S, Poulin E, Véliz D, Harrod C, Méndez MA. 2013. Phylogenetic and phylogeographic analysis of the genus Orestias (Teleostei: Cyprinodontidae) in the southern Chilean Altiplano: the relevance of ancient and recent divergence processes in speciation. *Journal of Fish Biology*. 82:927–943.

Vila I, Scott S, Méndez M, Valenzuela F, Iturra P, Poulin E. 2011. *Orestias gloriae*, a new species of cyprinodontid fish from saltpan spring of the southern high Andes (Teleostei: Cyprinodontidae). *Ichthyological Exploration of Freshwaters*. 22:345–353.

Villagrán C. 1993. Una interpretación climática del registro palinológico del Ultimo Máximo Glacial-Postglacial en Sudamérica. *Bulletin de l'Institut Francais d'Études Andines*. 22:243–258.

Xia X, Xie Z. 2001. DAMBE. Software package for data analysis in molecular biology and evolution. *Journal of Heredity*. 92:371–373.

Zupan L, Cabeza M, Maiorano L, Roquet C, Devictor V, Lavergne S, Mouillot D, Mouquet N, Renaud J, Thuiller W. 2014. Spatial mismatch of phylogenetic diversity across three vertebrate groups and protected areas in Europe. *Diversity and Distributions*. 20:674–685.