Amphibia-Reptilia 31 (2010): 85-96

Phylogeography of *Rhinella spinulosa* (Anura: Bufonidae) in northern Chile

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Abstract. The southern part of the Altiplano of the Andes Range is characterized by a complex hydrography, due to an intense geologic activity and the effects of the Pleistocene glaciations. This has produced a high degree of diversity at the species level in some aquatic taxa (e.g., fish and amphibians), which suggests that these same processes have produced divergence at the intraspecific level in co-distributed taxa. We investigated the genetic variation in populations of the anuran *Rhinella spinulosa* which represent its entire distribution in the extreme north of Chile (17°44′S-23°47′S). Haplotype networks of the mitochondrial control region recognized two main lineages, one of which is distributed from the northern boundary of Chile to the Salar de Alconcha and the other from the Salar de Carcote to the locality of Tilomonte. The northern lineage showed little phylogeographic structure; a few very frequent haplotypes are widely distributed. The southern lineage had greater structure, due principally to the high divergence of the populations from the eastern springs of the Salar de Atacama. Fu's *Fs* test and the mismatch distributions suggested that most of the populations of both lineages are in the process of demographic expansion. The spatial distribution of the genetic variability was correlated with the hydrography and the paleoclimatological data available for the region, which suggested that geographic expansions followed by periods of contraction of population ranges, together with sporadic floods may explain the observed phylogeographic patterns.

Keywords: anuran, Chilean Altiplano, mtDNA, phylogeographic structure.

Introduction

The Altiplano or Puna is an extensive high-altitude tableland located in the central Andes range, which includes parts of Peru, Bolivia, Chile and Argentina. This region has been affected by profound geological and climatic changes produced by the lifting of the Andes, volcanism and Pleistocene glaciations (Trumbull et al., 2006; Strecker et al., 2007), whose combined influence has determined the biogeographic and diversification patterns of its flora and fauna (Veloso et al., 1982; Arroyo et al., 1988; Ezcurra, 2002; Palma et al., 2002; Koscinski et al., 2008). In a wider biogeographic context, this region is considered to form part of an Andean transition zone where tropical and austral elements have mixed, which would also have contributed to the diversification of its flora and fauna (Morrone, 2004).

In Chile, the Altiplano occupies a narrow strip in the northeast corner of the country from approximately 17°30′S to 24°S, limited on the west by the eastern Andes Range. With a mean altitude of 4200 m, this region is characterized by a dry, cold climate which has produced mainly steppe vegetation (Veloso and Arroyo, 1982). The scarce precipitation in this zone comes from the tropical area to the East, but does not reach beyond the western Andes slopes to the Atacama Desert (Garreaud et al., 2003). Hydrographically, the majority of the northeast corner of Chile is occupied by endorheic drainage systems, which are limited to the west by endorheic or arheic basins which originate in the western slopes of the Andes and enter the Atacama Desert (e.g., Pampa del Tamarugal; fig. 1). The only exorheic systems of this zone are the Lluta and Azapa rivers, located in the

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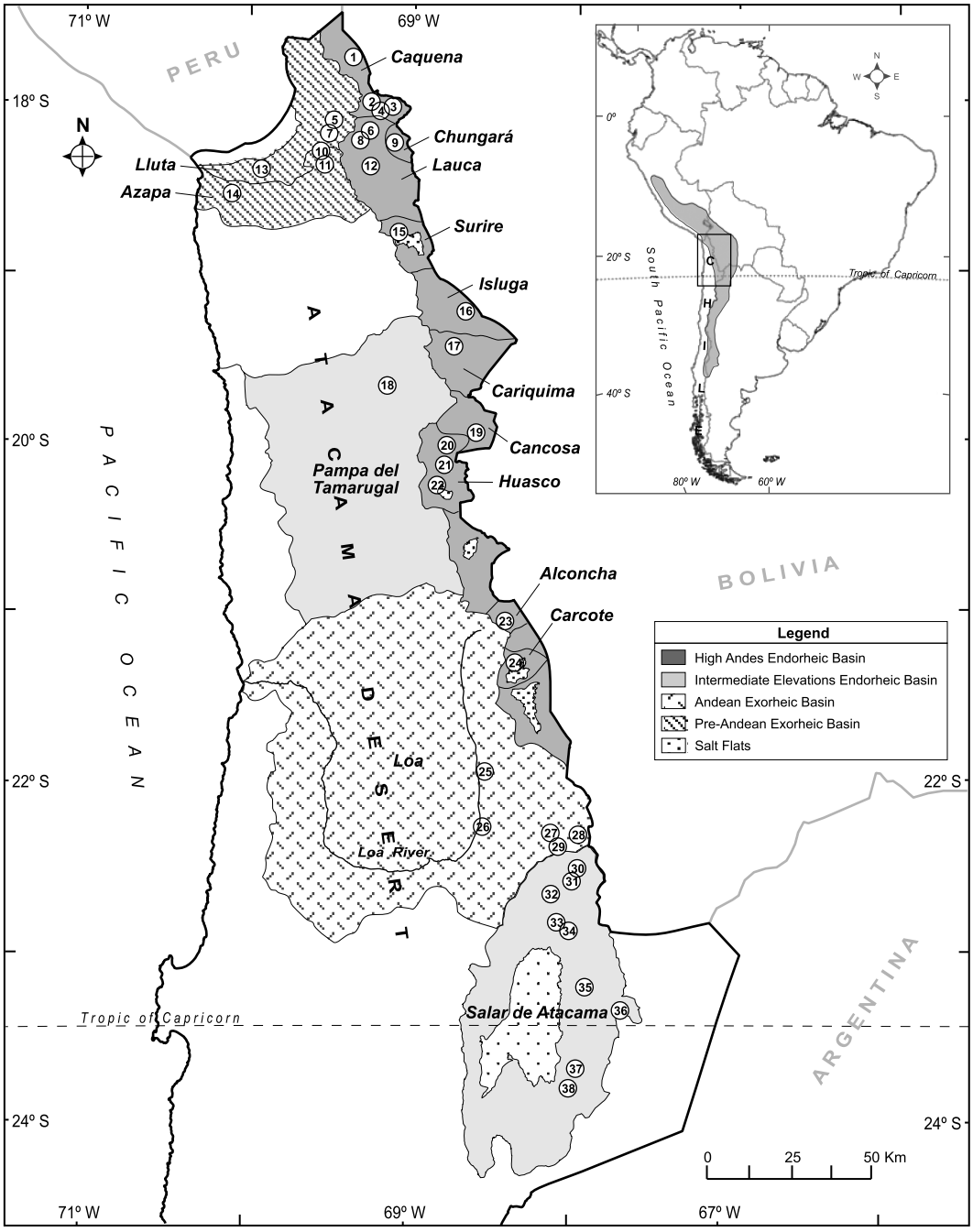

Figure 1. Map of northern Chile showing the localities (numbered circles) of *Rhinella spinulosa* included in this study (table 1 specifies the number of each locality). The names and limits of the hydrographic basins within Chile where the species is found are shown (thin solid lines). The different types of drainage basins are indicated with different gray tones and patterns. The map in the upper right shows the geographic distribution of *R. spinulosa* in South America (gray area).

extreme north of the country, and the Loa River (Niemeyer and Cereceda, 1984; fig. 1). Associated with the complex hydrography of this part of Chile, a high richness has been described at the species level for some aquatic taxa, such as the amphibians of the genus *Telmatobius*

Table 1. Number of specimens utilized (n) and geographic data of the localities of *Rhinella spinulosa* included in this study. The first column indicates the number (N) which represents each of the localities in the map of fig. 1. The last column indicates the haplotypes found in each population, numbered according to the network (fig. 2).

N	Locality	n	Basin name	Latitude (S)	Longitude (W)	Altitude (m)	Haplotypes
1	Umaqui	3	Caquena	17°44′07″	69°23′13″	4132	1, 2
2	Vioco	4	Caquena	18°02′31″	69°16′45″	4383	1, 3
3	Caquena	4	Caquena	18°03′33″	69°12′16″	4398	1, 4-6
4	Colpa	1	Caquena	18°03′34″	69°13′59″	4356	7
5	Pacollo	6	Lluta	18°10′46″	69°31′10″	4090	1, 8-9
6	Lauca	2	Lauca	18°11′39″	69°16′25″	4395	1
7	Putre	8	Lluta	18°11′47″	69°33′35″	3507	9-13
8	Parinacota	14	Lauca	18°12′52″	69°18′05″	4399	1, 9, 14-17
9	Chungará	5	Chungará	18°13′57″	69°10′53″	4583	1, 9, 18-19
10	Socoroma	5	San José de Azapa	18°15′48″	69°36′09″	3058	9, 12-13, 20
11	Zapahuira	9	San José de Azapa	18°19′50″	69°35′30″	3320	8, 11-13, 20
12	Chivatambo	4	Lauca	18°21′41″	69°16′38″	4329	1, 18
13	Lluta	2	Lluta	18°23′46″	69°58′23″	836	12
14	Azapa	3	San José de Azapa	18°31′14″	70°10′30″	287	12, 21-22
15	Surire	6	Surire	18°51′01″	69°08′25″	4081	1, 14, 23
16	Isluga	3	Isluga	19°15′10″	68°42′16″	3776	1, 14
17	Quebe	16	Cariquima	19°27′18″	68°48′34″	3958	1, 23-26
18	Chusmisa	8	Pampa del Tamarugal	19°40′45″	69°10′53″	3213	21-22
19	Cancosa	6	Cancosa	19°57′24″	68°41′59″	4007	1, 7, 23
20	El Piga	1	Huasco	20°01′57″	68°49′16″	3944	23
21	Collacagua	5	Huasco	20°07′50″	68°50′31″	3859	1, 23
22	Huasco	11	Huasco	20°16′45″	68°53′17″	3805	1, 23
23	Alconcha	2	Alconcha	21°03′39″	68°29′18″	4100	23
24	Carcote	7	Carcote	21°16′46″	68°19′21″	3688	27
25	Loa	5	Loa	21°56′50″	68°36′37″	3053	28
26	Lasana	1	Loa	22°16′22″	68°37′49″	2610	28
27	Caspana	6	Loa	22°20′03″	68°12′30″	3245	28-29
28	El Tatio	19	Loa	22°20′10″	68°00′59″	4264	28, 30-35
29	Chita	10	Loa	22°24′52″	68°10′21″	3741	28
30	Vado Río Putana	7	Salar de Atacama	22°31′42″	68°02′35″	4286	28-29, 31, 36-37
31	Machuca	6	Salar de Atacama	22°35′50″	68°03′52″	3979	28, 37-38
32	Río Grande	6	Salar de Atacama	22°39′56″	68°13′38″	3045	28-29
33	Katarpe	10	Salar de Atacama	22°50′02″	68°11′55″	2460	28-29, 37
34	Vilama	14	Salar de Atacama	22°51′49″	68°10′50″	2579	28-29, 37
35	Quebrada de Jere	23	Salar de Atacama	23°11′08″	67°59′27″	2513	29, 39-44
36	Tumbre	5	Salar de Atacama	23°19′13″	67°47′34″	3761	29
37	Peine	9	Salar de Atacama	23°41′00″	68°03′31″	2440	45
38	Tilomonte	17	Salar de Atacama	23°47′24″	68°06′34″	2365	46-48

(Veloso et al., 1982; Formas et al., 2005) and the fishes of the genus *Orestias* (Dyer, 2000; Lüssen et al., 2003).

There have been few studies that have investigated patterns of genetic differentiation at the specific or population level in the extreme north of Chile (Lüssen et al., 2003; Méndez et al., 2004; Palma et al., 2005). Lüssen et al. (2003) studied the phylogenetic relationships of the species of the genus *Orestias* in this area, suggesting a diversification of this group in the late Pleistocene. Palma et al. (2005), in a study of the phylogenetic relationships of species of sigmodontine rodents of the Chilean Altiplano and areas around the Atacama desert, found a high degree of phylogeographic differentiation between some of these species, suggesting a peripatric speciation model for this group of rodents. At the population level, Méndez et al. (2004) compared morphological and genetic variation among populations of the amphibian *Rhinella spinulosa* (as *Bufo spinulo-*

sus) in the north (18°12′S-18°31′S and 22°20′S-23°11′S) and central (32°51′S-33°21′S) zones of Chile. They found a positive correlation between morphological and genetic distances (obtained with nuclear RAPD markers) and a high level of genetic divergence between the two groups of populations of *R. spinulosa* from northern Chile, which was as great as between one of them and populations from central Chile. This last result suggested the presence of two highly divergent lineages in the north of Chile, but since the sampling only included two groups of populations separated by more than 450 km, it was not possible to obtain more precise information on the geographic distribution of those lineages.

R. spinulosa is widely distributed in the central and southern Andes (fig. 1) and is found throughout the Chilean Altiplano and contiguous zones between 2000 and 4600 m. Only in the extreme north of Chile, at around 18°25′S, populations have been found below 1000 m (Veloso et al., 1982; fig. 1). This is a polytypic species which includes several morphologically differentiated populations whose taxonomic status still is not clear (Cei, 1972). However, there still is no comprehensive phylogeographic study that clarifies the relationships among its populations and their levels of genetic divergence. The objective of the present study was to establish the distribution limits and patterns of genetic variation of the lineages detected by Méndez et al. (2004) in northern Chile by analyzing DNA sequences from the mitochondrial control region in an extensive sampling that included all the known distribution range in this region. The intra- and inter-population genetic variability and levels of phylogeographic structure of the main intra-specific lineages were examined in relation to the complex paleoclimatic and hydrogeographic history of this area of Chile to identify the historical and current processes that may explain this differentiation.

Materials and methods

We included a total of 273 specimens of *Rhinella spinulosa* from 38 Chilean localities located between 17°44′07″S and 23°47′24″S and between 67°47′34″W and 70°10′30″W (table 1, fig. 1). The samples included adults, juveniles, post-metamorphics and larvae; specimens are deposited in the Herpetological Collection of the Departamento de Biología Celular y Genética de la Universidad de Chile (DBGUCH). All individuals used in this study were treated in accordance with procedures approved by the Ethics Committee of the Universidad de Chile and the Chilean Comisión Nacional de Investigación Científica (CONICYT), which are based on the recommendations of the National Research Council (USA) (1996).

Total DNA was isolated from samples of toe, liver or muscle, using a modification of the salt extraction method of Jowett (1986). A fragment of the control region of mitochondrial DNA was amplified using primers CytbA-L (5′-GAATYGGRGGWCAACCAGTAGAAGACCC-3′) and ControlP-H (5′-GTCCATAGATTCASTTCCGTCAG-3′) reported by Goebel et al. (1999). The reaction mixtures included 3 mM of $MgCl_2$, 0.16 mM of each dNTP, 0.26 μM of each primer, 1.5 U of Taq polymerase (Invitrogen, Carlsbad, CA) and 50-100 ng of total DNA. The thermal profile for PCR was: 94°C for 2 min, followed by 36 cycles of 94°C for 30 s, 56°C for 45 s and 72°C for 90 s, with a final extension at 72°C for 10 min. The PCR products were sequenced in both directions in an ABI3730XL automatic sequencer (Applied Biosystems).

The DNA sequences of each individual were edited using the program BioEdit v.7.0.7.0 (Hall, 1999). Multiple alignment of the edited sequences was performed with the ClustalX v.1.81 program (Thompson et al., 1997) using the default parameters; alignments were then inspected visually. Haplotypes were obtained using the DnaSP v.4.50.3 program (Rozas et al., 2003).

The genetic variation patterns of the populations of *R. spinulosa* were studied using a combination of phylogeographic tools:

1. Haplotype networks were obtained using the median-joining network method (Bandelt et al., 1999), with the Network v.4.5.1.0 program (http://www.fluxus-engineering.com/sharenet.htm). The haplotypes for this analysis were generated including the sites with indels.
2. The phylogeographic structure of the populations was also investigated using spatial molecular analysis of variance using the SAMOVA v.1.0 program (Dupanloup et al., 2002). This program finds groups of contiguous populations that maximize the between-groups variance.
3. Two types of approximations were used to obtain information on the demographic history of the populations, using the groups recognized in the haplotype networks as units. The neutrality of the sequences was evaluated with Tajima's D statistic, and additionally the Fu's Fs test, to examinate whether the populations are in a process of population expansion. Finally, we obtained the mismatch distribution

of the groups of sequences, comparing them with a model of population expansion (Rogers and Harpending, 1992). All these analyses were performed with the DnaSP v.4.50.3 program, excluding the sites with indels.

4. We calculated the following diversity indices for the main groups defined in the haplotype network and for populations with sample size of $N \geqslant 5$: number of haplotypes, haplotype diversity and nucleotide diversity, using the program DnaSP v.4.50.3. We also calculated the uncorrected p-distances within and between the main groups defined by the haplotype network using the program MEGA4 (Tamura et al., 2007). The standard errors of the distances were estimated using bootstrap (100 pseudoreplicates).

Results

Sequence analysis

A total of 863 nucleotide sites were obtained as the final result of the multiple alignment, of which 70 were polymorphic and seven had indels. From the 273 sequences analyzed, 48 haplotypes were recovered when the indel sites were considered. The sequences were deposited in GenBank, with accession numbers AY663444-AY663517 and FJ643078-FJ643276.

Phylogeographic analyses

The network analysis showed two principal groups of highly divergent haplotypes (fig. 2): the northern lineage, composed of haplotypes 1 to 26, corresponding to the populations from the endorheic systems of the high Andes, pre-Andean exorheic systems and the Pampa del Tamarugal (17°44′S-21°04′S), and the southern lineage, composed of haplotypes 27 to 49, from the drainage basins of Carcote, the Loa River and the Salar de Atacama (21°17′S-23°47′S). The haplotype network of the northern lineage showed a low level of phylogeographic structure (i.e., the majority of the haplotypes are separated by few mutational steps), where the most frequent haplotypes have a very wide geographic distribution (table 1). By contrast, in the network of the southern lineage a high level of phylogeographic structure was found, due to the high divergence beetwen two groups of haplotypes (or haplogroups): (1) Populations from the drainage systems of Carcote, the Loa River and the northeast of the Salar de Atacama; (2) Quebrada de Jere, Peine and Tilomonte. The first haplogroup has the widest geographic distribution, reaching the locality of Tumbre (23°19′13″S), which is in the Salar de Atacama drainage system southeast of Quebrada de Jere (haplogroup 2). A singular aspect of the southern lineage is the overlapping of haplogroups 1 and 2 (fig. 2), since haplotype 29, one of the most frequent and widely distributed of haplogroup 1, is present in Quebrada de Jere (11 sequences) together with haplotypes restricted to this locality (12 sequences).

The SAMOVA analysis revealed a greater phylogeographic structure (i.e., the variance component among groups was greater than within groups or within populations) for the southern lineage than for the northern lineage (table 2). The four groups of populations found for the northern lineage were: (1) Pacollo, Putre, Socoroma, Zapahuira and Lluta, located between 800 and 3800 m; (2) Chusmisa (3213 m) and Azapa (287 m); (3) Vioco, Caquena, Colpa, Lauca, Parinacota, Chungará, Chivatambo, Surire, Isluga, Quebe, Cancosa, El Piga, Collacagua, Huasco and Alconcha, all over 3800 m; and (4) Umaqui (4132 m), which is the northernmost population included in this study. Five groups were found for the southern lineage: (1) Carcote (3688 m); (2) Loa, Lasana, Caspana, El Tatio, Chita, Vado Río Putana, Machuca, Río Grande, Katarpe and Vilama, located in the drainage system of the Loa River and in the springs of the northeast of the drainage basin of the Salar de Atacama (2579-4286 m). The other three groups are populations from the currently isolated eastern springs of the Salar de Atacama basin: (3) Quebrada de Jere (2513 m); (4) Peine (2440 m); and (5) Tilomonte (2365 m).

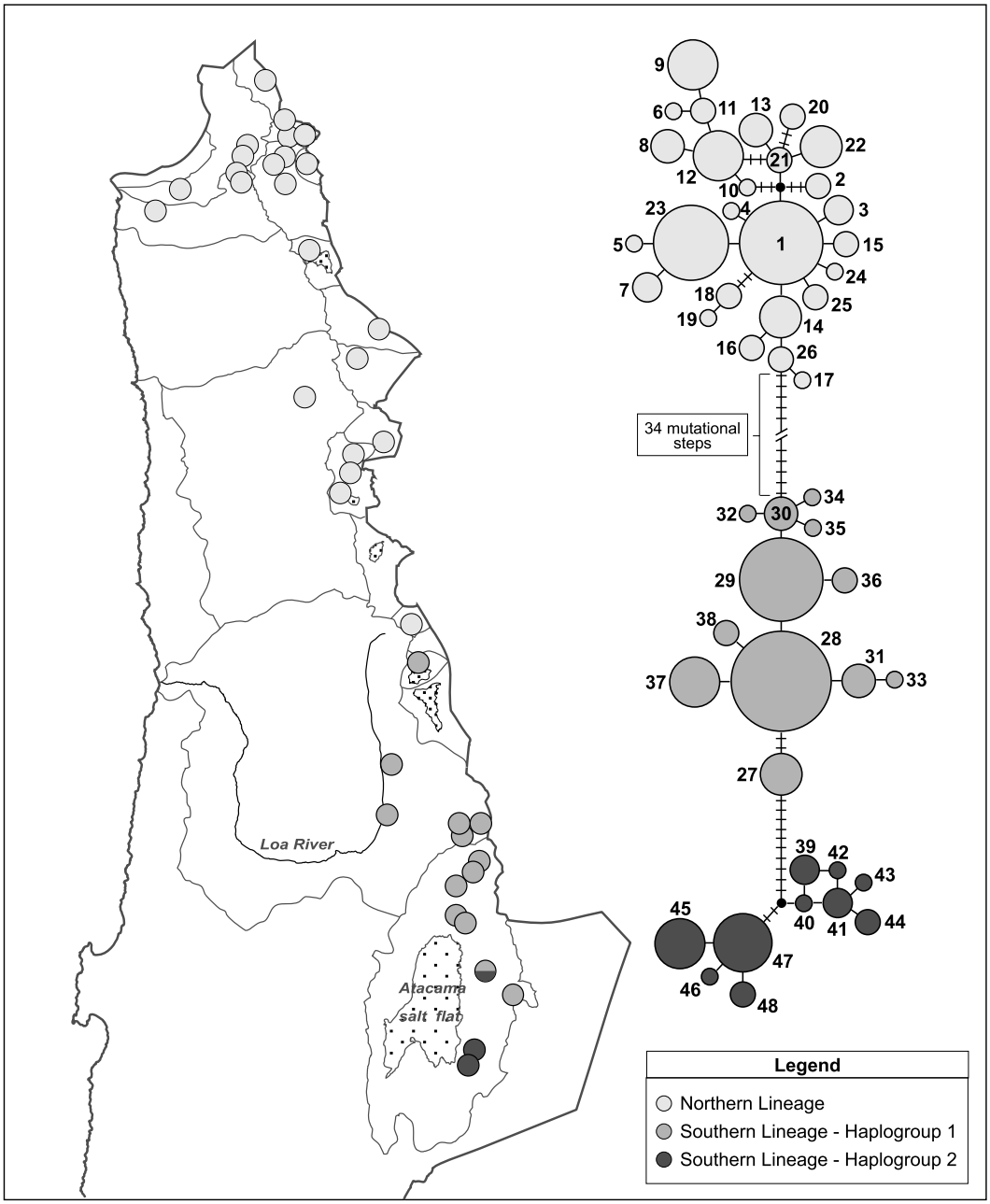

Figure 2. Haplotype network constructed using the median-joining method for the 48 mitochondrial haplotypes found in 273 individuals of *Rhinella spinulosa* from northern Chile. Haplotypes 1 to 26 are from the northern lineage; haplotypes 27 to 28 are from the southern lineage. The different degrees of shading of the localities (circles in the map) indicate the three main haplotype groups observed in the network. The size of circles in the network is proportional to the sampling frequency of haplotypes. The short transverse lines on the branches indicate the inferred mutational steps between the haplotypes (omitted when there was only one mutational step).

Demographic analyses

The definition of the groups of sequences to use in the tests of Tajima's *D* and Fu's *Fs* and the analysis of mismatch distributions was based on the groupings detected in the haplotype network and SAMOVA. For the northern lineage

we defined two groups, taking into account the principal divisions found in SAMOVA: (1) the haplotypes of populations mainly from over 3800 m (81 sequences) and (2) the haplotypes of populations mainly from below 3800 m (45 sequences). Two sequences from the population of Umaqui (haplotype 2) were excluded from this analysis because they showed the same degree of divergence with respect to the two groups just mentioned. Three groups were defined for the southern lineage: (1) haplotypes present in the drainage systems of Carcote, the Loa River and the springs from the northern part of the Salar de Atacama (107 sequences); (2) Quebrada de Jere (12 sequences); and (3) Peine and Tilomonte (26 sequences). Excluding the sites with indels, the analyses were performed on 858 sites for the groups of the northern lineage and 859 for the southern lineage.

Tajima's D indicated that all the groups of sequences mentioned above conform to a model of neutral evolution ($P > 0.1$). Although the five groups showed negative values for Fu's Fs test, these were only significant for Group 1 of the northern lineage and Group 1 of the southern lineage (table 3). The mismatch distribution showed a unimodal pattern that fits a model of expansion for the five groups of sequences defined above, suggesting that the corresponding populations are in a process of population expansion (table 3). A detail of uncorrected p-distances within and among lineages and haplogroups is shown in table 4. Additionally, diversity indeces for lineages, haplogroups and populations are specified in table 5.

Table 2. Percentages of variance for the components (sources of variation) of the spatial analysis of molecular variance (SAMOVA) for the two principal lineages of *Rhinella spinulosa* recognized in the haplotype network.

Source of variation	Northern lineage (four groups)	Southern linage (five groups)
Among groups	50.09*	78.55*
Among populations within groups	7.28*	0.99*
Within populations	42.64*	20.45*

* ($P < 0.05$).

Discussion

The mitochondrial evidence obtained in this study shows that the Chilean Altiplano and adjacent areas to the west, between 17°44′S and 23°47′S, are inhabited by two highly divergent lineages of *Rhinella spinulosa* (fig. 2; table 4). The limit between the two lineages is between the drainage systems of the Salar de Alconcha and the Salar de Carcote, approximately between 21°05′S and 21°15′S. Méndez et al. (2004) analyzed the genetic variation of this species in northern and central Chile with nuclear RAPD markers, suggesting the existence of two lineages in the north. Given that Méndez et al. (2004) included only a few populations from the north, it was not possible to discount the possibility that genetically interme-

Table 3. Results of Tajima's D [P] and Fu's Fs [P] tests and the mismatch distributions for the groups of haplotypes of *Rhinella spinulosa* recognized in this study. Pi = nucleotide diversity. Theta/site = theta per site, calculated using Eta (total number of mutations); Unimodal = unimodal distribution; Expansion = model of population expansion.

	Pi	Theta/site	Tajima's D	Fu's Fs	Mismatch distribution
Northern lineage					
Group 1	0.00126	0.00329	−1.73611 [NS]	−9.139 [0.000]	Unimodal, Expansion
Group 2	0.00312	0.00267	0.49961 [NS]	−0.967 [NS]	Unimodal, Expansion
Southern lineage					
Group 1	0.00118	0.00178	−0.80379 [NS]	−2.823 [0.034]	Unimodal, Expansion
Group 2	0.00158	0.00115	1.22248 [NS]	−1.132 [NS]	Unimodal, Expansion
Group 3	0.00081	0.00091	−0.28338 [NS]	−0.583 [NS]	Unimodal, Expansion

[NS] = not significant ($P > 0.05$).

Table 4. Uncorrected p-distances between (below the diagonal) and within (on the diagonal) the main lineages distinguished in the haplotype network. The values are mean ± standard error. The number of sequences used for each group is indicated (n). The sequences from Quebrada de Jere, which include haplotypes of the two haplogroups of the southern lineage, were considered in their respective haplogroups (see details in Results).

Lineage	n	Northern	Southern (haplogroup 1)	Southern (haplogroup 2)
Northern	128	0.00347 ± 0.00109		
Southern (haplogroup 1)	107	0.0398 ± 0.0066	0.00118 ± 0.00061	
Southern (haplogroup 2)	38	0.0414 ± 0.0069	0.0147 ± 0.0037	0.00290 ± 0.00107

Table 5. Number of haplotypes, haplotype diversity and nucleotide diversity for the main lineages distinguished in the haplotype network and populations with a sample size of 5 or more. The diversity values are mean ± standard deviation (sd). Also indicated are the number of sequences used for each group or population, the total number of nucleotide sites occupied and the number of polymorphic sites; the last two include the sites with indels.

Group or population	n° sequences	n° haplotypes	n° sites	Polymorphic sites	Haplotype diversity ± sd	Nucleotide diversity ± sd
Total	273	48	863	70	0.938 ± 0.006	0.0252 ± 0.0123
Northern lineage	128	26	859	24	0.892 ± 0.016	0.0037 ± 0.0021
Pacollo	6	3	858	7	0.800 ± 0.122	0.0044 ± 0.0029
Putre	8	5	858	6	0.857 ± 0.108	0.0024 ± 0.0017
Parinacota	14	6	859	10	0.846 ± 0.061	0.0044 ± 0.0027
Chungará	5	4	858	9	0.900 ± 0.161	0.0047 ± 0.0033
Socoroma	5	4	858	6	0.900 ± 0.161	0.0037 ± 0.0027
Zapahuira	9	5	858	7	0.861 ± 0.087	0.0030 ± 0.0020
Surire	6	3	859	2	0.600 ± 0.215	0.0010 ± 0.0009
Quebe	16	5	859	5	0.608 ± 0.130	0.0011 ± 0.0009
Chusmisa	8	2	858	1	0.250 ± 0.180	0.0003 ± 0.0004
Cancosa	6	3	858	2	0.733 ± 0.155	0.0010 ± 0.0009
Collacagua	5	2	858	1	0.600 ± 0.175	0.0007 ± 0.0008
Huasco	11	2	858	1	0.327 ± 0.153	0.0004 ± 0.0005
Southern lineage	145	22	863	28	0.863 ± 0.017	0.0077 ± 0.0040
Haplogroup 1	107	12	862	11	0.771 ± 0.026	0.0015 ± 0.0011
Carcote	7	1	861	0	0.000 ± 0.000	0.0000 ± 0.0000
Loa	5	1	862	0	0.000 ± 0.000	0.0000 ± 0.0000
Caspana	6	2	862	1	0.533 ± 0.172	0.0006 ± 0.0007
Tatio	19	7	862	6	0.819 ± 0.054	0.0022 ± 0.0015
Chita	10	1	862	0	0.000 ± 0.000	0.0000 ± 0.0000
Vado Río Putana	7	5	862	4	0.905 ± 0.103	0.0021 ± 0.0016
Machuca	6	3	862	2	0.733 ± 0.155	0.0010 ± 0.0009
Río Grande	6	2	862	1	0.533 ± 0.172	0.0006 ± 0.0007
Katarpe	10	3	862	2	0.733 ± 0.076	0.0012 ± 0.0010
Vilama	14	3	862	2	0.626 ± 0.105	0.0010 ± 0.0009
Tumbre	5	1	862	0	0.000 ± 0.000	0.0000 ± 0.0000
Haplogroup 2	38	10	863	10	0.804 ± 0.046	0.0030 ± 0.0018
Quebrada de Jere (total)	23	7	863	16	0.743 ± 0.081	0.0095 ± 0.0051
Quebrada de Jere (haplogroup 1)	11	1	862	0	0.000 ± 0.000	0.0000 ± 0.0000
Quebrada de Jere (haplogroup 2)	12	6	863	4	0.849 ± 0.074	0.0018 ± 0.0013
Peine	9	1	862	0	0.000 ± 0.000	0.0000 ± 0.0000
Tilomonte	17	3	862	2	0.324 ± 0.136	0.0004 ± 0.0005

diate populations might link those two groups. The present study suggests that this is not the case, and that there is a well-defined limit between these two intra-specific lineages, located between 21°05′S and 21°15′S, although the available information cannot exclude a possible zone of secondary contact.

The two main lineages differ in their degree of phylogeographic structure (fig. 2; table 2). Whereas the northern lineage (17°44′S-

21°04′S) showed little structure and haplotypes with a wide geographic distribution, the southern lineage (21°17′S-23°47′S) includes a very divergent group of haplotypes from the southeast of the Salar de Atacama (Quebrada de Jere, Peine and Tilomonte, 23°11′S-23°47′S), which would account for its greater degree of structure (fig. 2). This corroborates the genetic pattern observed by Méndez et al. (2004) with RAPD markers, where one of the populations of the southeast of the Salar de Atacama (Quebrada de Jere) appears separated in a UPGMA tree from some of the populations near the Loa River (Tatio, Chita and Vilama) included in this study. The concordance between the levels of divergence found using mitochondrial DNA sequences and nuclear RAPD markers for these populations and for the northern and southern lineages mentioned above suggests that the phylogeographic patterns found with the mitochondrial control region are a good approximation to the genetic differentiation of this species in this region of Chile.

The northern lineage is distributed in a north-south series of small endorheic drainage systems (from Caquena to Alconcha; fig. 1) which are closed or drain towards the east in hydrographic systems of Bolivia (Niemeyer and Cereceda, 1984). A common point for all these systems is that they were connected to extensive paleolakes which were formed at the end of the Pleistocene and whose remains currently form the lakes or salt flats of Poopó, Coipasa and Uyuni in Bolivia (Placzek et al., 2006). However, additional data are needed to determine if the population expansion of the northern lineage, inferred from Fu's *Fs* test and the mismatch distribution (table 3), may be associated with the expansion of one of these paleolacustrine systems. On the other hand, part of the genetic differentiation observed within this group may be due to the more recent formation of some of the drainage systems it occupies (e.g., Chungará, Surire) due to volcanic activity (Ochsenius, 1974), which may have isolated some populations.

A singular aspect of the northern lineage is that it includes the only known populations of *R. spinulosa* that live below 1000 m (Azapa and Lluta). With respect to the geographic distribution of the haplotypes of the Azapa population, it is important to note that two of them were also found in Chusmisa, located more than 160 km to the southeast in the arheic drainage basin of Pampa del Tamarugal (fig. 1). A possible explanation for the genetic affinity of these two populations, which are in the western border of the distribution of the species (and were distinguished as a group by SAMOVA), is that they belong to an old colonization front which expanded to the west when the climate of this area was more humid. One possible mechanism to explain the presence of the species in these marginal localities, particularly Chumisa, is the infrequent events of water discharge towards the arheic drainages in the Atacama Desert (Nester et al., 2007). Aditionally, the peripheral populations of the extreme north may be the result of range expansions aided by the floods produced during ENSO events (Garreaud et al., 2003), facilitated by the exorheic nature of the rivers Lluta and San José de Azapa.

As mentioned above, the greater degree of phylogeographic structure of the southern lineage is due principally to the high degree of divergence of the haplogroup present to the east of the Salar de Atacama (Quebrada de Jere, Peine and Tilomonte; fig. 2). The other haplogroup is present in populations located in the drainage system of the Loa River (the largest river which traverses the Atacama Desert) and the area north and northeast of the Salar de Atacama. This haplogroup is in expansion according to Fu's *Fs* test and the mismatch distribution (table 3), which may explain its presence both in the highest parts of the drainage systems of Loa River and the Salar de Atacama (over 3000 m) and in the lower altitude localities located in the western border (Loa River) and in the south of its distribution (north and east of the Salar de Atacama). A population expansion process supports the idea of a secondary contact

to explain the presence of haplotypes of the two haplogroups of the southern lineage in the Quebrada de Jere locality (fig. 2). However, nuclear evidence is required to evaluate other explanations, for example recent translocation of individuals due to human activity.

According to the demographic analyses, the majority of the populations included in this study represent lineages that are expanding (table 3). Although it has been documented that the Altiplano zone of Chile has been in a period of drought since the late Holocene (e.g., Latorre et al., 2003; Valero-Garcés et al., 2003), several authors have described changes in precipitation patterns which have resulted in expansions and contractions of the plant formations since the end of the Pleistocene and during all the Holocene (Betancourt et al., 2000; Bobst et al., 2001; Latorre et al., 2003; Fritz et al., 2004; Latorre et al., 2006; Quade et al., 2008). Thus it is necessary to consider these climatic changes, which have produced expansions and contraction of the aquatic habitats of this species, to explain its presence in areas below 1000 m in the foothills of the drainage systems of Lluta, Azapa and Pampa del Tamarugal (fig. 1) and the existence of lineages not related phylogenetically in contiguous geographic zones.

The most relevant result of this study is the high genetic divergence found between the two lineages of *R. spinulosa* that inhabit in the Chilean Altiplano and adjacent areas. However, we do not know the extension of these lineages in the Altiplano areas of neighboring countries and the causes that originated this differentiation, especially considering the absence of current geographical barriers. The other studies performed in the same area at the species level have attributed speciation patterns to climatic and orogenic processes of the Pleistocene which may also have produced differentiation at the intraspecific level (Lüssen et al., 2003; Palma et al., 2005). Lüssen et al. (2003) found two lineages of *Orestias* (composed of various species) in the Chilean Altiplano, whose geographic limit is at about 19°30′S. The limit established between the two main lineages of *R. spinulosa* does not coincide with the limit described for the species of *Orestias*, which suggests that the diversification of these two taxa was not produced by the same historical events. On the other hand, Palma et al. (2005) provided evidence in support of a peripatric speciation model for sigmodontine rodents of the Altiplano and Atacama Desert. This evidence may support a model of peripatric differentiation for some populations of *R. spinulosa*, particularly those of Quebrada de Jere, Peine and Tilomonte, which may be relict lineages that were isolated when the species had a wider distribution. The high level of nucleotide variation in these populations, compared to those from farther north (table 5), as well as their location in the southwest border of the known distribution range of the species in Chile supports this last interpretation.

Due to their low vagility and their philopatry, amphibians tend to present highly genetically structured populations over short geographic distances; thus they may retain the signals of historical events that have generated their current distributions (Vences and Wake, 2007; Zeisset and Beebee, 2008). Thus phylogeographic studies in amphibians may provide valuable information on the events of the late Pleistocene and Holocene those have influenced biogeographic processes (Zeisset and Beebee, 2008). The phylogeographic patterns described in *R. spinulosa*, together with information about other taxa, will help to understand the patterns of differentiation of the taxa which inhabit this part of the Altiplano, one of the regions in the Southern Hemisphere in which phylogeographic studies are still scarce (Beheregaray, 2008).

Acknowledgements. The authors thank financial support provided by FONDECYT grant 1061256 and Proyecto Domeyko, Iniciativa Transversal 1, Universidad de Chile. SAG (Servicio Agrícola y Ganadero) supplied collecting permits (Resolution numbers 3085/2000, 2105/2004 and 13/2006). C. Correa thanks the Beca CONICYT for doctoral studies.

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Received: January 28, 2009. Accepted: July 29, 2009.