Population cytogenetics of the "Northern Mod 1" chromosomal race of *Liolaemus monticola* Müller & Helmich (Iguanidae) from Central Chile

Citogenética poblacional de la raza cromosómica "Norte mod 1" de *Liolaemus monticola* Muller & Helmich (Iguanidae) en Chile Central

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ABSTRACT

We report the "Northern 2n=38-40, modification 1" race from three localities of the *Liolaemus monticola* complex in Chile that appears to be chromosomally and geographical intermediate between two previously described "Northern 2n=38-40" and "Multiple Fission, 2n=40-44 (MF)" races. This race retains the same "Northern" chromosomal features, but differs by being both polymorphic for an enlarged chromosome pair 6 and for a pericentric inversion in chromosome pair 7; these rearrangements are present in the "MF" race. At the population cytogenetics level, the mean proportion of polymorphic chromosomes in the "Northern, mod 1" race is relatively high and intermediate between the Northern" and "MF" races, while the chromosome "alleles" in the "Northern, mod 1" race deviate from the Hardy-Weinberg ratio higher than the aforementioned races. The Roger's distance between samples are in concordance with the chromosome races and the proposals barriers (The Colorado river and the Juncal river). The lowest chromosome "alleles" flux values (Nm) are between the chromosomal races, suggesting a low chromosomal introgression. These population cytogenetics patterns, plus the origin of the chromosomal rearrangements, and the recombination patterns resulting from chromosomal heterozygosity are compared with the situation of other populations of the "Northern" and the "MF" races previously described. We discuss the possible "Northern, mod 1" hybrid status in the evolution of this complex in central Chile.

Keywords: hybridization, *Liolaemus monticola*, "Northern modification 1, 2n = 38-40" chromosomal race, population cytogenetics.

RESUMEN

Reportamos la citogenética poblacional de la raza "Norte 2n=38-40, modificada 1" para tres localidades del complejo de *Liolaemus monticola* en Chile, que resulta ser cromosómica y geográficamente intermedia entre dos razas previamente descritas la "Norte, 2n=38-40" y la "Múltiple Fisiones, 2n=40-44 (MF)". Esta raza, retiene las mismas características cromosómicas de la raza "Norte", pero difiere de ésta dada la condición polimórfica para un cromosoma alargado del par 6 y una inversión pericéntrica del par cromosómico 7, rearreglos que están presentes en la raza "MF". A nivel citogenético poblacional, la proporción promedio de los cromosomas polimórficos en la raza "Northern, mod 1" is relativamente alta e intermedia entre las razas "Norte" y "MF", en tanto los "alelos" cromosómicos de la raza "Norte, mod 1" se desvían grandemente de los valores Hardy-Weinberg comparados a las razas antes mencionadas. Las distancias de Roger entre las muestra concuerdan con las razas cromosómicas y con las barreras propuestas (El Río Colorado y El Río Juncal). Los valores más bajos de flujo ("alelos") cromosómico (Nm) se encuentran entre las razas cromosómicas, sugerentes de una baja introgresión. Estos patrones de citogenética poblacional, más el origen de los rearreglos cromosómicos y de los patrones de recombinación resultantes de la heterocigosidad cromosómica son comparados con la situación que presentan otras poblaciones de las razas "Norte" y "MF", previamente descritas. Discutimos el posible status híbrido de la raza "Norte, mod 1" en la evolución de este complejo en Chile central.

Palabras clave: hibridizacion, *Liolaemus monticola*, raza cromosómica "Norte Modificada 1, 2n = 38-40", citogenética poblacional.

INTRODUCTION

Liolaemus monticola Müller and Helmich (1932) is a highly variable, endemic montane lizard species distributed along the temperate mountain Ranges in Chile, between latitudes 30º and 40ºS and at altitudes between 400 and 2300m (Donoso-Barros 1966, Peters & Donoso-Barros 1970). This species displays a latitudinal gradient of karyotypic diversity and complexity (Lamborot 1993) and offers an ideal system for studying the possible evolutionary roles of various kinds of karyotypic modifications and modes of evolution in the differentiation of species. At present we can distinguish the following chromosomal races: 1-The "Primitive 2n=32" with 12 macrochromosomes and 20 microchromosomes, considered ancestral in *Liolaemus* (Lamborot 1991, 1993, 2001, 2008; Lamborot & Alvarez-Sarret 1989; Lamborot *et al*. 1979, 1981) and other iguanids (Gorman 1973; Paull *et al*. 1976), 2- The "Southern 2n=34", 3- The "Northern 2n=38 to 40" and, 4- The "Multiple Fission 2n=40 to 44" (Lamborot 1998, 2008). In previous manuscripts we (Lamborot 1991, 1993, 2008; Lamborot & Alvarez-Sarret 1993) hypothesized that the "Northern 2n=38 to 40" ("Northern") race is derived from the "Southern 2n=34" and the "Multiple Fission 2n=42-44" ("MF") is derived from "Northern" race. These races are clearly separated by rivers: the Maipo River and one of its affluent the Yeso River separate the "Southern, 2n=34 and the "Northern" races; in turn the Aconcagua River separated the latter race from the "MF" (Fig 1). These results provided evidence that these rivers have been important in the chromosomal and morphological differentiation (Lamborot & Eaton 1992, 1997; Lamborot *et al*. 2003) by interrupting gene flow, and by limiting the re-expansion of spatially disjoint differentiates.

The Colorado River, one tributary of the Aconcagua River, presents *L. monticola* lizards of the chromosomal race named "Northern modification 1, 2n=38-40" (" Northern mod 1") on both sides, briefly mentioned in Lamborot (2008), Lamborot *et al*. (2003) and Vásquez *et al*. (2007).
In this study, we assess the degree of chromosomal divergence that occurred between these populations compared with some representative localities of the "Northern" and the "MF" races, previously analyzed. Also we estimate the distribution of chromosome variation within and among these chromosomal races of the *L. monticola* complex, and compare these patterns of chromosome divergence for concordance with patterns of genetics (Vásquez *et al*. 2007; Torres-Pérez *et al*. 2007) and morphological differentiation (Lamborot & Eaton 1992, 1997; Lamborot *et al*. 2003). Concordance of divergence patterns among these separate data sets would provide strong evidence of population divergence associated with glaciation barriers of major river valleys.

MATERIALS AND METHODS

All *L. monticola* were collected from spring to autumn (with the permission to collect from SAG) from one locality north the Colorado River and one locality south the Colorado River and from Blanco River (Fig. 1 and Appendix along with the sample size). Lizards were sacrificed by urethane injection in the pineal eye and the chromosomes from these localities were prepared from bone marrow, liver, spleen and testes, using the colchicine-hypotonic pre-treated air-drying technique similar to Lamborot (1993) and stained with Giemsa. Voucher specimens are deposited in the collection of the Evolutive Cytogenetics Laboratory, Facultad de Ciencias, Universidad de Chile.

We selected metaphase plates from each specimen were photographed with a Leitz-Ortholux microscope, and several karyotypes were constructed from enlarged prints to score the chromosomal "genotype". The population chromosomes of each aforementioned locality were compared with representative samples from the "Northern, 2n=38-40" and the "MF, 2n=42 - 44" previously described. Additional observations of spermatocytes at diakinesis, chiasmata, and metaphases II were also made.

We followed the abbreviation of Lamborot (2001): the ancestral biarmed non-fission chromosome morphology was coded "A" and the fission rearrangements "B". Inversions of ancestral biarmed chromosomes were coded as "C", whereas inversions of the fission product in chromosome pair 2 were noted as "D". The enlarged chromosome was noted as "E". We used the chi-square test to analyse the frequencies of the chromosomal heterocygotes expected under Hardy-Weinberg equilibrium, using the program BIOSYS-1 (Swofford & Selander 1989), and an UPGMA dendrogram based on genetic distances (Nei 1972, 1978; Rogers 1972) was generated with BIOSYS-1 and the exact Levene's formula correction for small sample size was used to calculate expected values (*fide* Li 1955).

The distribution of chromosome variation within and among samples was assessed using Wright's *F*-statistics (Wright 1978), and the modified by Weir and Cockerham (1984). We focused our analysis on the F_{ST} estimator, which is the genetic subdivision among populations, based on each locus's departure from heterocygosity levels expected for panmictic populations. We first calculated F_{ST} for all populations together, and then for the three chromosomal races, and within races. From the F_{ST} (theta) of Weir & Cockerham (1984) we estimated the number of migrants per generation (Nm).

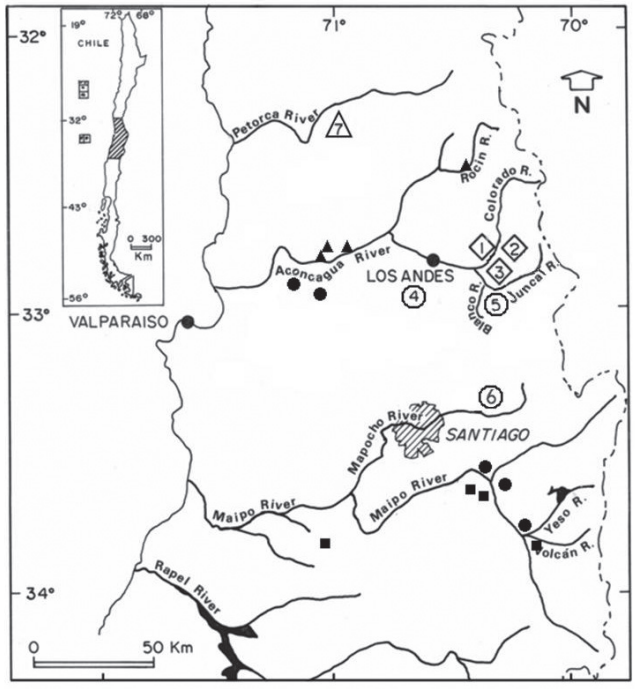

Figure 1. Comparative distribution of the *Liolaemus monticola* chromosomal races across a part of Central Chile. The numbers correspond to the localities sampled for the chromosomal analysis at both sides of the Aconcagua River. Open symbols: Diamonds, "Northern, mod 1, 2n= 38-40" race: 1. Colorado North; 2. Colorado South; 3. Blanco River. Circles, "Northern, 2n= 38-40"race: 4. Cuesta Chacabuco; 5. Saladillo; 6. Farellones. Triangle: "Multiple Fission, 2n= 42-44" race: 7. Hierro Viejo. Solid symbols corresponds to other *L. monticola* localities sampled previously, not included in the analyses: Squares: "Southern, 2n=34" race; Circles: "Northern, 38-40"race.

Figura 1. Distribución comparada de las razas cromosómicas de *Liolaemus monticola* en una parte de Chile central. Los números corresponden a las localidades muestreadas para los análisis cromosómicos a ambos lados del Río Aconcagua. Símbolos abiertos: Diamantes, raza "norte, mod 1, 2n=28-40: 1. Colorado North; 2. Colorado South; 3. Blanco River. Circulos, raza "Norte, 2n= 38-40": 4. Cuesta Chacabuco; 5. Saladillo; 6. Farellones. Triángulos: raza "Multiple Fisiones, 2n= 42-44": 7. Hierro Viejo. Los símbolos sólidos corresponden a otras localidades de *L. monticola* previamente muestreadas, no incluidas en los análisis: Cuadrados, raza "Sur, 2n=34"; Círculos, raza "Norte, 38-40".

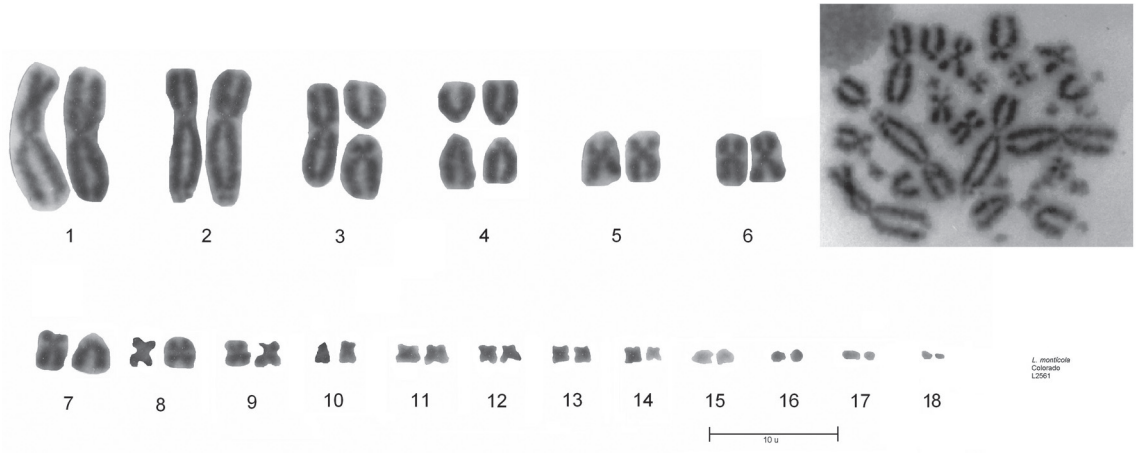

Figure 2 A representative karyotype of a lizard (L-2561) from the "Northern mod 1", 2n=39. Pair Heterozygote for pair 3 (P_3F_4) and pair 7.

Figura 2. Cariotipo representativo de una lagartija (L-2561) de la raza "Norte, mod 1" con 2n=39 y heterocigoto para el par 3 (P_3F_4) y para el par 7.

RESULTS

Pattern of Chromosomal Variation. The "NORTHERN MOD 1" RACE KARYOTYPE. The three locality samples: Colorado North (sample 1), Colorado South (sample 2) and Blanco River (sample 3) present intra population and inter population variation in chromosome number. The Appendix summarizes our results. The diploid chromosome number (2n) in this race ranges from 38 to 40 for both sexes in different variants as the "Northern" race previously described for several localities (Lamborot 1991, 2001; Lamborot & Alvarez-Sarret 1993), but differs by a polymorphic enlarged pair 6 and/or a pericentric inversion in chromosome pair 7 (Fig. 2). All the variants (Fig. 3) exhibit the replacement of the biarmed macrochromosome pair 4 by four acrocentric chromosomes and bear 24 microchromosomes. The 2n variants result from the fission polymorphic condition for the macrochromosome pair 3. Some individuals were homozygous for two metacentric pair 3 (St_3F_4) and 2n=38 or heterozygous for one metacentric 3 (P_3F_4) (Fig. 2 and 3D) and more or less 27% of the lizards sampled from this race were P_3F_4 plus two acrocentrics and 2n=39 (Fig. 2), or homozygous for the fissioned pair 3 (F_3F_4) and 2n=40 (Fig. 3 A, B and C). One fissioned product of chromosome 3 presents the subtelocentric condition instead of the acrocentric one as for the other fissioned products. A polymorphic condition was presents for an enlarged chromosome 6 (Fig. 3D) and for a pericentric inversion in chromosome 7 (Fig. 2 and 3C and 3D). Only one individual from Blanco River (sample 3), the male 2139, appears to be a mosaic, polymorphic with fission in chromosome pair 1 (see Appendix). In general, some degree of aneuploidy was encountered in the diploid metaphase plates concerning the polymorphic pairs 3, 6 and 7 (not quantified).

In diakinesis arrays chiasmata were always terminal in males, such that bivalents were ring-shaped or linear (Fig. 4). In the homozygotes for the metacentric chromosome 3 (St_3F_4), five classes may be reliably determined among macrochromosomal bivalents. Pairs 1 and 2 are close enough in size that their identification could be equivocal and in this class, two terminal chiasmata per bivalent were observed. In the polymorphic males for pair 3 (P_3F_4), there was a linear trivalent (Fig. 4B and 4C), with two terminal chiasmata. Otherwise the same classes as the St_3F_4 may be reliably determined among the macrochromosome bivalents. In diakinesis of the F_3F_4 homocygotes, four classes may be reliably determined among macrochromosomal bivalents (Fig. 4D). Pairs 1 and 2 were similar to St_3F_4 and P_3F_4, with two chiasmata per bivalent; the fissioned pairs 3 and 4 presented linear bivalents with one terminal chiasmata, and bivalents 5, 6, and 7 with two chiasmata each. In good plates when pair 6 is polymorphic an unequal ring can be observed; the same when pair 7 is polymorphic (Fig. 4C).

THE "NORTHERN, 2n =38-40" AND THE "MULTIPLE FISSION, 2n=42-44" RACES. The karyotypes that characterize the "Northern" and "MF" races populations analysed are coincident with those previous reported (Lamborot 1991, 1993,1998, 2001). But a re-examination of 27 lizard karyotypes from Cuesta Chacabuco ("Northern" race, sample 4) previously reported (Lamborot 2001), showed that two of them (Lizards 2083 (AE) and 2086(EE), see appendix) exhibited enlarged chromosome for pair 6.

Population Cytogenetics. When the chromosome "genotypes" were tested for conformance to Hardy-Weinberg expectations, most of them were found to have a genotypic ratio consistent with Hardy-Weinberg equilibrium, except the following population races and chromosome pairs, that exhibited significant deviations ($P < 0.05$*; $P < 0.01$**) in the direction of heterocygote deficiency (Table 1): "Northern mod 1" race: sample 2, pair 3 (0.008**), and pair 7 (0.013*); "Northern" race: sample 4, pair 6 (0.000**). The Chi-square tests for the population samples of the "MF" race showed no significant deviation from the frequencies of the heterocygotes and homocygotes for the fissioned pairs 1, 2, 3 and 7. Some deviation was found for the polymorphism for the enlarged pair 6 (0.038*) (Table 1).

The measures of genetic variability for all populations are given in Table 2. The overall mean number of "alleles" per chromosome pair in the *L. monticola* races was 1.3, ranging from 1.1 ("Northern" race, samples 5 and 6) to 1.7 ("MF" race, sample 7). The overall mean observed heterocygosity (Ho) was 0.060 (sample 5) to 0.177 (sample 7). The mean value of percent polymorphism (P) is 36.7 and the expected heterocygosity (He) is 0.132 (Table 2).

The mean F_{ST} value is 0.421, indicating that 42.1 per cent of the genetic variation in *Liolaemus monticola* is attributable to differentiation among populations. The inbreeding coefficient (F_{IS}) is low (0.0918), presumably because Hardy-Weinberg proportions are maintained within populations by random mating (data not shown). Table 3 presents the estimate Nm values among populations and between chromosome races.

For all population of *L. monticola* the Rogers' distances were estimated among samples (fig 5), ranging from 0.026 (sample 4 *vs* 5, from the "Northern" race) to 0.518 (sample 4 from "Northern" *vs* 7, from "MF" race). The UPGMA phenogram (Fig 5) showed a congruence of the chromosome races and revealed two main clusters: one corresponds to the "MF" sample and the second includes the "Northern" and the "Northern mod 1" subclusters clearly separated. Within the second group, two subclusters are recognized: one includes all three "Northern mod 1" (samples 1, 2 and 3) and the other the "Northern" (sample 4, 5 and 6).

Table 1. Chromosome frequencies and cytogenetic variability parameters for five polymorphic chromosomes in 7 locality samples of three *Liolaemus monticola* chromosomal races in Central Chile. For the sample locality number, see Fig 1 and appendix. HW= Hardy-Weinberg equilibrium; $p < 0.05$*and $p < 0.01$** value with 1 df.

Tabla 1. Parámetros de las frecuencias cromosómicas y de la variabilidad citogenética para cinco polimorfismos cromosómicos en muestras de 7 localidades de tres razas cromosómicas de *Liolaemus monticola* en Chile central. Vea la Fig. 1 para ver a que localidad corresponden los números de muestra y el apéndice para los equilibrios de HW= Hardy-Weinberg; $p < 0.05$*y $p < 0.01$** con 1 gl.

Samples	1	2	3	4	5	6	7
PAR 1							
A	1.000	1.000	0.929	1.000	1.000	1.000	0.086
B	0.000	0.000	0.071	0.000	0.000	0.000	0.914
H-W χ^2			0.000				0.203
p			1.000				0.652
PAR 2							
A	1.000	1.000	1.000	1.000	1.000	1.000	0.069
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.931
H-W χ^2							0.117
p							0.732
PAR 3							
A	0.119	0.350	0.429	0.370	0.327	0.512	0.190
B	0.881	0.650	0.571	0.630	0.673	0.488	0.810
H-W χ^2	0.300	6.949	0.570	0.118	0.089	0.295	1.425
p	0.584	0.008**	0.811	0.731	0.765	0.587	0.233
PAR 6							
A	0.738	0.775	1.000	0.944	1.000	1.000	0.569
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.262	0.225	0.000	0.056	0.000	0.000	0.431
H-W χ^2	3.616	0.010		16.993			4.302
p	0.057	0.919		0.000**			0.038*
PAR 7							
A	0.690	0.700	0.171	1.000	1.000	1.000	0.776
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.310	0.300	0.429	0.000	0.000	0.000	0.224
H-W χ^2	0.865	6.169	3.214				3.136
p	0.352	0.013*	0.073				0.077

DISCUSSION

ORIGIN OF THE "NORTHERN MOD 1, 2n=38-40" CHROMOSOME RACE. This race, as its name implies, probably originated from the "Northern" race Andean Range, and is geographical and chromosomally intermediate between the "Northern" and the "MF" race (Fig. 1), with two novel chromosomal mutations: a polymorphism for an enlarged chromosome 6 and a polymorphic pericentric inversion in chromosome 7 (Lamborot *et al.* 2003). The enlarged chromosome 6 could be explained by heterochromatin addition. In turn the "Northern mod 1" race gave rise to the "MF" race, that retains all the characteristic rearrangements as well as the same number of microchromosomes of the former, but is also polymorphic for fissions in pairs 1 and 2 (Lamborot *et al*. 2003) and is considered the most derived and the most polymorphic of the *L. monticola* complex, that it could represent a late colonisation and differentiation events in more xeric habitats (Lamborot 1998, 2001, 2008; Vásquez *et al.* 2007).

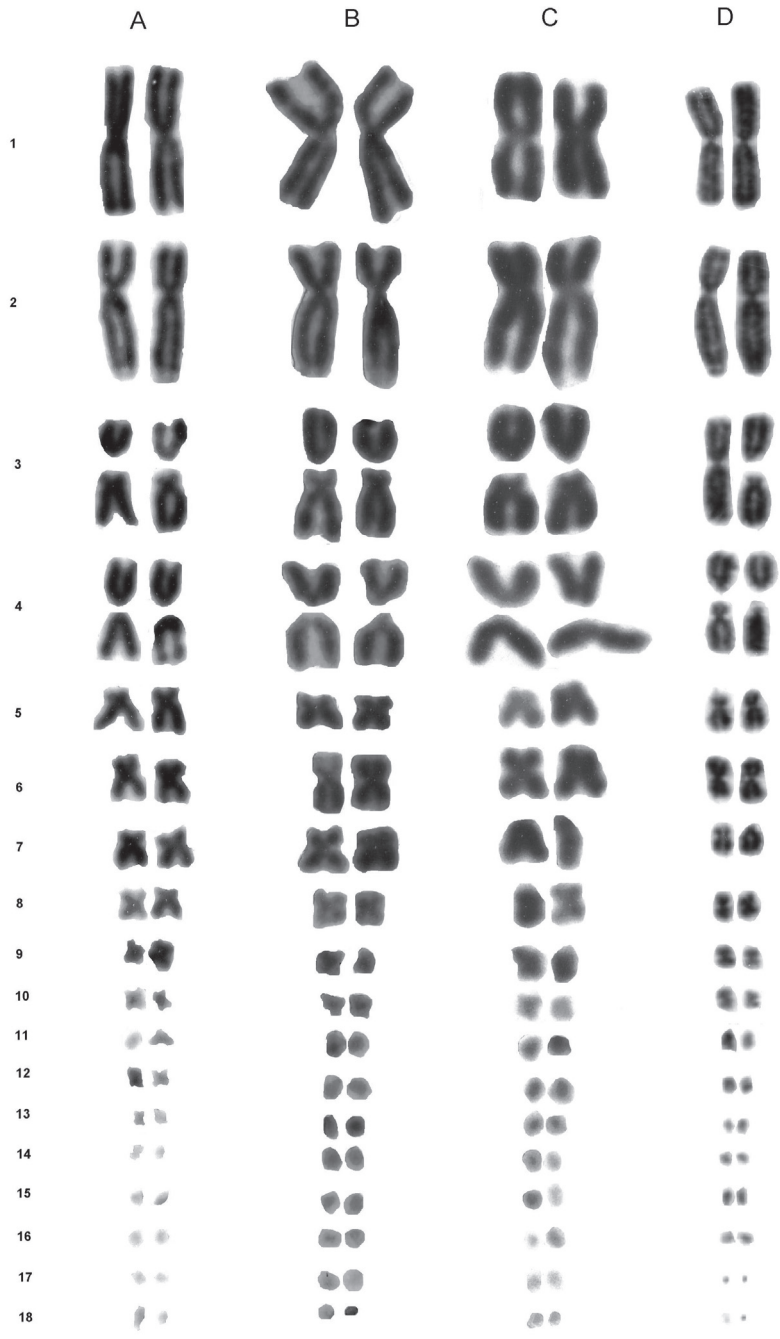

Figure 3. Representative karyotypes of *Liolaemus monticola* "Northern mod 1, 2n=38-40" race, from sample 1. A and C. Lizards 2545 and 2553: homozygotes for fissioned pairs 3 and 4 (F_3F_4) and polymorphic pair 7. B. Lizard 2552: homozygote for fissioned pairs 3 and 4 (F_3F_4); and polymorphic pair 6 (AE). D. Lizard 2623: Polymorphic for fission in chromosome pair 3, homozygote for fission 4 (P_3F_4) and enlarged 6 and heterozygous for a pericentric inversion in chromosome 7.

Figura 3. Cariotipos representativos de la raza "Norte mod 1, 2n=38-40" de la muestra 1. A y C: Lagartijas 2545 y 2553: homocigotas para las fisiones de los pares 3 y 4 (F_3F_4) y polimórfico para el par 7. B. Lagartija 2552: homocigoto para la fisión de los pares 3 y 4 (F_3F_4) y polimórfica para el par 6 (AE). D. Lagartija 2623: Polimórfica para la fisión del cromosoma 3, homocigota para la fisión del par 4 (P_3F_4) y para el alargamiento del par 6, y heterosigota para una inversión pericéntrica en el cromosoma 7.

Recently, based on alloenzymes variation, Vásquez *et al*. 2007 proposed an "hybridization hypothesis" where the "Northern mod 1" race could represent a primary hybrid zone for two locality samples (in this study samples 1, 2 and 3) based on the intermediate geographic location between the "Northern" and the "MF" races, the large number of loci that deviate from the Hardy-Weinberg genotypes ratios, and the large number of private alleles among the samples analysed; such alleles of mutational origin are called "hybridzymes" (Woodruff 1989). In this study we document: i) The presence of polymorphisms for two novel chromosome rearrangements present in the "MF" race, and absent in the "Northern", except in sample 4. ii) The mean proportion of polymorphic chromosomes (P) in the "Northern mod 1" race, is relatively high P= 42.9 and intermediate compared with the "Northern" race: samples 5 and 6 P=14.3 and sample 4, P= 28.6; but less than the average of the "MF" race, P= 71.4 (Table 2). iii) The overall mean number of "alleles" per chromosome pair in the *L. monticola* "Northern, mod 1" race is intermediate (A=1.4) between the "Northern" race (from A=1.1 to A= 1.3) and the "MF" race (A=1.7). iv) A 50% of the chromosome "alleles" deviate from the Hardy-Weinberg ratios in the "Northern mod 1" race, meanwhile a 25% is for the "Northern" and a 25% is for the "MF" races. v). The somatic mutation for a fissioned pair 1 found in a mosaic lizard from sample 3, increases the chromosomal mutation rate. This private novel chromosome mutation is a convenient feature of how distinct the Blanco River population (sample 3) from other two "Northern mod 1", population samples (1 and 2) at the Andean Range. It has been demonstrated that elevated mutation rates are presumably precipitated by hybridization events (Naviera & Fontdevila 1985).

The documented aforementioned points support the hybrid origin for the "Northern, mod 1" race, corresponding to a primary hybrid zone.

Our results confirm previous reports of linearly arranged karyotypic variation, with increased complexity from south to north, but with the presence of the new "Northern mod 1" race geographically intermediate between two other derived races. The clinal pattern of chromosomal variation is in several aspects concordant with Hall's "Cascade Model of Speciation" (Hall 1973, 1980, 1983) or the "Chain process" (White 1978) or "Primary chromosomal allopatry" (King 1981) hypotheses.

Pattern of Chromosomal Variability and Gene Flow. The Rogers's chromosomal distances (Fig. 5) are in concordance with the chromosome races and with the hypothesized riverine barriers. The distances obtained are higher than those obtained by allozyme variation by Vásquez *et al*. 2007, and by morphology (Lamborot *et al*. 2003) and mitochondrial cytochrome b sequences (Torres-Pérez *et al*. 2007).

The genetic distances and theta estimates show that, at the scale of this study, the chromosomal variation is distributed into geographical coherent chromosome races and some riverine barriers separate them (Fig. 1 and 5).

The estimates of gene flow (Nm) based on Wright's method (Table 3A) between all populations, tend to be high within each chromosomal race and geographical proximity. For example, within the "Northern mod 1", the Nm between samples 1 and 2 separate by the Colorado River (at a similar altitude, near the head water) is Nm=29. Between samples 3 and 2, both at the south shore and at different altitudes, the Nm is 26.06, mean while; the Nm between samples 3 and 1, across the Colorado River is low (Nm=2.0). The lowest Nm values are between the chromosome races (Table 3B), suggesting a low chromosomal introgression. When these Nm values are compared with those obtained using alloenzyme electrophoresis to estimate the pattern of genetic variability, the Nm for the genetic markers are concordant but higher than the chromosomal markers. We suggest that chromosomal and morphological markers may reflect the effects of natural selection, whereas alloenzymes are considered nearly genetic markers and are best for estimating gene flow, thus reflecting the effect of history and chance.

Riverine Barriers. Based on data obtained in this study, the Juncal River, and affluent of the Aconcagua river, separates the "Northern" and "Northern Mod 1" races; in turn the Aconcagua river acts as strong barrier to gene flow between the "Northern" and the "MF" races, supporting the suggestion of Pounds & Jackson (1981), that mayor rivers can facilitate differentiation, or at least limited the re-expansion for genetically divergence lizards populations. However, the upper reach of the Colorado River, another tributary of the Aconcagua River do not separate chromosome races, suggesting at least limited post glacial expansion of lizards across smaller headwater streams, but some differentiation between the south and the north shores is noticed among the "Northern mod 1", samples 1 and 2 and 3 (Fig. 5) with high Nm values in between (Table 3). The same pattern has been shows for mammals groups in Amazonian tributary basins (Patton *et al.* 1994)

Geological data for the region in central Chile demonstrate that glaciations in the Pleistocene period were extensive (Brüggen 1950, Vuilleumier 1971). The Aconcagua Valley (lat. 32º 50'S) is located transitionally between the arid zone of the so-called "Norte Chico" and the temperate winter humid part of Middle Chile.

Table 2. Chromosome variability parameters in 7 *Liolaemus monticola* populations from central Chile. N: Population sample size , A: mean number of "alleles" per karyotype, P: mean proportion of polymorphic chromosomes, Ho: observed mean heterozygosity, He: expected mean heterozygosity (unbiased estimate; Nei, 1978) (se, standard error). The numbers of samples correspond to geographic localities plotted in Fig. 1 and Appendix.

Tabla 2. Parámetros de la variabilidad cromosómica en 7 poblaciones de *Liolaemus monticola* de Chile central. N: Tamaño de la muestra poblacional, A: promedio del número de "alelos" por cariotipo, He: promedio de la heterocigocidad esperada (estimativo no sesgado; Nei, 1978) (se, error estándar). El número de muestra corresponde a la ubicación geográfica de cada localidad detallada en la Fig. 1 y en el Apéndice.

Races	"Northern Mod 1"			"Northern"			"MF"
Sample	1	2	3	4	5	6	7
N	21	20	7	27	26	42	29
A	1.4	1.4	1.4	1.3	1.1	1.1	1.7
(se)	(0.2)	(0.2)	(0.2)	(0.2)	(0.1)	(0.1)	(0.2)
P	42.9	42.9	42.9	28.6	14.3	14.3	71.4
Ho	0.143	0.107	0.224	0.069	0.060	0.078	0.177
(se)	(0.076)	(0.054)	(0.132)	(0.063)	(0.060)	(0.078)	(0.055)
H-W He	0.150	0.179	0.171	0.083	0.064	0.072	0.208
(se)	(0.075)	(0.085)	(0.094)	(0.067)	(0.064)	(0.072)	(0.071)

Table 3. Estimates of gene flow (Nm) based on Wright's Theta method: A. Among all population samples. B. Among chromosomal races

Tabla 3. Estimativos del flujo génico (Nm) basados en el método Teta de Wright: A. Entre todas las muestras de las poblaciones. B. Entre las razas cromosómicas.

A.

Races	"Northern Mod 1"			"Northern"		"MF"
Population samples	1	2	3	4	5	6	7
1	----	29.0	2.0	1.03	0.94	0.56	0.19
2		----	26.05	2.20	1.68	1.16	0.20
3			----	1.09	0.86	0.85	0.20
4				----	9999.0	13.51	0.14
5					----	4.49	0.13
6						----	0.11

B.

Races	"Northern Mod 1"	"MF"
"Northern"	1.12	0.09
"Northern Mod 1"		0.19

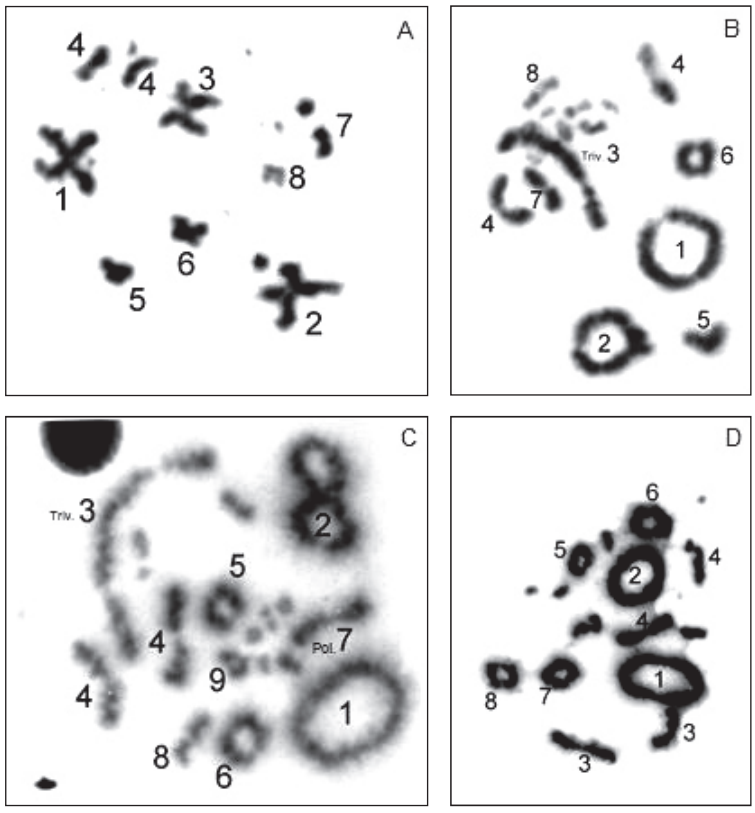

Figure 4. Representative meiotic arrays of *Liolaemus monticola* "Northern mod 1. 2n=38-40" race". A and B lizard 2561 (P_3F_4): A-Metaphase II: Chromosomes 1, 2, 3 and 7 are metacentrics, plus two acrocentric chromosomes from pair 4. B. Diaquinesis with a trivalent pair 3 and a polymorphic bivalent pair 7. C. Lizard 2562 (P_3F_4) is polymorphic for chromosome 3 and 7. D. Lizard 2539 (F_3F_4) is homozygote for fissioned pairs 3 and 4.

Figura 4. Placas meióticas representativas de la raza "Norte mod 1, 2n=38-40" de *Liolaemus monticola.* A y B lagartija 2561(P_3F_4): A-Metafase II: Los cromosomas 1, 2, 3 and 7 son metacéntricos, más dos cromosomas acrocentricos del par 4. B. Diaquinesis con un trivalente para el par 3 y un bivalente polimórfico para el par 7. C. Lagartija 2562 (P_3F_4) polimórfica para los cromosomas 3 y 7. D. Lagartija 2539 (F_3F_4) es homocigota para los pares fisionados 3 y 4.

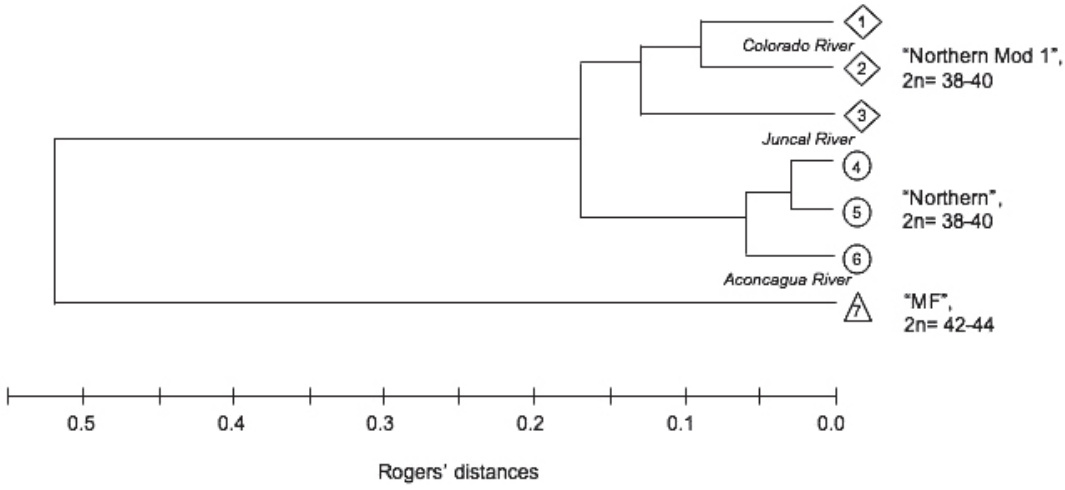

Figure 5. Phenogram generated by UPGMA cluster analysis based on Rogers's genetic distance matrix (Appendix) among all pairwise *Liolaemus monticola* samples from central Chile. For geographic origin, see Fig. 1.

Figura 5. Fenograma generado mediante análisis de cluster basado en una matriz de distancias genéticas de Rogers (apéndice) entre todos los pares de muestras de *Liolaemus monticola* de Chile central. Para el origen geográfico, ver Fig. 1

The upper zone of the Aconcagua is a tectonic calm zone in comparison with other montane valleys of Central Chile, where it is possible to distinguish the traces of three glacial advances, plus a debatable fourth one. The traces demonstrated that as the Pleistocene progress, the glaciations retried each time to highest altitude, coupled to an increasing aridity. The chronology accepted by Caviedes (1972) allows the presumption that the glaciations of the Middle Chilean Andes occurred "in phase" with those of the Northern Hemisphere, but they never reached a similar intensity.

In summary, this allow a better understanding of the possible steps and directions of the sequence of mitotic and meiotic chromosomal changes in the divergence of the *L. monticola* complex and the importance of hybridization events as well as the importance of riverine barriers as the Aconcagua Rivers and some of its tributaries as the Juncal River compared to the Colorado River, to gene flow and divergence of the present populations.

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APPENDIX

Locality, individual and chromosomal "genotype" analyzed by BIOSYS-1 for all lizards of *Liolaemus monticola* chromosomal races examined in this study. The seven "loci" correspond to the first 7 pair of chromosomes.

Localidad, individuo y genotypo cromosómico analizados por BIOSYS-1 de todas las razas cromosómicas de *Liolaemuls monticola* analizadas en este estudio. Los siete "loci" corresponden a los 7 primeros pares de cromosomas.

			Chromosomal pair number										Chromosomal pair number						
Sample	individual	sex	1	2	3	4	5	6	7	Sample	individual	sex	1	2	3	4	5	6	7
1. Colorado North	2539	m	AA	AA	BB	BB	AA	AA	AA	5. Saladillo	2286	f	AA	AA	AA	BB	AA	AA	AA*
32° 52' S 70° 22' W	2543	m	AA	AA	BB	BB	AA	EE	AA	32° 58' S 70° 18' W	2287	m	AA	AA	BB	BB	AA	AA	AA*
1550m	2544	m	AA	AA	BB	BB	AA	AA	AC	1450	2288	m	AA	AA	BB	BB	AA	AA	AA*
	2545	m	AA	AA	BB	BB	AA	AA	AC		2289	m	AA	AA	AB	BB	AA	AA	AA*
	2546	m	AA	AA	BB	BB	AA	AE	AC		2290	m	AA	AA	BB	BB	AA	AA	AA*
	2551	m	AA	AA	AB	BB	AA	AA	AC		2447	f	AA	AA	AB	BB	AA	AA	AA*
	2552	m	AA	AA	BB	BB	AA	AE	AA		2448	m	AA	AA	BB	BB	AA	AA	AA*
	2553	f	AA	AA	BB	BB	AA	AA	AC		2449	m	AA	AA	AB	BB	AA	AA	AA*
	2554	f	AA	AA	BB	BB	AA	AA	AA		2450	m	AA	AA	BB	BB	AA	AA	AA*
	2555	f	AA	AA	BB	BB	AA	AA	AC		2451	f	AA	AA	AB	BB	AA	AA	AA*
	2556	f	AA	AA	BB	BB	AA	AA	AC		2452	m	AA	AA	AB	BB	AA	AA	AA*
	2557	m	AA	AA	BB	BB	AA	AA	AA		2453	m	AA	AA	AA	BB	AA	AA	AA*
	2613	m	AA	AA	BB	BB	AA	AA	AA		2454	m	AA	AA	AA	BB	AA	AA	AA*
	2614	m	AA	AA	AB	BB	AA	AA	AA		2455	f	AA	AA	BB	BB	AA	AA	AA*
	2615	m	AA	AA	BB	BB	AA	AA	AA		2456	m	AA	AA	BB	BB	AA	AA	AA*
	2616	m	AA	AA	BB	BB	AA	AE	AC		2457	f	AA	AA	AB	BB	AA	AA	AA*
	2617	m	AA	AA	BB	BB	AA	AE	AC		2458	f	AA	AA	AB	BB	AA	AA	AA*
	2619	f	AA	AA	AB	BB	AA	EE	CC		2459	f	AA	AA	BB	BB	AA	AA	AA*
	2620	f	AA	AA	BB	BB	AA	AA	AA		2460	f	AA	AA	AB	BB	AA	AA	AA*
	2622	m	AA	AA	AB	BB	AA	AE	AC		2463	f	AA	AA	AB	BB	AA	AA	AA*
	2623	f	AA	AA	AB	BB	AA	EE	AC		2465	f	AA	AA	AB	BB	AA	AA	AA*
											2466	f	AA	AA	BB	BB	AA	AA	AA*
2. Colorado South	2540	m	AA	AA	AA	BB	AA	AA	AA		2467	m	AA	AA	AB	BB	AA	AA	AA*
32° 53' S 70° 20' W	2541	m	AA	AA	BB	BB	AA	AE	AA		2468	m	AA	AA	BB	BB	AA	AA	AA*
1550 m	2542	m	AA	AA	BB	BB	AA	AA	AA		2474	f	AA	AA	BB	BB	AA	AA	AA*
	2548	f	AA	AA	BB	BB	AA	EE	AA		2475	f	AA	AA	BB	BB	AA	AA	AA*
	2549	m	AA	AA	BB	BB	AA	AE	AA										
	2550	m	AA	AA	BB	BB	AA	AA	CC	6.Farellones	0166	m	AA	AA	AA	BB	AA	AA	AA
	2559	m	AA	AA	AA	BB	AA	AA	AC	33° 20' S 71 ° 20' W	0262	m	AA	AA	AA	BB	AA	AA	AA
	2560	m	AA	AA	BB	BB	AA	AA	CC	1400 m	0259	m	AA	AA	AA	BB	AA	AA	AA
	2561	m	AA	AA	AB	BB	AA	AA	AC		0233	f	AA	AA	AB	BB	AA	AA	AA
	2562	m	AA	AA	AA	BB	AA	AA	AC		0239	f	AA	AA	AB	BB	AA	AA	AA
	2564	m	AA	AA	AA	BB	AA	AA	AA		0237	m	AA	AA	AB	BB	AA	AA	AA
	2565	f	AA	AA	AB	BB	AA	AE	AA		0238	f	AA	AA	AB	BB	AA	AA	AA
	2567	f	AA	AA	AB	BB	AA	AE	CC		0235	m	AA	AA	AB	BB	AA	AA	AA
	2569	m	AA	AA	AA	BB	AA	AE	AA		0234	m	AA	AA	BB	BB	AA	AA	AA
	2570	m	AA	AA	AB	BB	AA	AA	AA		0228a	*	AA	AA	AA	BB	AA	AA	AA
	2571	m	AA	AA	BB	BB	AA	AA	AA		0228b	*	AA	AA	AB	BB	AA	AA	AA
	2572	m	AA	AA	BB	BB	AA	AA	AA		0220	m	AA	AA	AB	BB	AA	AA	AA
	2573	m	AA	AA	BB	BB	AA	AA	AA		0217	m	AA	AA	BB	BB	AA	AA	AA
	2579	m	AA	AA	BB	BB	AA	AE	CC		0216	m	AA	AA	AB	BB	AA	AA	AA
	2582	f	AA	AA	BB	BB	AA	AE	AC		0215	f	AA	AA	AA	BB	AA	AA	AA
											0214	f	AA	AA	AB	BB	AA	AA	AA
3. Río Blanco	0387	m	AA	AA	AB	BB	AA	AA	AC		0174	m	AA	AA	BB	BB	AA	AA	AA
32° 55' S 70° 16' W	0388	f	AA	AA	BB	BB	AA	AA	AA		0175	m	AA	AA	BB	BB	AA	AA	AA
1450 m	2137	f	AA	AA	AB	BB	AA	AA	AC		0176	m	AA	AA	AB	BB	AA	AA	AA
	2138	m	AA	AA	BB	BB	AA	AA	AC		1422	f	AA	AA	BB	BB	AA	AA	AA
	2139*	m	AA	AA	AB	BB	AA	AA	AC		1669	m	AA	AA	AA	BB	AA	AA	AA
	2445	f	AA	AA	AB	BB	AA	AA	AC		1670	m	AA	AA	AB	BB	AA	AA	AA
	2446	f	AA	AA	AA	BB	AA	AA	AC		1671	m	AA	AA	BB	BB	AA	AA	AA
											1672	f	AA	AA	BB	BB	AA	AA	AA
4. Cuesta Chacabuco	2078	m	AA	AA	BB	BB	AA	AA	AA		1673	f	AA	AA	AB	BB	AA	AA	AA
32° 56' S 70° 44' W	2079	m	AA	AA	AB	BB	AA	AA	AA		1675	f	AA	AA	AB	BB	AA	AA	AA
1100	2080	m	AA	AA	AB	BB	AA	AA	AA		1677	f	AA	AA	AB	BB	AA	AA	AA
	2081	m	AA	AA	BB	BB	AA	AA	AA		1678	m	AA	AA	AB	BB	AA	AA	AA
	2082	m	AA	AA	AB	BB	AA	AA	AA		1679	m	AA	AA	AB	BB	AA	AA	AA
	2083	m	AA	AA	BB	BB	AA	AE	AA		1681	m	AA	AA	BB	BB	AA	AA	AA
	2084	f	AA	AA	AA	BB	AA	AA	AA		1690	m	AA	AA	BB	BB	AA	AA	AA

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