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Original Investigation

Integrated analyses of chromosome, molecular and morphological variability in the Andean mice *Eligmodontia puerulus* and *E. moreni* (Rodentia, Cricetidae, Sigmodontinae)

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ABSTRACT

Patterns of evolution and systematics of sigmodontine rodents are matters of continuous revision and debate. The silky mouse, *Eligmodontia*, is a phyllotine rodent adapted to arid environments. Chromosomal and molecular data have identified six species in this genus. Among these *E. puerulus* and *E. moreni* are sister taxa from the high Andean and lowland deserts, respectively, with large chromosomal differences and intermediate levels of molecular divergence. The purpose of our study was to quantify the degree of variability (morphological, cytogenetic, and molecular) and to analyze its evolutionary implications within, and between, these sister species in the Monte and Puna biomes of Argentina. Our results show a high variability at the chromosomal and molecular level, but low morphological differentiation among populations of *E. puerulus*. Diploid numbers vary from 31 to 37 due to a complex Robertsonian system, whereas cytochrome-b distances range from 0.15% to 5.75%. On the other hand, *E. moreni* shows high morphological differentiation between populations, but low intraspecific differentiation at the molecular (from 0.73% to 1.4%) and chromosomal level ($2n = 52$). Comparison of *E. puerulus* with *E. moreni* reveals high morphological and chromosomal distinction between them, but absence of molecular differentiation. Our results suggest that: (1) the high genetic variability of *E. puerulus* could be associated to its geographic distribution in the complex topography of the high Andean Puna; (2) the high morphological differentiation between *E. moreni* and *E. puerulus* could be the result of natural selection; and (3) molecular polyphyly between *E. puerulus* and *E. moreni* could be due to introgression or a recent split of these taxa. Finally, our results emphasize the need to integrate different datasets in the analysis of species variability and diversification, as tools for understanding their evolutionary histories.

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Introduction

Taxonomy, systematics and evolutionary processes driving the divergence in South American sigmodontine rodents are subject to continuous review and debate (Hershkovitz 1962; Reig 1986; Steppan 1998; Smith and Patton 1993, 1999; D'Elía 2003). Like in many other taxa, species delimitation and relationships among sigmodontines have been historically based on different characteristics of the organisms. Morphological traits were the first to be used and have been widely utilized in a broad variety of organisms. However, genetic data have sometimes revealed a higher variability than that detected by morphological approaches alone (King 1993; Avise 1994). It has been noted that the strong incongruence between different datasets is indicative of different evolutionary processes, which can only be identified through an integrative approach (Brower et al. 1996; Doyle 1997; Modi 2003; Renaud et al. 2007 and references therein; Robovský et al. 2008).

Among the most important evolutionary processes promoting species separation, chromosomal speciation occurs rapidly, with little differentiation in DNA sequences and partial or complete absence of morphological divergence (King 1993; Dobigny et al. 2005). Speciation may also be a byproduct of rapid evolution by directional natural selection, caused by local adaptation to different environments (i.e. ecological speciation) that promotes morphological differentiation (Schluter 2001). Mechanisms proportional to the time of separation between taxa can be identified in DNA sequences that have no strong selective pressures and have a more or less neutral kind of evolution (Avise 1994).

Sister species of recent origin constitute valuable biological models for empirical investigations in evolutionary processes. They offer the opportunity to identify the main events promoting the divergence, because some of the other variables associated with natural populations can be in part controlled (Dobigny et al. 2005; Martínez et al. 2010).

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Representatives of the genus *Eligmodontia* are highly desert-adapted rodents, distributed from Peru and Bolivia to the south of Argentina and Chile (Lanzone 2009; Lanzone et al. 2007). Several species and subspecies were described in the genus in the basis of morphologic variation (rev. in Hershkovitz 1962). However, Hershkovitz (1962), synonymized all forms within *E. typus* with two subspecies: *E. t. typus* and *E. t. puerulus*. Later cytogenetic studies identified six species (Lanzone and Ojeda 2005 and references therein).

At molecular level, studies of cytochrome b have shown good correspondence between cytogenetically delimited taxa and the differences expected in this gene for distinctive mammalian species (Lanzone et al. 2007; Mares et al. 2008). Among them, *E. puerulus* (Philippi, 1896) and *E. moreni* (Thomas, 1896) are sister species with relatively low levels of molecular divergence and clear chromosomal differences (Spotorno et al. 1998; Lanzone et al. 2007; Mares et al. 2008). However, samples of *E. puerulus* were underrepresented in previous molecular studies (Lanzone et al. 2007; Mares et al. 2008) and chromosomal data was reported from a few populations (Spotorno et al. 1994, 1998). Furthermore, the morphological distinctiveness of these sister species are unknown.

The purpose of our study was to characterize the intra and interspecific differentiation of *E. puerulus* and *E. moreni* occurring in Northwestern Argentina, in the contiguous biomes of the high Andean (Puna) and lowland deserts (Monte), respectively (for a detailed characterization of these biomes see Cabrera 1976). In particular, we analyze their morphology, chromosomes and molecular genetics in an attempt to recognize the potential evolutionary processes involved in the variability and diversification of these sister taxa.

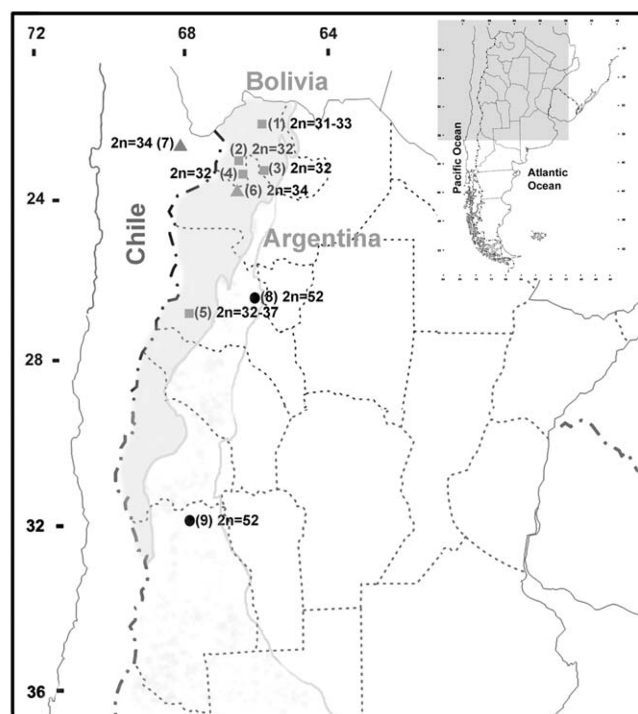

Fig. 1. Map of Argentina and Chile showing the populations of *Eligmodontia puerulus* (1–7) and *E. moreni* (8–9) studied. Triangles correspond to populations (6 and 7) analyzed by Spotorno et al. (1994, 1998). 1: Abra Pampa (Jujuy Province); 2: Susques (Jujuy Province); 3: Saladillo (Jujuy Province); 4: 18 km E of Salinas Grandes (Jujuy Province); 5: Pastos Largos (Catamarca Province); 6: Cauchari (Salta Province; Spotorno et al. 1994); 7: San Pedro de Atacama (Chile; Spotorno et al. 1998); 8: Campo Arenal (Catamarca Province); 9: Telteca (Mendoza Province). The biomes are outlined in gray: on the left the Puna and on the right the Monte.

Material and methods

We collected 90 individuals of *Eligmodontia* along the Puna and Monte desert biomes (Fig. 1). Localities, number of collected individuals (*N*), coordinates (longitude/latitude/altitude) and Provinces are as follows: Abra Pampa (*N* = 22), −65.7028/−22.7680/3518 m asl, Jujuy Province; 26 km W of Susques (*N* = 11), −66.5377/−23.3858/4198 m asl, Jujuy Province; 8 km E of Saladillo (*N* = 2), −64.9068/−24.4177/705 m asl, Jujuy Province; 18 km E of Salinas Grandes (*N* = 1), −23.6880/−65.7008/3980 m asl, Jujuy Province; Cortaderas (*N* = 19), −68.1492/−27.5844/3338 m asl, Catamarca Province; Campo Arenal, Los Nacimientos (*N* = 15), −66.6785/−27.1474/2139 m asl, Catamarca Province; Telteca Reserve (*N* = 21), −68.0463/−32.3932/520 m asl, Mendoza Province (Fig. 1). The first five populations correspond to the Puna desert biome and the last two to the Monte desert biome. Voucher specimens are housed in the mammal collection of the Instituto Argentino de Zonas Aridas (CMI – IADIZA), CCT-Mendoza, CONICET (Appendix A).

Chromosomal preparations of specimens were obtained using the standard hypotonic technique for bone-marrow (Ford and Hamerton 1956) with small modifications. Chromosomes were stained with Giemsa (pH = 6.8). Ten metaphase spreads were counted for each specimen.

For quantitative morphology, six external and 22 standard cranial features (Tables 1 and 2) were measured using a caliper rounded to the nearest 0.1 mm (Martin et al. 2001). Only animals with fully erupted dentition were used for the analyses. Univariate, multivariate principal component (MPC) and discriminant analyses (MDA) were implemented using InfoStat and Statistica programs. Means, Standard deviation (SD), and level of statistical significance (*p*) of Kruskal–Wallis or Mann–Whitney *U*-test were calculed for comparisons among populations of *E. puerulus* and *E. moreni*, respectively. For comparison between *E puerulus* and *E. moreni* the range were included and the Mann–Whitney *U*-test were implemented to detected significant morphological differences. For multivariate analyses the variables were used without further transformation. In the discriminant analysis, groups were classified according to the chromosome data. For posterior classification the calculation of a priori classification probability was proportional to the size of the groups.

For the molecular analyses, total DNA from liver tissues was extracted following the standard Phenol–Chloroform method (Sambrook et al. 1989). A fragment of 687pb of the cytochrome-b gene was amplified by polymerase chain reaction (PCR) using the primers MVZ 05 and MVZ 16 (Smith and Patton 1993). Amplifications were performed with the following parameters: denaturation at 95 °C (3 min), annealing at 50 °C (1 min), and extension at 72 °C (1 min) for 35 cycles. Negative controls were included in all experiments. PCR products were purified with Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced in both directions by Macrogen Inc. (Seoul, South Korea; www.macrogen.com). Sequencing reactions were carried out under BigDyeTM terminator cycling conditions using an ABI PRISM 3700 DNA automatic analyzer (PE Applied Biosystems, Forster City, California).

Fifteen individuals belonging to different species and localities, and with different diploid number, were sequenced (Table 3). Additionally, 15 sequences were obtained from the GenBank of *Eligmodontia* (Table 3) and related species *Graomys griseoflavus* (AY275117), *G. domorum* (AF159291), *Calomys laucha* (AY033190), *C. lepidus* (AF159294) and *C. musculinus* (AF385603) used as outgroup species. Sequence alignment was carried out with Clustal X (Thompson et al. 1997) and corroborated by eye inspection. Sequences were translated to proteins to proof for stop codons or other unusual substitutions using MEGA 4.1 (Tamura et al. 2007). Jukes–Cantor distances (JC) were calculated. Phyloge-

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netic relationships among *Eligmodontia* species were analyzed using minimum evolution (ME), maximum parsimony (MP) and maximum likelihood (ML) in PAUP 4.0B10 (Swofford 2002). The distance used in the ME tree was JC with all three nucleotide positions. MP trees were inferred with 200 replicates of heuristic search with tree-bisection-reconnection (TBR) branch swapping and random addition sequence. Variable nucleotide positions were equally weighted and treated as unordered characters. Node support was tested using 1000 bootstrap replicates in the ME and MP analyses. Branches with <50% support were allowed to collapse. ML model and parameters values were estimated with Modeltest 3.04 (Posada and Crandall 1998). The model selected by AIC (minimum theoretical information criterion) was GTR + I + G ($-\ln L = 3402.458$) with the following parameters: percentage of invariable sites = 0.523; gamma distribution shape parameter = 1.66; assumed base frequencies $A = 0.311$, $C = 0.285$, $G = 0.116$, $T = 0.288$; substitution matrix: $(A-C) = 0.8926$, $(A-G) = 6.0091$, $(A-T) = 1.1909$, $(C-G) = 0.0001$, $(C-T) = 9.0944$, $(G-T) = 1.0000$, and node support was assessed with 100 replicates.

Results

Chromosomes

Individuals of *E. puerulus* from Susques ($N = 10$), Saladillo ($N = 2$) and from 18 km E of Salinas Grandes ($N = 1$) showed $2n = 32$, FN = 48 (Fig. 2). The karyotype is composed of nine pairs of biarmed (meta-submetacentric) and six pairs of acro-telocentric chromosomes. The X is a medium-sized acrocentric chromosome and the Y a small acrocentric. Secondary constrictions were observed in interstitial position in two biarmed pairs (Fig. 2a). The population of Abra Pampa ($N = 16$) was polymorphic for two chromosome rearrangements with $2n = 31$, 32 and 33; FN = 48 (Fig. 2b). Secondary constrictions were observed in different positions in accordance with the diploid number in two biarmed pairs. In the population of Pastos Largos, Catamarca Province, two individuals showed the same diploid and fundamental numbers as previously described ($2n = 32$, FN = 48). However, eight individuals from Pastos Largos had diploid numbers ranging from 35 to 37, FN = 48 (Fig. 2c). Sec-

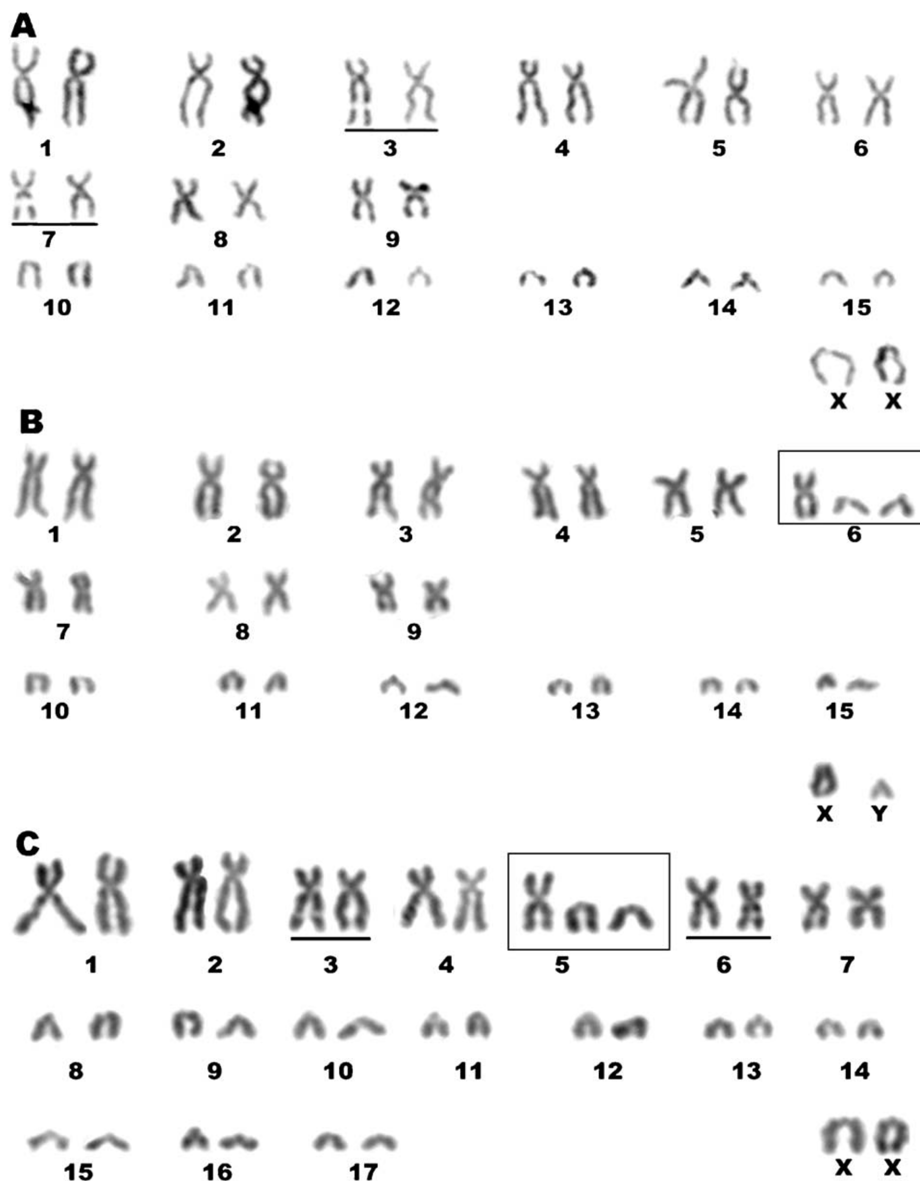

Fig. 2. Bone marrow standard Giemsa staining karyotype of *Eligmodontia puerulus* from (a) Susques with $2n = 32$/FN = 48; (b) from Abra Pampa with $2n = 33$/FN = 48 and (c) from Pastos Largos with $2n = 37$/FN = 48. Secondary constrictions are underlined. Chromosomes involved in the Robertsonian translocation in herozygotes are in boxes.

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ondary constrictions were observed in interstitial position in two biarmed pairs. Sexual chromosomes were identical to those observed in the other Puna populations. All karyotypes described can be transformed through successive Robertsonian translocations.

Typical trivalents were observed in the meiosis of heterozygote individuals (Fig. 3). Neither univalents nor other meiotic disturbances were observed.

All individuals of *E. moreni* from Telteca (Mendoza Province) and Campo Arenal (Catamarca Province) showed a constant chromosome complement with $2n = 52$ and FN = 50, which was presented in Lanzone et al. (2007).

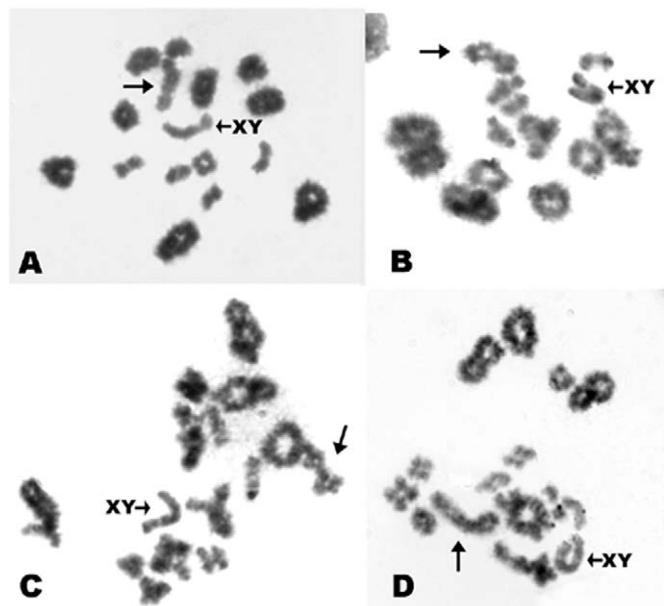

Fig. 3. Diakinesis cells from individuals of *E. puerulus* heterozygote for Robertsonian translocation. Trivalents are indicated by arrows; XY = sex pair.

Craniometrical analysis: intraspecific differences

In general, no significant differences between male and female *E. puerulus* were detected (univariate analyses, Kruskal–Wallis test). Both sexes differ only in nasal breadth ($p = 0.008$), with the nose larger in males than in females. The tendency to larger size in males is maintained in 24 metric characters, although in most cases these differences are very small and never significant. Because of these results, males and females were combined in later analyses.

The populations of *E. puerulus* from Susques (Jujuy), Abra Pampa (Jujuy) and Refugio Pastos Largos (Catamarca) in the Puna of Argentina show little variation (univariate analyses, Kruskal–Wallis test). Only five variables show significant differences among populations (Table 1). On the other hand, populations

Table 1
Means, standard deviation (SD), and level of statistical significance (*p*) of Kruskal–Wallis and Mann–Whitney *U*-test for specimens of *E puerulus* and *E. moreni*, respectively, from different populations.

	Species						
	E. puerulus				*E. moreni*		
Populations Variables	Abra Pampa (*N* = 22) Mean ± SD	Susques (*N* = 11) Mean ± SD	Pastos Largos (*N* = 19) Mean ± SD	*p*	Campo Arenal (*N* = 15) Mean ± SD	Telteca Reserve (*N* = 21) Mean ± SD	*p*
Total length	154.82 ± 12.54	160.09 ± 7.7	156.24 ± 11.72	0.58	183.64 ± 10.44	187.00 ± 12.94	0.447
Head and body length	83.5 ± 7.24	84.64 ± 5.84	80.79 ± 6.75	0.24	81.53 ± 5.19	77.75 ± 4.40	0.0226
Tail length	71.32 ± 6.85	75.45 ± 5.07	76.41 ± 6.24	0.1	102.36 ± 6.71	108.85 ± 10.23	0.016
Hind foot length	23.27 ± 1.11	23.73 ± 0.79	23.79 ± 1.13	0.42	24.27 ± 1.02	24.43 ± 1.12	0.812
Ear length	17.59 ± 1.37	18.36 ± 0.5	18.11 ± 1.21	0.17	19.69 ± 1.33	18.86 ± 1.37	0.047
Weight	18.73 ± 4.7	20.27 ± 1.95	16.53 ± 3.3	0.017*	19.90 ± 3.12	17.40 ± 3.76	0.024
Condylobasal length	21.68 ± 1.39	22.16 ± 0.56	21.72 ± 0.8	0.43	22.52 ± 0.59	21.56 ± 0.72	0.001
Least interorbital breadth	3.95 ± 0.1	4.03 ± 0.13	3.94 ± 0.13	0.22	4.29 ± 0.15	4.15 ± 0.14	0.012
Zygomatic breadth	12.84 ± 0.67	12.88 ± 0.17	12.66 ± 0.37	0.27	13.10 ± 0.43	12.43 ± 0.43	<0.001
Maximum cranial length	24.49 ± 1.39	24.92 ± 0.46	24.34 ± 0.73	0.12	25.42 ± 0.68	24.61 ± 0.80	0.004
Basal length	19.67 ± 1.4	20.07 ± 0.51	19.59 ± 0.77	0.34	20.55 ± 0.54	19.61 ± 0.69	<0.001
Breadth of braincase	11.75 ± 0.33	11.9 ± 0.26	11.58 ± 0.22	0.01**	11.96 ± 0.27	11.54 ± 0.21	<0.001
Length of maxillary toothrow	3.92 ± 0.16	3.89 ± 0.07	3.87 ± 0.13	0.5	4.08 ± 0.12	3.91 ± 0.14	0.003
Length of palate	11.77 ± 0.74	12.04 ± 0.22	11.81 ± 0.43	0.46	12.55 ± 0.31	12.05 ± 0.50	0.005
Width of bulla	5.04 ± 0.19	5.07 ± 0.12	5.09 ± 0.17	0.97	5.49 ± 0.12	5.36 ± 0.18	0.022
Length of bulla and tube	5.62 ± 0.32	5.59 ± 0.08	5.58 ± 0.19	0.46	6.32 ± 0.18	6.23 ± 0.22	0.212
Length of bulla	4.61 ± 0.24	4.73 ± 0.17	4.7 ± 0.23	0.33	5.51 ± 0.13	5.38 ± 0.21	0.047
Length of mandible toothrow	3.85 ± 0.12	3.8 ± 0.09	3.81 ± 0.12	0.38	3.95 ± 0.16	3.86 ± 0.13	0.081
Maximum mandible length	12.47 ± 0.72	12.61 ± 0.24	12.42 ± 0.47	0.51	12.85 ± 0.32	11.98 ± 0.50	<0.0001
Length of diastema	5.84 ± 0.48	5.84 ± 0.21	5.79 ± 0.23	0.59	5.73 ± 0.30	5.43 ± 0.31	0.0095
Palatal bridge length	5.16 ± 0.35	4.88 ± 0.21	4.75 ± 0.34	0.002***	5.12 ± 0.29	5.33 ± 0.24	0.0134
Palate breadth in m	3.03 ± 0.23	3.06 ± 0.11	2.96 ± 0.18	0.3	3.00 ± 0.15	2.88 ± 0.16	0.0260
Palate breadth in m^3	2.9 ± 0.22	2.95 ± 0.13	2.85 ± 0.19	0.35	2.73 ± 0.14	2.64 ± 0.17	0.0431
Incisive breadth	1.54 ± 0.1	1.62 ± 0.06	1.55 ± 0.1	0.11	1.65 ± 0.07	1.51 ± 0.07	<0.0001
Incisive foramen length	5.13 ± 0.27	5.25 ± 0.16	5.05 ± 0.19	0.04**	5.19 ± 0.35	4.72 ± 0.23	0.0003
Nasal breadth	2.37 ± 0.2	2.4 ± 0.08	2.38 ± 0.15	0.78	2.30 ± 0.15	2.19 ± 0.15	0.0436
Nasal length	9.28 ± 0.53	9.52 ± 0.33	9.22 ± 0.37	0.16	9.26 ± 0.24	8.90 ± 0.53	0.0256
Rostrum width	4.08 ± 0.25	4.18 ± 0.18	4.01 ± 0.19	0.06	3.95 ± 0.19	3.91 ± 0.17	0.5257
Tail % of head and body	85.63 ± 7.1	89.57 ± 9.43	95.92 ± 5.9	0.0003*	117.77 ± 33.55	140.05 ± 11.36	0.003

Asterisks indicate significant differences among populations.
N = sample size.
* Pastos Largos to Abra Pampa and Susques.
** Susques to Pastos Largos.
*** Abra Pampa to Susques and Pastos Largos.

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Table 2
Means, standard deviation (SD), range and level of statistical significance (*p*) of Mann–Whitney *U*-test for specimens of *E puerulus* and *E. moreni*. *N* = sample size.

Variables	Species		
	E. puerulus (*N* = 54) Mean ± SD (range)	*E. moreni* (*N* = 36) Mean ± SD (range)	*p*
Total length	156.13 ± 11.25 (132.00–178.00)	185.66 ± 11.96 (157.00–208.00)	<0.0001
Head and body length	82.57 ± 6.78 (67.00–95.00)	79.37 ± 5.05 (69.00–89.00)	0.0098
Tail length	73.81 ± 6.61 (59.00–92.00)	106.18 ± 9.40 (92.00–125.00)	<0.0001
Hind foot length	23.57 ± 1.07 (21.00–26.00)	24.36 ± 1.07 (22.10–27.00	0.0013
Ear length	17.91 ± 1.21 (15.00–21.00)	19.21 ± 1.40 (16.00–21.50)	<0.0001
Weight	18.07 ± 4.01 (10.00–27.00)	18.44 ± 3.68 (12.50–27.50)	0.7696
Condylobasal length	21.80 ± 1.05 (18.70–23.30)	21.96 ± 0.82 (20.3–23.4)	0.7168
Least interorbital breadth	3.97 ± 0.12 (3.60–4.30)	4.21 ± 0.16 (3.90–4.60)	<0.0001
Zygomatic breadth	12.79 ± 0.49 (11.80–13.80)	12.70 ± 0.55 (11.60–13.90)	0.4126
Maximum cranial length	24.52 ± 1.03 (21.70–26.20)	24.95 ± 0.85 (23.30–26.40)	0.0673
Basal length	19.73 ± 1.05 (16.70–21.10)	20.01 ± 0.78 (18.20–21.30)	0.3017
Breadth of braincase	11.72 ± 0.30 (11.10–12.30)	11.72 ± 0.31 (11.20–12.60)	0.8232
Length maxillary toothrow	3.89 ± 0.13 (3.60–4.20)	3.98 ± 0.15 (3.70–4.30)	0.0060
Length of palate	11.84 ± 0.56 (10.30–12.70)	12.26 ± 0.49 (11.20–13.00)	0.0012
Width of bulla	5.07 ± 0.17 (4.60–5.50)	5.41 ± 0.17 (5.10–5.80)	<0.0001
Length of bulla and tube	5.60 ± 0.23 (5.00–6.00)	6.27 ± 0.21 (5.90–6.70)	<0.0001
Length of bulla	4.67 ± 0.22 (4.20–5.20)	5.43 ± 0.19 (5.00–5.80)	<0.0001
Length of mandible toothrow	3.83 ± 0.11 (3.60–4.00)	3.90 ± 0.15 (3.60–4.30)	0.0234
Maximum mandible length	12.48 ± 0.55 (11.20–13.40)	12.34 ± 0.61 (11.20–13.20)	0.3080
Length of diastema	5.82 ± 0.34 (5.00–6.50)	5.56 ± 0.33 (4.90–6.10)	0.0008
Palatal bridge length	4.93 ± 0.38 (4.20–5.70)	5.24 ± 0.28 (4.60–6.00)	0.0001
Palate breadth in m	3.01 ± 0.19 (2.60–3.40)	2.93 ± 0.17 (2.60–3.30)	0.0288
Palate breadth in m^3	2.89 ± 0.19 (2.40–3.30)	2.68 ± 0.17 (2.40–3.10)	<0.0001
Incisive breadth	1.56 ± 0.10 (1.40–1.80)	1.57 ± 0.10 (1.40–1.80)	0.8967
Incisive foramen length	5.12 ± 0.24 (4.60–5.50)	4.92 ± 0.37 (4.20–5.70)	0.0025
Nasal breadth	2.38 ± 0.16 (2.00–2.80)	2.23 ± 0.16 (1.90–2.60)	0.0001
Nasal length	9.32 ± 0.45 (8.20–10.30)	9.05 ± 0.47 (8.00–10.00)	0.0098
Rostrum width	4.08 ± 0.22 (3.50–4.60)	3.93 ± 0.18 (3.60–4.30)	0.0009
Tail % of head and body	89.99 ± 8.49 (70.59–116.00)	134.34 ± 12.24 (109.33–162.16)	<0.0001

of *E. moreni* from the lowland Monte Desert sites of Telteca Reserve (Mendoza) and Campo Arenal (Catamarca) show significant differences in most external and cranial measures (*U* Mann–Whitney test, Table 1). In general, individuals from Campo Arenal are larger than those from the Telteca Reserve, and this is also true for all external and cranial variables, except for three. Total length and tail length (the first influenced by the second) and palatal bridge length show an inverse relation, being smaller in the Campo Arenal population (Table 1).

Craniometrical analysis: interspecific differences

E. moreni and *E. puerulus* show significant differences in most external and cranial measures (univariate analyses, *U* Mann–Whitney test, Table 2). Multivariate analyses of external and cranial measurements of all specimens of the two species correctly separate all individuals of *E. puerulus* from *E. moreni*. The MPC divides both species along the second axis with no species overlap (Fig. 4). In this analysis, the first component explains 43% of the variability, the second 23% and the third 5%. The MDA shows a clear separation between *E. puerulus* and *E. moreni* ($p < 0.0001$). Because of differences in sample size, prior probabilities were set equal to size of samples. This procedure accurately assigned 100% of the individuals to the correct species in a post hoc comparison. In a step-wise discriminant function analysis, the variables which most contributed to the discrimination were: total length, bulla length with and without tube, breadth of palate in m^3, nasal breadth, head and body length.

Molecular data

Molecular divergence in the sequences of *E. puerulus*, with identical fundamental numbers (FN = 48) but different diploid numbers ($2n = 32$–37), varied from 0.15% to 5.75% (J–C). Molecular divergence in the sequences of *E. moreni* varied from 0.73% to 1.47%. *E. puerulus* differed between 11 and 8% from the other recognized species of the genus, except when compared to *E. moreni* with which differentiation was low (1%). Some individuals of *E. puerulus* showed the lowest interspecific molecular distances in the group when compared to the sequences of *E. moreni*.

All phylogenetic analyses generated the same tree topology, where *E. puerulus* and *E. moreni* are not monophyletic. Haplotypes of both taxa are mixed in a clade that has good support. The most basal dichotomy of this clade included a group with most *E. puerulus* from Jujuy and a group including the remaining *E. puerulus* and all *E. moreni* (Fig. 5).

The MP analysis data set (*N* = 30) has 258 variable characters of which 209 are parsimony informative. Molecular analyses of

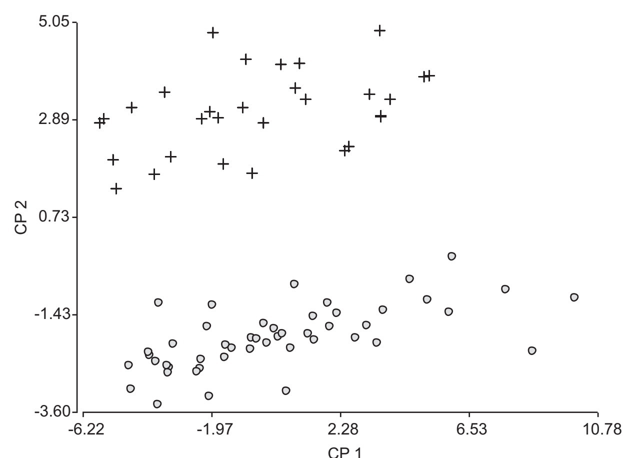

Fig. 4. Plot of principal component analysis using all cranial and external characters of both *Eligmodontia* species. Individuals of *E. puerulus* are indicated by circles and of *E. moreni* by crosses.

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Table 3
Specimens used in molecular analyses. Corresponding species, locality, access numbers to the GenBank (and in parentheses numbers of IADIZA's Mammal Collection), diploid (2*n*) and fundamental number (FN) and literature reference. Arg. = Argentina. In the second column, numbers in parentheses indicate the localities recorded in Fig. 5.

Species	Locality	No.	2*n*/FN	References
E. morgani	Las Leñas, Mendoza, Arg.	HQ706640 (CMI7429)	34/32	This study
E. morgani	4.2 km E de Bariloche, Río Negro, Arg.	AF108691	?	Smith and Patton (1999)
E. bolsonensis	Campo Arenal, Catamarca, Arg.	HQ706638 (CMI6838)	44/44	This study
E. typus	Telteca, Mendoza, Arg.	HQ706639 (CMI6828)	44/44	This study
E. typus	10 km S de Cmallo, Río Negro, Arg.	AF108692	?	Smith and Patton (1999)
E. hirtipes	?	AY341054	?	Palma and Marquet (unpublished data)
E. hirtipes	La Paz, Bolivia	AF159289	50/48	Anderson and Yates (2000)
E. puerulus	Cortaderas, Catamarca, Arg.	HQ706641 (CMI7172)	32/48	This study
E. puerulus	Cortaderas, Catamarca, Arg.	HQ706642 (CMI7171)	35/48	This study
E. puerulus	Cortaderas, Catamarca, Arg.	HQ706643 (CMI7173)	37/48	This study
E. puerulus	Cortaderas, Catamarca, Arg.	HQ706644 (CMI7162)	36/48	This study
E. puerulus	Abra Pampa, Jujuy, Arg.(1)	HQ706645 (CMI6938)	32/48	This study
E. puerulus	Abra Pampa, Jujuy, Arg. (1)	HQ706646 (CMI6936)	33/48	This study
E. puerulus	Abra Pampa, Jujuy, Arg. (1)	HQ706647 (CMI6947)	31/48	This study
E. puerulus	Salinas Grandes, Jujuy, Arg. (2)	HQ706648 (CMI6830)	32/48	This study
E. puerulus	25 km O de Susques, Jujuy, Arg. (3)	HQ706649 (CMI6970)	32/48	This study
E. moreni	Campo Arenal, Catamarca, Arg. (4)	HQ706650 (CMI6834)	52/50	This study
E. moreni	Telteca, Mendoza, Arg. (5)	HQ706651 (CMI7115)	52/50	This study
E. moreni	Campo Arenal, Catamarca, Arg. (4)	HQ706652 (CMI6835)	52/50	This study
E. moreni	Antofagasta de la Sierra, Catamarca, Arg. (6)	EU377653	?	Mares et al. (2008)
E. moreni	El Desmonte, Catamarca, Arg. (7)	EU377638	?	Mares et al. (2008)
E. moreni	Ea El Leoncito, Mendoza, Arg. (8)	EU377613	?	Mares et al. (2008)
E. hirtipes	La Paz, Bolivia	EU377635	?	Mares et al. (2008)
E. hirtipes	Laguna Colorada, Potosí, Bolivia	EU377634	?	Mares et al. (2008)
E. hirtipes	Colchane, Tarapacá, Chile	EU377636	?	Mares et al. (2008)

Eligmodontia and related rodent species, with the same weight for all nucleotide positions, generated one most parsimonious trees with a length = 554, consistence index CI = 0.60, retention index RI = 0.70 and homoplasy index HI = 0.40. This tree presents a lower resolution than the one recovered with distance methods, because *E. hirtipes* join at the base of the *Eligmodontia* tree as a non resolved polytomy. Again, in this analysis, *E. puerulus* and *E. moreni* are nested together in a clade with a high boostrap support (Fig. 5).

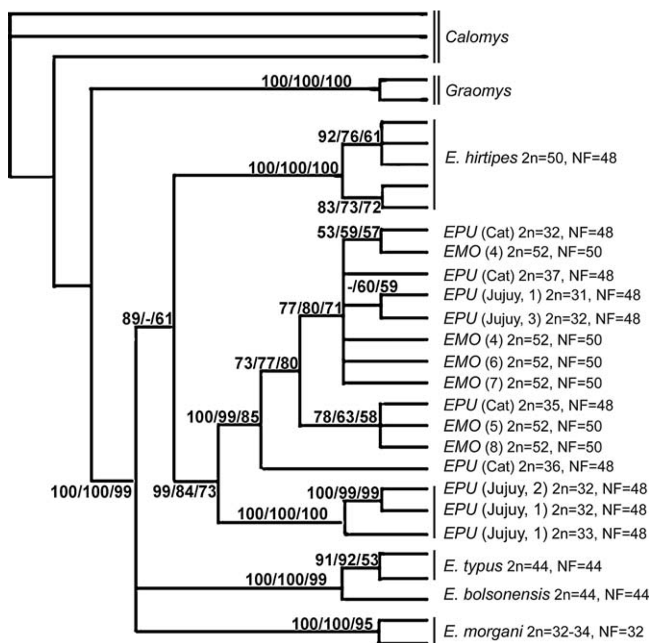

Fig. 5. Results of phylogenetic analyses for *Eligmodontia* species based on cytochrome-b sequences, diploid number and geographic origin of the Puna specimens. The numbers close to the branches are bootstrap support for the nodes: minimum evolution/maximum parsimony/maximum-likelihood analyses, respectively. *EPU* = *E. puerulus*, *EMO* = *E. moreni*; Cat = Catamarca Province; Jujuy Province. The hyphen (-) indicates relationships not recovered in some analyses. The numbers in parentheses correspond to the localities mentioned in Table 3.

The ML analysis corroborates the close relationship and confirms the absence of monophyly in *E. puerulus* and *E. moreni*, similar to that observed in the other analyses. The basal dichotomy of this clade separates a group containing the same three individuals of *E. puerulus* from Jujuy and a sister group with the remaining *E. puerulus* and *E. moreni*, as in the ME and MP analyses. In this tree, *E. hirtipes* appears as sister clade to the *E. moreni*–*E. puerulus* group, like in the ME tree, but with lower bootstrap support (Fig. 5).

Discussion

A taxonomic confusion has arisen about the species of *Eligmodontia* inhabiting the Puna desert. Initially, specimens with 2*n* = 34 (FN = 48) from Cauchari were assigned to *E. moreni* (Spotorno et al. 1994). Subsequently, these same authors found a cytotype identical to that of Cauchari in individuals from the type locality of *E. puerulus* (San Pedro de Atacama, Chile), indicating that this chromosome constitution belongs to *E. puerulus* (Spotorno et al. 1998). One of the individuals from Catamarca (this study) shows a conventional karyotype identical to those previously described by Spotorno et al. (1994, 1998). Additionally, our data from the Argentinean Puna reveal high intraspecific chromosome variability, with fixed chromosome races as well as polymorphic populations. All these karyotypes correspond to a system of multiple Robertsonian (Rb) translocations, evidenced by the constancy in the fundamental number of autosomic arms (FN = 48) and by the presence of typical trivalents in meiosis. Four Rb translocations are necessary to generate the Rb system described in this study. However, it is unknown whether karyotypes from different populations with the same diploid numbers have the same rearrangements. Further G-banding studies are required to determine homology of the cytotypes from Puna. This type of intraspecific chromosome variation is very common among rodents. It has been demonstrated that, in general, large Rb differences are required to promote reproductive isolation (Searle et al. 1990; Nachman 1992; Zambelli et al. 1994;

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Rogatcheva et al. 1998; Tiranti 1998; Piálek et al. 2005). Our results at morphologic, chromosomal and molecular levels, suggest that the *Eligmodontia* individuals from the Argentina Puna biome could belong to the same species.

At molecular level (cytb), the populations of *E. puerulus* have high genetic variability too, compared to other data for sigmodontine rodents (Smith and Patton 1993, 1999; Steppan 1998; Almeida et al. 2007; Mares et al. 2008). The maximum cytb value from paired comparisons is 5.75% (this study). Previous studies on *Eligmodontia* found lower values for intraspecific differences (Hillyard et al. 1997; Lanzone et al. 2007; Mares et al. 2008).

The high molecular variability observed in *E. puerulus* may be due to different causes. The first possibility is that they are two different species grouped under one taxon. This is supported by the large discontinuity observed in the genetic variability among haplotypes (there is a group of intra-clades paired distances ranging from 0.15 to 1.5% and another one between clades of 4.2–5.8%). However, the observed geographical distribution of haplotypes has cast some doubts about this possibility. Within the Abra Pampa population, one individual has more than 5% of divergence with others from Jujuy (one from its same locality and the other from Salinas Grandes). This may be due to a process of divergence in allopatry and subsequent migration. However, it is necessary to consider that the absence of intermediate genetic distances may be due to low sampling effort. Nevertheless, the great molecular variability detected in *E. puerulus* is striking.

Some authors (Neigel and Avise 1993; Avise 1994; Wakeley 2000) have suggested that the genetic variability of a lineage is primarily determined by the originally available diversity of its most immediate ancestor, its evolutionary age, selection, population subdivision, gene flow and mating system. Subdivision of the population could be one of the main factors involved in this differentiation. While *E. typus* and *E. morgani* from Patagonia show a low degree of genetic differentiation among populations (Lessa et al. 2010), the extent of population partitioning in *Eligmodontia* from the Puna is unknown. Nevertheless, there are some indications that could consist (or have consisted, at some point in their evolutionary histories) of semi-isolated populations. Topographically, the Puna is a highly complex and fragmented Andean landscape composed of valleys surrounded by mountains and volcanoes (Allmendinger et al. 1997). In addition, the marked differences observed in chromosome number among these populations indicate the presence of barriers to gene flow. Thus, cytogenetic and molecular data suggest that isolation and migration might have been important factors in promoting the genetic variability observed in *E. puerulus*.

At taxonomic and systematic level, most *Eligmodontia* species are monophyletic and possess interspecific differences in cytochrome b that are within the range of those observed for other mammals, and particularly for rodents (Smith and Patton 1993, 1999; Steppan 1998; D'Elía 2003; Baker and Bradley 2006). It has been suggested that a divergence higher than 5% for cytochrome b may be indicative of the presence of two species (Baker and Bradley 2006). Most *Eligmodontia* species far exceed this limit, which strongly supports their specific status (Lanzone et al. 2007; Mares et al. 2008). However, the data presented in this work for *E. puerulus* and *E. moreni* are conflicting. Validity of both species is supported by the high morphological and chromosomal differentiation between them. *E. moreni* is a species with high karyotypic constancy and distinctive morphology. In turn, the chromosome complement of *E. puerulus* is highly variable and is morphologically different from *E. moreni*. Moreover, seven chromosomal rearrangements are required at least (and at least one non-Rb) to derive the karyotype of one species into the other. Yet, it is important to remark that, including only molecular data of this gene, both species are indistinguishable.

The inconsistency between gene tree and species tree can be explained by distinct evolutionary mechanisms (Brower et al. 1996; Doyle 1997; Maddison 1997; Nichols 2001; Funk and Omland 2003; Rosenberg 2003). A possibility is that the evolutionary divergence of *E. puerulus* and *E. moreni* is of recent origin. When speciation has occurred in recent evolutionary time, a polyphyletic pattern similar to that observed between *E. moreni* and *E. puerulus* is expected as a result of incomplete lineage sorting (Maddison 1997; Funk and Omland 2003; Rosenberg 2003). If this is correct, the morphological differentiation between *E. puerulus* and *E. moreni* must have been acquired in a short period of time (i.e. by natural selection). Accelerated morphological changes were observed in some species pairs as a result of natural selection and adaptation to new environmental conditions (Modi 2003; Lecompte et al. 2005; Monteiro and Dos Reis 2005; Renaud et al. 2007). Both studied species occurr in different environmental conditions. While *E. moreni* occurs in scrublands and sand dune habitats of the warm temperate lowland Monte Desert, *E. puerulus* lives in the cold highland Puna desert (>3500 m) in sandy and grassland habitats (Cabrera 1976). This can also explain the interpopulation differentiation observed in *E. moreni*, which may be responding differentially to the habitats they occupy.

On the other hand, the same polyphyletic pattern is expected if the two species hybridize or have done so at some point in the past (Maddison 1997; Funk and Omland 2003). But, the large chromosomal differentiation has cast doubts about this possibility. The lack of monophyly between distinct species appears to be a relatively common phenomenon among animals (Funk and Omland 2003). However, it is very difficult to determine conclusively whether a particular case of polyphyly is due to incomplete lineage sorting or to hybridization, particularly when introgression is old (Funk and Omland 2003).

In several sigmodontine rodents with chromosome variations within-populations was observed absence of molecular divergence, as expected for chromosome polymorphisms (Nachman and Myers 1989; Silva et al. 2006; Martínez et al. 2010). In this group of rodents, chromosomally differentiated species by underdominant rearrangements showed low to high molecular divergence; but in general, molecular data are consistent with chromosomal results (Silva et al. 2006; Almeida et al. 2007; Lanzone et al. 2007; Martínez et al. 2010). A similar pattern as the one described here was reported for *Scapteromys* (D'Elía and Pardiñas 2004). In most populations, *S. aquaticus* and *S. tumidus* showed consistent differentiation in molecular and chromosome characters. However, in a population from Uruguay, specimens presented the chromosome complement of *S. tumidus* and haplotypes of *S. aquaticus*. Whether this observed pattern was due to incomplete lineage sorting or introgression could not be elucidated (D'Elía and Pardiñas 2004).

In conclusion, our results emphasize the need to integrate different datasets in the analysis of species variability and diversification, since different approaches can lead to different interpretations if they are analyzed in separate frameworks. This is particularly relevant when dealing with highly speciose groups (e.g. sigmodontine rodents) and heterogeneous landscapes, such as the Monte–Puna aridlands.

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Appendix A. Specimens examined are deposited in the mammal collection of IADIZA (CMI)

E. moreni: Rva. Telteca, Mendoza Prov.: CMI-6106, CMI-6213, CMI-6214, CMI-6219, CMI-6221, CMI-6826, CMI-6827, CMI-7108, CMI-7109, CMI-7110, CMI-7111, CMI-7112, CMI-7113, CMI-7114, CMI-7115, CMI-7116, CMI-07117, CMI-7122, CMI-7123, CMI-7124; 18 km N de Las Catitas, Mendoza Prov.: CMI-7235; Campo Arenal, Catamarca Prov.: CMI-6834, CMI-6835, CMI-6837, CMI-6839, CMI-6911, CMI-6912, CMI-6913, CMI-6914, CMI-6915, CMI-6916, CMI-6917, CMI-6918, CMI-6923, CMI-6924, CMI-6925

E. puerulus: Abra Pampa, Jujuy Prov.: CMI-6936, CMI-6937, CMI-6938, CMI-6939, CMI-6940, CMI-6942, CMI-6944, CMI-6945, CMI-6946, CMI-6947, CMI-6948, CMI-6949, CMI-6950, CMI-6951, CMI-6952, CMI-06954, CMI-6955, CMI-6956, CMI-6957, CMI-6958, CMI-6959, CMI-6960; Susques, Prov. Jujuy: CMI-6961, CMI-6962, CMI-6963, CMI-6964, CMI-6965, CMI-6966, CMI-6967, CMI-6968, CMI-6969, CMI-6970, CMI-7010; Cortaderas (Refugio Pastos Largos, a 95 km O de Fiambalá), Catamarca Prov: CMI-0338, CMI-3362, CMI-3364, CMI-3374, CMI 3376, CMI-3381, CMI-7162, CMI-7163, CMI-7170, CMI-7171, CMI-7172, CMI-7173, CMI-7174, CMI-7179, CMI-7180, CMI 03366, CMI 3378, CMI-3370, CMI-3377; Saladillo, Jujuy Prov.: CMI-6102, CMI-6104

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