

## Phylogeographic Structure of the Fossorial Long-Clawed Mouse *Chelemys macronyx* (Cricetidae: Sigmodontinae)

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**Oriet Alarcón, Guillermo D'Elía, Enrique P. Lessa, and Ulyses F.J. Pardiñas (2011)** Phylogeographic structure of the fossorial long-clawed mouse *Chelemys macronyx* (Cricetidae: Sigmodontinae). *Zoological Studies* 50(5): 682-688. We present a phylogeographic study of the fossorial sigmodontine mouse *Chelemys macronyx*. Analyses were based on mitochondrial DNA sequences of specimens collected over most of distributional range of the species. Results showed that *C. macronyx* has a shallow genealogy that is geographically structured into 2 main clades: one in the northern part of the species distribution, at high-Andean localities in the Argentinean provinces of Mendoza and northern Neuquén, and the other covering the majority of its distributional range at medium- to low-elevation localities from northwestern Neuquén to the south. The northern clade appears to have been demographically stable, while the southern clade presents signals of demographic expansion. These results suggest that current genetic variation of *C. macronyx* may have originated from 2 refugia. <http://zoolstud.sinica.edu.tw/Journals/50.5/682.pdf>

**Key words:** Abrotrichini, Andes, Cytochrome *b*, Taxonomy, Patagonia.

Among the diverse rodent subfamily Sigmodontinae, the clade of long-clawed mice within the tribe Abrotrichini (Patterson 1992, D'Elía et al. 2007) stands out because of adaptations to fossoriality (Pearson 1984). One of these is *Chelemys* Thomas, a genus widely distributed along the Southern Andes (from about 30°S to the northern shore of the Strait of Magellan) including an important area of the Patagonian steppe of Argentina (Pardiñas et al. 2003).

First described as a subgenus of *Akodon* Meyen, *Chelemys* was later subsumed under *Notiomys* Thomas by Osgood (1925, see also Osgood 1943) in his influential revision of long-clawed mice, a classificatory scheme followed in the influential catalogs of Ellerman (1941) and

Cabrera (1961). However, other authors (Thomas 1927, Gyldenstøpe 1932) retained *Chelemys* as a valid genus. Later, given its degree of morphological differentiation from other long-clawed abrotrichines, Pearson (1984) and Reig (1987) set the basis for its current classification as a distinct genus (Musser and Carleton 2005, D'Elía et al. 2007).

The specific diversity of *Chelemys* remains poorly understood as a consequence of the lack of revisionary studies, a scenario common for most South American rodent taxa (Bezerra et al. 2009). Currently (Musser and Carleton 2005), 3 species are recognized: *C. megalonyx* (Waterhouse), *C. macronyx* (Thomas), and *C. delfini* (Cabrera). The distinction of *C. macronyx*

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from *C. megalonyx*, type species of the genus, is beyond doubt as these are clearly morphologically distinct; moreover, a phylogenetic analysis of DNA sequences indicated that these 2 might not be sister species, which would render *Chelemys* polyphyletic (Rodríguez et al. 2008, Feijoo et al. 2010). Distinction between *C. delfini* and *C. macronyx* is not as clear and currently is mostly sustained in the statement of Johnson et al. (1990), who claimed both species are distinguishable. Unfortunately, the holotype of *C. delfini* is lost, further complicating an assessment of the status of this taxon (Musser and Carleton 2005).

In addition, 3 other nominal forms are associated with *C. macronyx*: *Akodon* (*Chelemys*) *vestitus* Thomas, *Notiomys vestitus alleni* Osgood, and *N. vestitus fumosus* Thomas. Currently, these taxa are considered synonyms of *C. macronyx*, although their distinction has never been evaluated by means of an extensive assessment of morphologic variation. Recently, Lessa et al. (2010) reported that a divergent haplotype from Mendoza, Argentina was sister to a seemingly unstructured clade containing all Patagonian samples of *C. macronyx*. This assessment, however, was based on a limited sample size. In addition, between the Mendocinian locality and the closest Patagonian locality sampled, there is a large gap of ca. 580 km; therefore, it was not possible to confidently state if *C. macronyx* exhibits phylogeographic structure. Herein, we extend the phylogeographic analysis of this species by expanding the geographic sampling analyzed, including specimens collected at 4 localities within the aforementioned geographic gap. The aims of the study were to assess the phylogeographic pattern of *C. macronyx* to further evaluate the recent demographic history of the species and preliminarily evaluate the distinction of the different nominal forms associated with *C. macronyx*.

## MATERIALS AND METHODS

Analyses were based on mitochondrial DNA sequences of the cytochrome (Cyt) *b* gene (801 bp) of 29 specimens collected at 16 Argentinean and Chilean localities covering the entire latitudinal range of the species (Table 1, Fig. 1). Importantly, our sampling included specimens collected at the type localities of *Acodon macronyx* (per the geographic placement of its locality, see Pearson and Lagiglia 1992, and also Ojeda et al. 2005) and *N. vestitus alleni*. Sequences of the

remaining species of the fossorial clade of the Abrotrichini were used to conform the outgroup: *C. megalonyx* (GenBank accession no.: DQ309559), *Geoxus valdivianus* (U035312), *N. edwardsii* (U035371), and *Pearsonomys annectens* (AF108672). Sequences of *C. macronyx* were acquired from GenBank or obtained by us following protocols in D'Elía et al. (2008); the latter were submitted to GenBank (Table 1).

Sequences were aligned using Clustal X (Thompson et al. 1997) with default values for all alignment parameters. Observed percentages of genetic divergence ( $p$ -distances) were calculated with MEGA 4 (Tamura et al. 2007). The genealogy was reconstructed with a Bayesian approach using Mr. Bayes 3.1 (Ronquist and Huelsenbeck 2003). The analysis consisted of 2 independent runs, each with 3 heated and 1 cold Markov chains. The model used included 6 categories of base substitutions, a gamma-distributed rate parameter, and a proportion of invariant sites; all model parameters were estimated in Mr. Bayes 3.1. Uniform-interval priors were assumed for all parameters except the base composition and GTR parameters, which assumed a Dirichlet prior process. Runs were allowed to proceed for  $10^7$  generations with trees sampled every 1000 generations. Convergence on a stable log-likelihood value was checked by plotting log-likelihood values against the generation time. The 1st 25% of trees were discarded as burn-in; the remaining trees were used to compute a 50% majority rule consensus tree and obtain posterior probability (PP) estimates for each clade. The spatial genetic structure was further evaluated by an analysis of molecular variance (AMOVA; Excoffier et al. 1992) using Arlequin (Schneider et al. 2000) with haplotypes grouped by collection localities and the main clades found in the genealogical analysis.  $D$  (Tajima 1989) and  $F_s$  (Fu 1993) statistics were estimated for each of these main clades.

## RESULTS

Sequences of *Chelemys macronyx* had 46 variable sites that defined 20 haplotypes. Six haplotypes were present in more than 1 specimen; of these, 3 were shared among specimens at different localities (Table 1). The average divergence between haplotype pairs was 1.2% (range, 0.0%-2.9%). The locality of Cerro Colorado was the most variable, while Laguna

Varvaco Tapia and El Manzano were invariant.

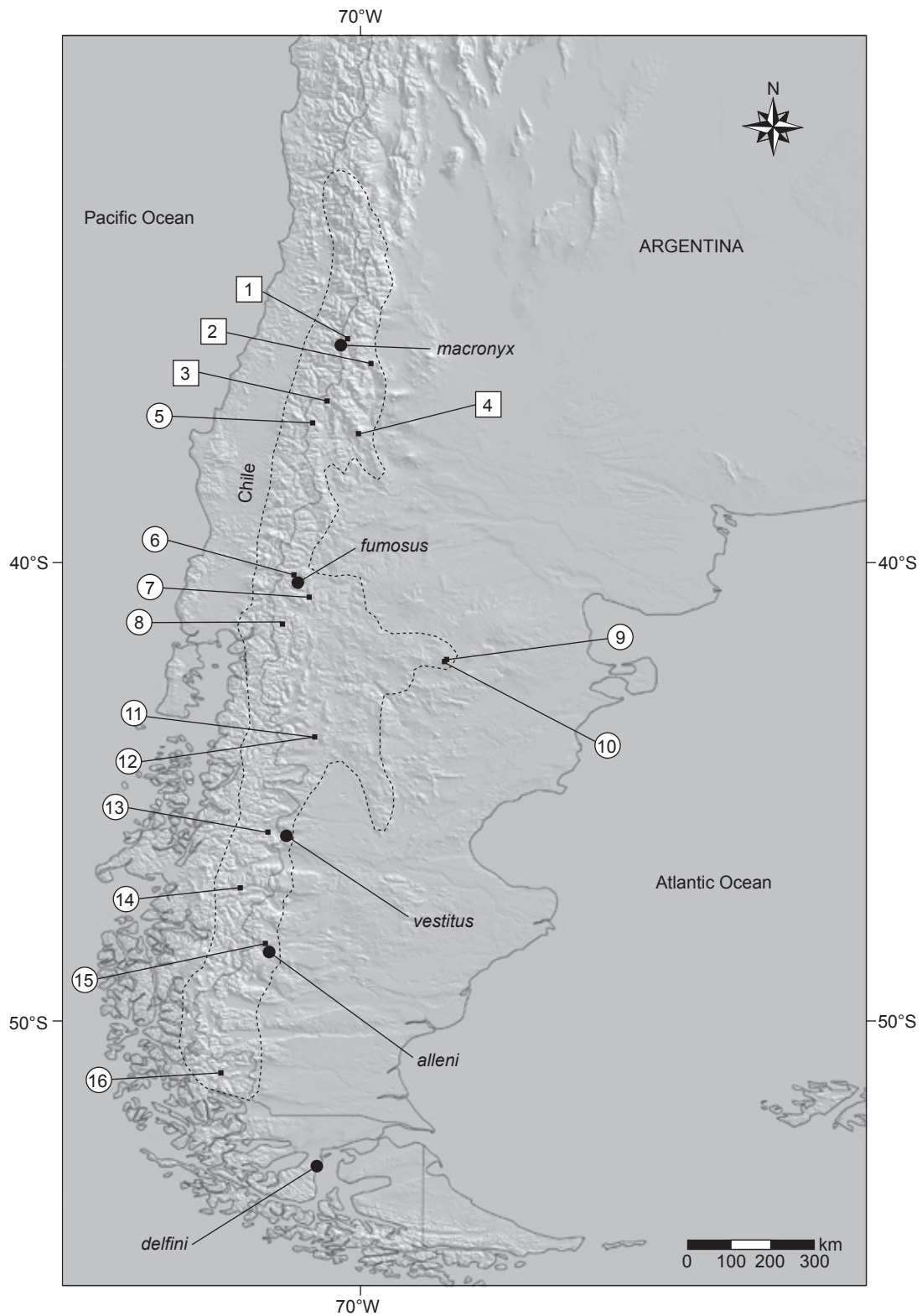
*Chelemys macronyx* showed a shallow genealogy that was nonetheless geographically structured into 2 main, strongly supported allopatric clades (Fig. 2). One clade (PP = 0.91) grouped haplotypes recovered from specimens collected in the northern range of the distribution, at Argentinean high-Andean localities in the provinces of Mendoza and northern Neuquén. The haplotype recovered from a topotype of *C. macronyx* fell in this clade. The other main clade (PP = 0.96) included variants recovered from specimens of the remaining distributional range covering medium to

lowland localities from northwestern and southern Neuquén, Chubut, and Santa Cruz Provinces in Argentina and the Aysén and Magallanes regions in Chile. Haplotypes recovered from topotypes of *alleni* and quasi-topotypes of *vestitus* fell in this widely distributed clade. The observed average divergence between the main clades was 2.3%. In addition, results of the AMOVA indicated that most (75.56%) of the genetic variation observed in the entire sample that was analyzed was due to differences between these 2 phylogeographic units.

**Table 1.** Sampling localities, numbers of specimens studied, and haplotypes found in the phylogeographic analysis of *C. macronyx*. Locality numbers follow those used in figure 1

	Country	Province/ Region	Locality	Geographic coordinates	Specimens analyzed	GenBank accession nos.	Haplotypes found
1	Argentina	Mendoza	Valle Hermoso	35.097940°S, 70.102470°W	1: RAO 114	HM167839	1
2			Cerro Colorado	35.514583°S, 69.926833°W	2: GBD1134, CNP 1896	JF706710*, JF706711*	1, 2
3		Neuquén	Laguna Varvarco Tapia	36.426389°S, 70.619167°W	2: CNP 442, CNP 447	JF706712*, JF706713*	3
4			Laguna Tromen	37.078889°S, 70.118333°W	1: CNP 823	JF706714*	4
5			Laguna Epu Lauquen	36.816667°S, 71.079722°W	1: CNP 441	JF706715*	5
6			Mirador del arroyo Pii Pii	40.213333°S, 71.361667°W	1: CNP 440	HM167830	6
7			Estancia Paso Coihue	40.890000°S, 71.290000°W	4: MVZ 184941, MVZ 184943, MVZ 184942, MVZ 184938	HM167832, HM167834, HM167833, HM167835	7, 8, 8, 9
8		Río Negro	44 km W of Bariloche	41.650000°S, 71.790000°W	1: MVZ 155800	U03533	10
9		Chubut	Paso de la Vaca	42.155566°S, 68.265050°W	1: CNP 1249	HM167823	11
10			Sierra de Talagapa	42.234444°S, 68.240556°W	2: CNP 822, AT 42	HM167831, HM167825	11, 12
11			1.5 km N Puesto de Tepuel	43.851667°S, 70.728056°W	1: CNP 2374	JF706716*	13
12			Puesto Tepuel	43.865278°S, 70.726111°W	1: CNP 2375	JF706717*	14
15		Santa Cruz	Estancia La Ensenada	48.365000°S, 72.088889°W	3: PNG 750, CNP 445, CNP 446	JF706708*, JF706707*, HM167824	15, 15, 16
13	Chile	Aysén	Coyhaique	46.000000°S, 72.000000°W	1: KG 004	HM167838	17
14			Sector el Manzano	47.150000°S, 72.650000°W	4: GD 926, GD 931, GD 935, GD 951	HM167826, HM167827, HM167828, HM167829	15, 15, 15, 15
16		Magallanes	Torres del Paine	51.166667°S, 72.950000°W	3: JG 101, NK 105734, TK 110079	JF706709*, HM167837, HM167836	18, 19, 20

\*indicates those sequences gathered in this study. Sequenced specimens are at the Colección de Mamíferos del Centro Nacional Patagónico (CNP), Chubut, Argentina or will be deposited there (GBD and PNG); or will be deposited at the Museo de Zoología de la Univ. de Concepción, Concepción, Chile (JG) or Colección de Mamíferos, Univ. Austral de Chile, Valdivia, Chile (GD). Haplotype numbers are those used in figure 2.



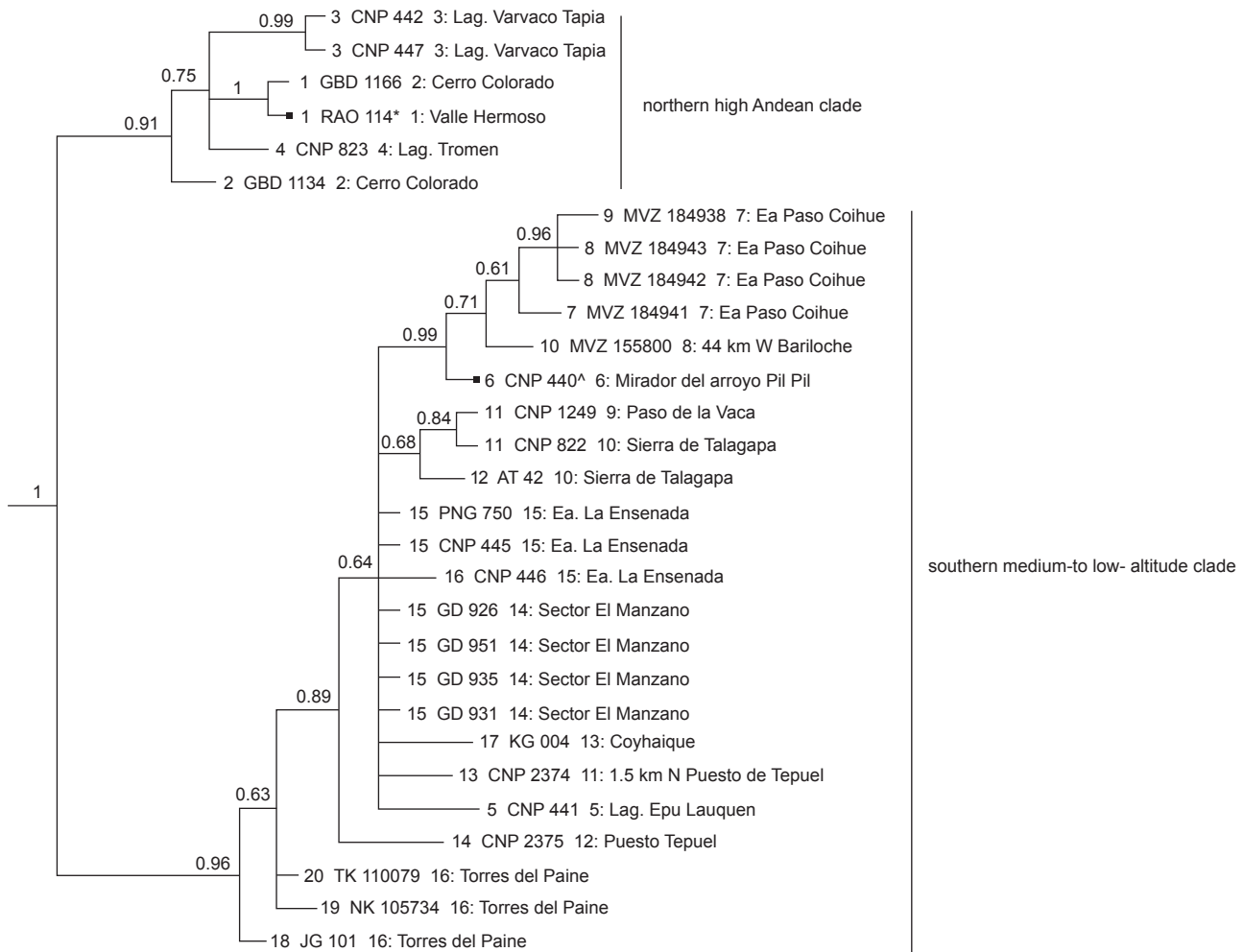
**Fig. 1.** Known geographic distribution (dotted line) of *Chelemys macronyx* showing collection localities of specimens analyzed in the phylogeographic study. Locality numbers follow those of table 1. Haplotypes recovered at localities marked with squares fall into the northern high-Andean clade. Haplotypes recovered at localities marked with circles fall in the mid-lowland clade mostly associated with *Nothofagus* forests (see text and Fig. 2). Type localities of taxa discussed in the text are indicated by black circles.

DISCUSSION

The genealogical structure described for *Chelemys macronyx* suggests a geographical subdivision into 2 areas of differentiation. Divergence between these is limited (i.e., 2.3% at the Cyt *b* gene) and awaits further analyses, including a detailed morphological assessment and examination of variation in nuclear loci. If such analyses corroborate the structure uncovered here, the subspecific names which should be applied are *C. m. macronyx* for the northern, high-Andean clade and *C. m. vestitus* for the southern, mid-elevation-lowland clade, mostly associated with *Nothofagus* forests. Under this scenario, *alleni* would be a junior synonym of *vestitus*; presumably

the same would apply to *fumosus*, although we have no topotypes, but specimens were collected close to its type locality. A study of specimens collected at the type locality of *delfini*, Punta Arenas in southernmost continental Chile (Fig. 1), is needed to test the distinction of this form from *C. macronyx*.

Our results indicate that *C. macronyx* presents phylogeographic structure consisting of 2 parapatric clades that mostly replace the latitudinal and elevational dimensions: a rather restricted northern high-Andean clade and a wide southern medium- to low-elevation clade that covers most of the distributional range of the species. The phylogeographic break shown by *C. macronyx* coincides with the location of the main Patagonian



**Fig. 2.** Genealogical relationship, reconstructed in the Bayesian analysis, of cytochrome *b* gene haplotypes recovered from 29 specimens of *Chelemys macronyx* (outgroup not shown). Numbers next to the nodes correspond to the posterior probabilities. Details of the terminal labels are as follows: haplotype number (1 to 20; see Table 1); specimen catalog number; locality number and name (see details in Table 1 and Fig. 1). \*Indicates the haplotype recovered from the topotype of *Acodon macronyx*. ^Indicates the haplotype recovered from a specimen collected at the type locality of *Notiomys vestitus alleni* (considered a synonym of *Chelemys macronyx*).

highlands around the Domuyo and Tromen volcanoes. Besides this geographical coincidence, the cause of this phylogeographic break remains to be investigated. This phylogeographic pattern is unique among Patagonian sigmodontines. *Akodon iniscatus* Thomas, *Calomys musculinus* (Thomas), *Eligmodontia morgani* J.A. Allen, *E. typus* F. Cuvier, *Graomys griseoflavus* Thomas, *Oligoryzomys longicaudatus* (Bennett), *Phyllotis xanthopygus* (Waterhouse), and *Reithrodon auritus* (G. Fischer) lack phylogeographic structure (Lessa et al. 2010 fig. 1; see also Belmar et al. 2009). *Abrothrix longipilis* (Waterhouse) and *A. olivaceus* (Waterhouse) present deeper (as measured by means of genetic distances) and more-northerly phylogeographic breaks than does *C. macronyx*. In addition, *A. longipilis* shows another break at middle latitudes of Patagonia, which is absent in *C. macronyx*, and that is also displayed by *Euenomys chinchilloides* (Waterhouse), *Geoxus valdivianus* (Philippi), and *Loxodontomys micropus* (Waterhouse) (Cañon et al. 2010, Lessa et al. 2010).

Finally, the main clades of *C. macronyx* show contrasting signals of demographic history. Neutrality tests showed non-significant values for  $D$  and  $F_s$  (1.02753 NS and 1.55426 NS, respectively) for the high-Andean clade. These results are indicative of demographic stability of the species in the sampled area; suggesting that during the last glacial phase, *C. macronyx* persisted in refugia located in the sampled area. Meanwhile, for the lowland clade, the conservative  $D$  statistic was negative, but not significant, while  $F_s$  was negative and significant (-0.55953 NS and -20.48419\*). These results suggest that southern populations are younger. Together with the lack of geographic structure within the broadly distributed southern clade, this fact suggests that *C. macronyx* recently colonized its southern distributional range, although not necessarily after the Last Glacial Maximum (see Lessa et al. 2010). In this sense, isolated populations of *C. macronyx* on the Patagonian steppe may be relicts of a past distribution from where the current distributional area was colonized. A similar scenario was suggested by Cañon et al. (2010) for the short-footed mouse *Loxodontomys micropus*. This scenario suggests that at least 2 refugia, one for each main geographic clade, may be needed to account for the current geographic structure of the genetic variation of *C. macronyx*. However, a scenario invoking a single refugium in the north that served as a source of colonization for the

southern distribution cannot, for the moment, be ruled out. Further analyses based on additional specimens and nuclear DNA variation are needed to test the taxonomic and demographic hypotheses advanced here.

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