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Molecular systematics of *Abrothrix longipilis* (Rodentia: Cricetidae: Sigmodontinae) in Chile

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We evaluated systematic relationships among most of the subspecies of the long-haired sigmodontine mouse *Abrothrix longipilis* in Chile. We sequenced the complete cytochrome-*b* gene and 634 base pairs of intron 7 of the β-fibrinogen gene from specimens from 17 localities along the distributional range of *A. longipilis*. Phylogenetic analyses confirm *Abrothrix sanborni*, once considered a subspecies of *A. longipilis*, as a valid species. In the latter taxon we obtained a structured phylogenetic pattern that recovered most of the geographic races traditionally recognized for this mouse. However, strong differentiation was found between *A. l. longipilis* from Mediterranean Chile and subspecies from the Temperate and Patagonian Forests in the south, suggesting that the former could constitute a different species. We concluded that historical events (glaciations), coupled with local selection pressures (different ecogeographic zones), might account for the current geographic pattern of variation in *A. longipilis*. DOI: 10.1644/10-MAMM-A-031.1.

Key words: *Abrothrix longipilis*, β-fibrinogen, Chile, cytochrome-*b* gene, subspecies, systematics

Abrothrix (Waterhouse, 1837) is a genus of small to medium-sized mice that is part of the recently diagnosed tribe Abrotrichini, formerly known as the Andean clade, a monophyletic group of South American sigmodontine rodents (D'Elía et al. 2007; Rodríguez-Serrano et al. 2008b). Abrotrichini includes several species once included in Akodontini. Cabrera (1961), Osgood (1943), Thomas (1916), and Waterhouse (1837) considered *Abrothrix* as a subgenus of *Akodon*, and subsequent revisions raised this taxon to the genus level. It is restricted to the southwestern portion of the Andes, although at very high latitudes it is widespread in Chilean and Argentinean Patagonia (Mann 1978; Osgood 1943; Redford and Eisenberg 1989). Currently, 8 species are recognized within *Abrothrix*, because *A. markhami* recently was assigned as a subspecies of *A. olivacea* (Rodríguez-Serrano et al. 2008a).

One of the most common species within the genus is the nominotypic *Abrothrix longipilis* (Waterhouse 1837). It is a long-haired, volelike, medium-sized mouse, with tail shorter than the body, pelage dark brown dorsally and gray ventrally, and a large and heavy skull with long and tapered nasals (Osgood 1943). *A. longipilis*, like *A. sanborni*, has a single baculum (as in neotomines), in contrast to the complex ones described for other *Abrothrix* species (Gallardo et al. 1988; Spotorno 1992). Phylogenetically, the species has been recovered as the sister taxon of the remaining *Abrothrix* species (D'Elía 2003; Rodríguez-Serrano et al. 2008b).

Abrothrix longipilis inhabits a variety of vegetation types, ranging from cloud forests to brushy areas and marshes, but it is most common in moister areas with a high proportion of shrub and litter cover (Mann 1978; Redford and Eisenberg 1989). It occurs in a great portion of Chile, from Coquimbo to Patagonia and Tierra del Fuego, including Argentina in the latter 2 areas. The distribution of *A. longipilis* encompasses the most diverse environments in Chile, from the Mediterranean ecoregion in the north to the Temperate and Patagonian Forests of southern Chile and Argentina. The southern distribution of *A. longipilis* occupies an area that was affected by glacial cycles of Pleistocene, including the Last Glacial Maximum (LGM) between 25,000 and 16,000 years ago (Glasser et al. 2008; Rabassa et al. 2005).

Along the wide geographic range of *A. longipilis* a variety of subspecies are recognized based on subtle morphologic and coloration patterns, although some conflicts exist regarding the status of some of these taxa (Mann 1978; Osgood 1943). The northernmost subspecies is *A. l. longipilis* (Waterhouse, 1837), which corresponds to the nominotypic subspecies and comparatively is the largest form, with a range between 30° and 33°S (Fig. 1). *A. l. hirta* (Thomas, 1895) is found in the eastern Andes, in the Argentinean provinces of Mendoza and

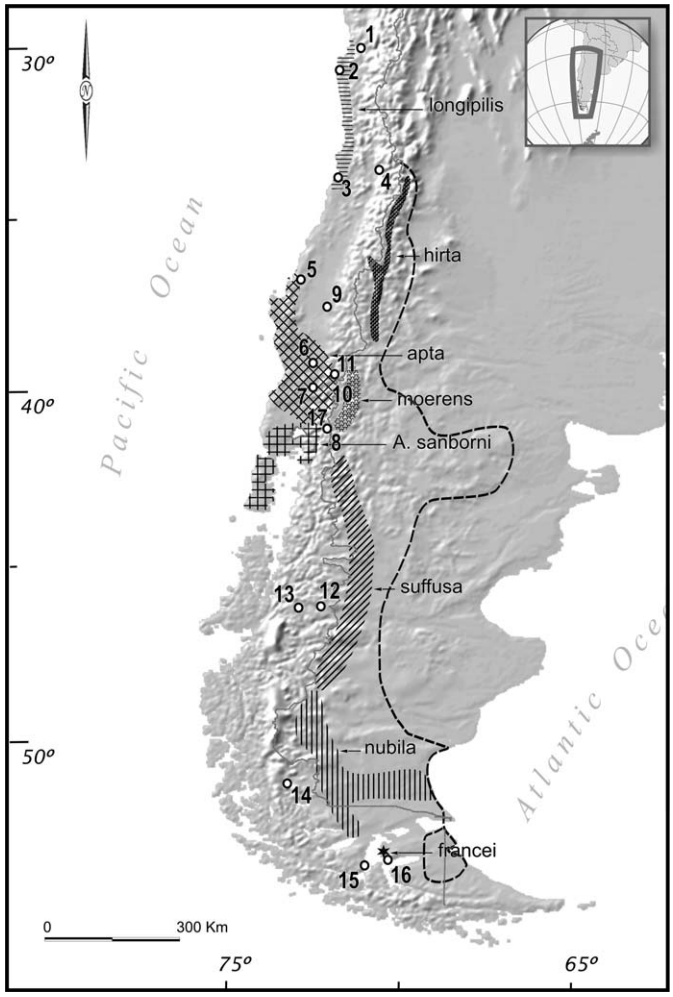

Fig. 1.—Geographic distribution of *Abrothrix longipilis* subspecies and *A. sanborni*. The Chilean distribution is modified from Osgood (1943). The dashed black line is the approximate distributional limit of *A. longipilis* in Argentina according to Pardiñas et al. (2003, 2008), Udrizar Sauthier (2009), and U. Pardiñas (Centro Nacional Patagónico, Argentina; pers. comm.). The numbers on the map represent sampled localities (see Appendix I for details).

Neuquén (34°–37°S), but apparently crosses to the Chilean side at about the latitude of the province of Talca (35°S—Osgood 1943). In the Temperate Forests ecoregion we distinguish *A. l. apta* (Osgood, 1943), which ranges from the coast of Concepción southward to Los Ríos and Los Lagos regions (36°–42°S, Fig. 1). *A. l. castaneus* (Osgood, 1943) is restricted to Mocha Island off the coast of Bío-Bío region (38°S). *A. l. moerens* (Thomas, 1919) might not occur in Chile but is found in forests on the Argentinean side and next to the Chilean border at Lake Nahuel Huapi (41°S). In the transition area to the Patagonian Forests (41°–47°S) *A. l. suffusa* (Thomas, 1903) is found in the Aysén region and contiguous areas of Argentina, whereas *A. l. nubila* (Thomas, 1929) is found in the Patagonian Forests and steppe areas of the Magallanes region and adjacent Argentina (47°–52°S). *A. l. francei* (Thomas, 1908) is restricted to Tierra del Fuego (53°S). Two other taxa are recognized by Mann (1978) as subspecies of *A. longipilis*: *A. l. sanborni* (Osgood, 1943), distributed in the Temperate Forests of Los Ríos and Los Lagos regions including the Chiloé Island (39°–44°S); and *A. l. lanosa* (Thomas, 1897) from the vicinities of Punta Arenas and Tierra del Fuego in Patagonia (53°S). Osgood (1943), Musser and Carleton (2005), Iriarte (2008), and Muñoz-Pedreros and Yañez (2009) considered *A. sanborni* a valid species, although Spotorno et al. (2000) considered it a synonym of *A. longipilis* based on a single specimen and partial sequences of the cytochrome-*b* gene (*Cytb*). Yañez et al. (1978), Reise and Venegas (1987), Tamayo et al. (1987), Galliari and Pardiñas (1999), Musser and Carleton (2005), and Teta et al. (2006) recognized *A. lanosa* as a species. A recent work based on nucleotide sequences, cytogenetics, and morphology validates the specific status of *A. lanosa* and shows it to be closely related to *A. longipilis* (Feijoo et al. 2010).

The major goal of this paper was to evaluate systematic relationships in most of the subspecies of *A. longipilis* throughout its geographic range, between 30° and 52°S in Chile. We specifically evaluated the systematics of most subspecies within recognized ecogeographic regions of the country. We also evaluated the relationships of *A. sanborni* with respect to *A. longipilis*, because several current authors recognize the former as a species based on morphology, and no molecular phylogenetic analysis is yet available. To accomplish these goals we sequenced the complete mitochondrial *Cytb* and intron 7 of the nuclear β-fibrinogen gene (FGB).

Materials and Methods

Tissues and specimens analyzed.—Voucher specimens for the individuals sequenced in this study were deposited in the Colección de Flora y Fauna ''Profesor Patricio Sánchez Reyes'' (SSUC), Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile, and the Museum of Southwestern Biology (MSB), Department of Biology, University of New Mexico, Albuquerque, New Mexico. Tissues and other data associated with each specimen were cross-referenced directly to each voucher specimen and stored in the collection using a special field catalog number, the NK number used by the SSUC and MSB; MUSA corresponds to ''Museo de Historia Natural de la Universidad Nacional de San Agustín de Arequipa,'' Arequipa, Perú, and ER to the field catalog of Enrique Rodríguez. A detailed list of the specimens sequenced per locality is given in Appendix I. We followed guidelines of the American Society of Mammalogists during the collection and handling of the animals used in this work (Gannon et al. 2007). The a priori assignment of subspecies for the specimens collected along the geographic range of *A. longipilis* followed the geographic distribution proposed by Osgood (1943; Appendix I). Thus, this a priori assignment was evaluated through phylogenetic analyses. The localities included in our study are very close to the type localities of some Chilean and Argentinean forms

(Appendix I), and for *A. l. francei* we sampled the type locality (Porvenir, Tierra del Fuego). The scientific names used in this paper follow the new nomenclature for *Abrothrix* because this genus is now considered a feminine genus; thus, *olivacea* instead of *olivaceus*, *lanosa* instead of *lanosus*, and *suffusa* instead of *suffusus* (Patterson et al., in press).

Nucleotide sequence analyses.—DNA was extracted from frozen liver samples treated with the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin). The complete *Cytb* (1,144 base pairs [bp]) was amplified for 79 individuals representing 17 localities throughout the range in Chile. In addition, we amplified the nuclear intron 7 of FGB (634 bp) for 27 of the above 79 individuals from 12 localities. Primers used to amplify *Cytb* were 14724 (L—Irwin et al. 1991) and MVZ14 (H—Smith and Patton 1993), and the thermal cycle was performed using the following protocol: initial denaturation for 5 min at 95°C, followed by 32 cycles of 93°C (1 min 30 s), 46°C (1 min 5 s), and 72°C (2 min). A final extension at 72°C for 5 min terminated the reaction. Primers to amplify FBG were β17-mammL and βfib-mammU (Matocq et al. 2007), and the thermal cycle was performed using the following protocol: initial denaturation for 5 min at 94°C, followed by 28 cycles of 94°C (1 min), 64°C (15 s), and 72°C (40 s). The final extension was at 72°C for 4 min. Double-stranded polymerase chain reaction products were purified with Qiaquik (Qiagen, Valencia, California). Cycle sequencing (Murray 1989) was performed using primers 14724, MVZ14, and 15162 (Irwin et al. 1991) for *Cytb*, and β17-mammL and βfib-mammU for FGB, labeled with the Big Dye Terminator kit (Perkin Elmer, Norwalk, Connecticut). Sequencing reactions were analyzed on an Applied Biosystems Prism 3100 automated sequencer (Applied Biosystems, Foster City, California). Sequences were aligned using the CLUSTAL_X program (Thompson et al. 1997) and by eye. All sequences have been deposited in GenBank under accession numbers GU564005–GU564084 and HM004435 for *Cytb* and GU564085–GU564113 for FGB.

Phylogenetic analyses.—Phylogenetic analyses were conducted using maximum-parsimony, maximum-likelihood, and Bayesian methodology on the *Cytb*, FGB, and concatenated *Cytb*–FGB sequences. For maximum parsimony we used PAUP* 4.0 b10 (Swofford 2002), treating all characters as unordered with 4 possible states (A, C, G, T) and using only those characters that were phylogenetically informative. For parsimony, a heuristic search was performed with 500 random additions and branch swapping via tree-bisection-reconnection (Nei and Kumar 2000). A strict consensus tree was estimated when more than one equally parsimonious tree was obtained, and we obtained the consistency index (CI) and the retention index (RI) for the most-parsimonious tree. The reliability of nodes was estimated by nonparametric bootstrapping (Felsenstein 1985) with 1,000 pseudoreplications. Maximum-likelihood searches were performed with the TREEFINDER version of October 2008 (Jobb 2008). We selected the best-fitting model of nucleotide substitution using the corrected Akaike information criterion (AIC_c—Akaike 1974) in TREEFINDER. We evaluated support for the nodes with 1,000 bootstrap replicates (Felsenstein 1985). For *Cytb* sequences the AIC_c identified the TIM + Γ model (Tavaré 1986) as the best model of base substitution. The gamma shape parameter was = 0.1554, and the proportions of nucleotides were A = 0.2806, C = 0.2867, G = 0.1287, and T = 0.3038. For the FGB sequences the AIC_c identified the GTR + I model (Tavaré 1986) of nucleotide substitution, the percentage of invariant sites was 0.9239, and the proportions of nucleotides were A = 0.2854, C = 0.1868, G = 0.2125, and T = 0.3153. For the concatenated sequences the AIC_c identified the GTR + Γ as the substitution model. The gamma shape parameter was 0.10, and the proportions of nucleotides were A = 0.2816, C = 0.2496, G = 0.1595, and T = 0.3091. Sequences also were analyzed in a Bayesian framework to estimate the posterior probabilities of phylogenetic trees. Ten million phylogenetic trees were generated, sampling every 1,000 trees to assure that successive samples were independent. The first 1,000 trees of the sample were removed to avoid including trees before convergence of the Markov chain. Given that we used 2 independent molecular markers, we applied a general likelihood-based mixture model as described by Pagel and Meade (2004, 2005), based on the general time-reversible (GTR) model (Rodríguez et al. 1990) of sequence evolution. This model accommodates cases in which different sites in the alignment evolved in qualitatively distinct ways but does not require prior knowledge of these patterns or partitioning data. These analyses were conducted using the BayesPhylogenies software (http://www.evolution.rdg.ac.uk/SoftwareMain.html). To find the best mixture model of evolution we estimated the number of GTR matrices by using a reversible-jump Markov chain Monte Carlo method (Pagel and Meade 2006). The reversible-jump Markov chain Monte Carlo method visits the different mixtures of GTR matrices in proportion to their posterior probabilities, ''jumping'' from simple to complex models or vice versa, making a direct estimate of the support of 1GTR, 2GTR, 3GTR, and so on. Only the combination of matrices with the fewest number of parameters that significantly increased the likelihood was used (1GTR + Γ for *Cytb* data; 2GTR + Γ for concatenated data) to compute a 50% majority rule consensus tree. The percentage of samples that recover any particular clade on this tree represents the posterior probability of that clade; these are the p values, and $p \geq 95\%$ was considered evidence of significant support for a clade (Huelsenbeck and Ronquist 2001). Phylogenetic trees were rooted with the outgroup criterion using *A. lanosa* and *A. jelskii*, because these taxa constitute part of the sister clade to *A. longipilis* (Smith and Patton 1999; Feijoo et al. 2010).

Results

Phylogeny based on Cytb sequences.—The maximum-parsimony analysis generated 192 equally parsimonious trees with 547 steps, CI = 0.6307, and RI = 0.9564. The strict consensus tree is similar to that generated with maximum-likelihood and Bayesian methods, with high bootstrap and

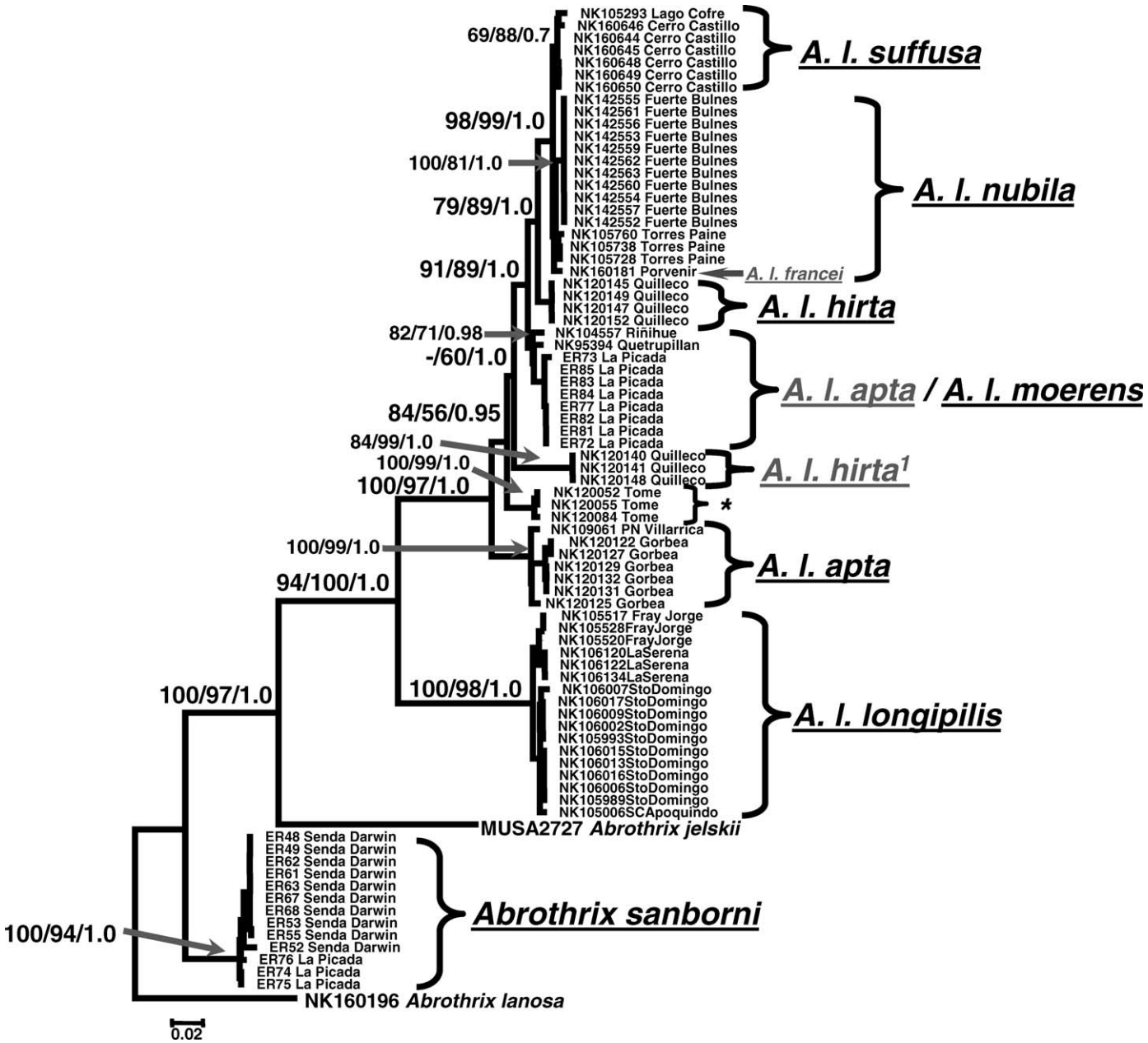

Fig. 2.—Phylogeny of *Abrothrix longipilis* and *A. sanborni* based on the complete cytochrome-*b* mitochondrial gene. Numbers on the nodes represent parsimony and likelihood bootstrap values (1,000 pseudoreplications for each) and the posterior probability. Names in gray reflect the taxonomy of Osgood (1943); those in black show our proposition. An asterisk (*) represents a taxonomically unnamed population, and 1 denotes a form sympatric with, but genealogically distinct from, *A. l. hirta* (sensu stricto).

posterior probability support values; thus, we present a single tree as shown in Fig. 2. The 1st split in the phylogenetic tree recovered a well-differentiated and supported clade that includes specimens from La Picada and Senda Darwin, both from the Temperate Forests ecoregion in Chile representing *A. sanborni* (Fig. 2). Three individuals from La Picada (Vicente Pérez Rosales National Park, 41°S) fall in the *A. sanborni* clade together with those of Senda Darwin (Chiloé Island), whereas other specimens from La Picada were recovered in the *A. l. moerens* clade (Fig. 2). Next in the tree is a series of clades representing *A. longipilis*, where the grouping that included specimens of the northernmost subspecies (*A. l. longipilis*) is strongly differentiated from other subspecies (Fig. 2). The latter northern clade included populations from the Mediterranean ecoregion: Fray Jorge, La Serena, Santo Domingo, and 1 specimen from San Carlos de Apoquindo. The next part of the phylogenetic tree recovered southern subspecies *A. l. apta*, *A. l. moerens*, *A. l. hirta*, *A. l. suffusa*, and *A. l. nubila*. The former 2 are from the southern temperate forests, whereas the latter 2 appeared as slightly differentiated in the Patagonian forests of mainland Chile (Fig. 2). *A. l. hirta*, represented by specimens from the Andean locality of

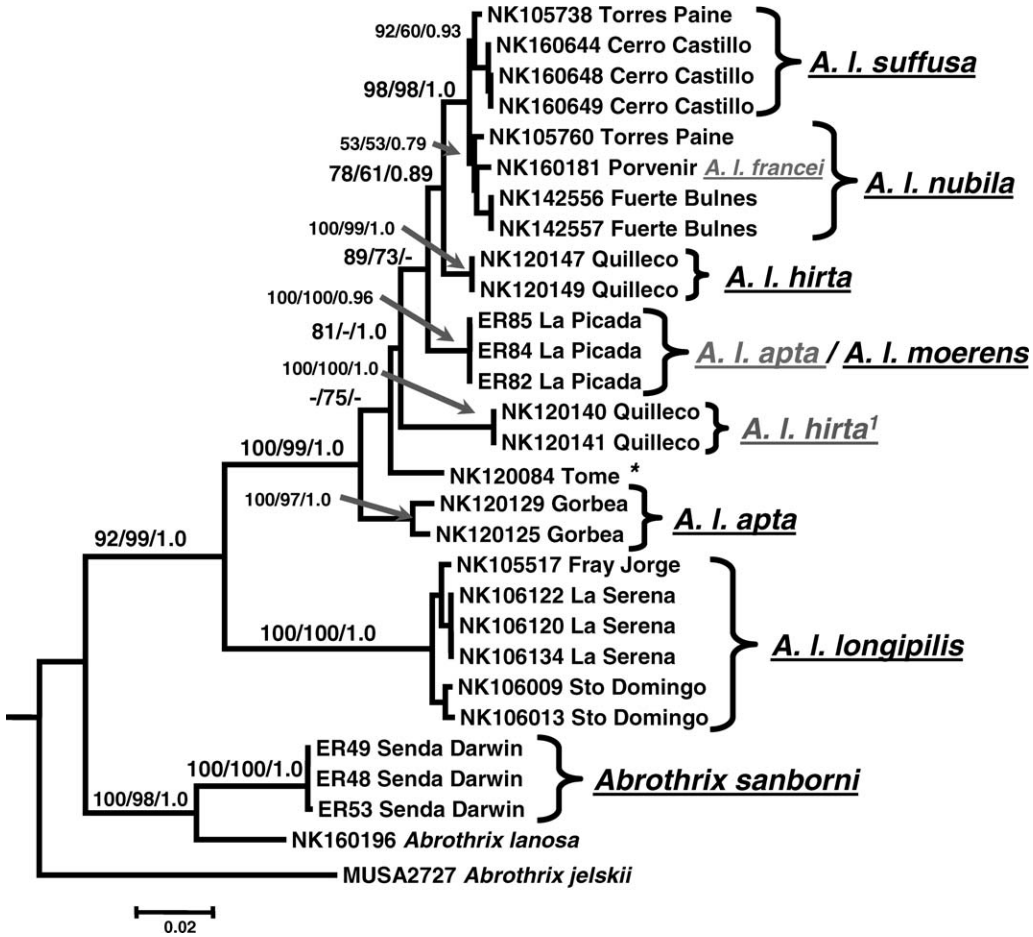

Fig. 3.—Phylogeny of *Abrothrix longipilis* and *A. sanborni* based on concatenated cytochrome-*b* and β-fibrinogen intron sequences. Numbers on the nodes represent parsimony and likelihood bootstrap values (1,000 pseudoreplications each) and the posterior probability. Names in gray reflect the taxonomy of Osgood (1943); those in black show our proposition. An asterisk (*) represents a taxonomically unnamed population, and 1 denotes a form sympatric with, but genealogically distinct from, *A. l. hirta* (sensu stricto).

Quilleco in the Bío-Bío region (about 37°S), appeared as polyphyletic. Regarding *A. l. francei*, the single specimen trapped 25 km southeast of Porvenir in Tierra del Fuego, the type locality of this taxon, appeared as indistinguishable from *A. l. nubila* from the continent (see ''Discussion''). Finally, the phylogeny (parsimony and likelihood analyses) based on FGB was similar to and recovered the same grouping of subspecies as that of *Cytb* and therefore is not shown here.

Phylogeny based on selected concatenated sequences.—The maximum-parsimony analysis generated 12 equally parsimonious trees 490 steps long, CI = 0.6796, and RI = 0.8865. The parsimony strict consensus, the likelihood, and Bayesian analyses recovered similar trees (Fig. 3), comparable to that obtained with *Cytb*. The trees recovered a basal clade representing *A. sanborni* from Senda Darwin (Chiloé) and clades corresponding to *A. l. longipilis*, *A. l. moerens* (La Picada), *A. l. apta* (locality of Gorbea), the polyphyletic *A. l. hirta*, and the Patagonian forms *A. l. suffusa* and *A. l. nubila* (Fig. 3).

It is important to notice the variable position of outgroups when analyzing *Cytb* and concatenated sequences. In the 1st data set *A. jelskii* constitutes the 1st outgroup of *A. longipilis*, and these lineages form a polytomy between *A. sanborni* and *A. lanosa* at the base of the tree. In the concatenated analysis *A. jelskii* was recovered at the base of the tree, and *A. lanosa* was the outgroup of *A. sanborni*.

Discussion

The 1st major result of our work is the validation of *A. sanborni* as a good species based on molecular data with strong bootstrap and posterior probability support values. Although some authors recognized *A. sanborni* as a valid species (Feijoo et al. 2010; Gallardo et al. 1988; Osgood 1943; Spotorno 1992) based on morphology, others considered it a synonym of *A. longipilis* based on a single specimen and partial *Cytb* sequences (Spotorno et al. 2000). Our work makes an important contribution through sampling and molecular analyses to reaffirm the taxonomic status of this form. The clade that recovered *A. sanborni* included specimens from Senda Darwin in the Chiloé Island (Ancud County) and La Picada in Vicente Pérez Rosales National

Park (Llanquihue Province). These localities are more than 150 km apart and separated by a marine barrier. How can we explain the association between these populations? The most-parsimonious hypothesis is the connection between Chiloé Island and mainland Chile during glacial periods of the Pleistocene (Clapperton 1994; Glasser et al. 2008). The Chacao channel, which currently separates the island and the continent, was a land bridge that allowed the passage of mainland biota to the island and vice versa. Given that the LGM occurred about 18,000 years ago (Glasser et al. 2008), it seems likely that not enough time has elapsed for differentiation between populations from mainland and island localities. A similar pattern of relationships between biota of the mainland and Chiloé has been found in lizards of the genus *Liolaemus* (Vidal 2007), the freshwater crayfish *Samastacus spinifrons* (Castro et al., in litt.), the sigmodontine olive field mouse *Abrothrix olivacea* (Rodríguez-Serrano et al. 2006), and the marsupial *Dromiciops gliroides* (Himes et al. 2008).

Regarding the disagreement between the phylogenetic position of the outgroups used in the mitochondrial and concatenated data, this could be due to the short time span of *Abrothrix* diversification (1 million years—Rodríguez-Serrano et al. 2008b). If rapid diversification favored incomplete lineage sorting of nuclear genes with higher effective population sizes than mitochondrial ones (Avise 1989), gene trees based on concatenated data might not produce accurate species trees. Further studies on the phylogenetic relationships of Abrotrichini based on different nuclear markers could improve our understanding of diversification in these sigmodontines.

The phylogeny of *A. longipilis* recovered several clades that represent mostly traditional subspecies (Mann 1978; Osgood 1943). However, *A. l. longipilis*, the type for *A. longipilis*, is strongly differentiated from other geographic races (Figs. 2 and 3). The northernmost subspecies is represented here by specimens from the coast of Chile such as Fray Jorge, La Serena, and Santo Domingo, the former 2 localities part of the Coquimbo region where the type form occurs (Osgood 1943; Waterhouse 1837). According to Osgood (1943), *A. l. longipilis* is distributed mainly along the coast of Coquimbo and Valparaíso regions. However, the clade recovering *A. l. longipilis* also included a pre-Andean specimen from the cordillera of Santiago, San Carlos de Apoquindo. Thus, *A. l. longipilis* is a taxon restricted mostly to the central Chilean Mediterranean ecoregion, with populations distributed along coastal areas and the central valley as hypothesized in our phylogeny. The *A. l. longipilis* clade showed a level of differentiation comparable to that between *A. sanborni* and the other subspecies. The sequence divergence for *Cytb* between *A. l. longipilis* and the other subspecies in our analysis is about the same order of magnitude (11%) as that between *A. sanborni* and other subspecies (13%). In contrast, sequence divergence between those subspecies is 3.5–4%, in the range of distance values reported for abrotrichine subspecies by Smith and Patton (1999).

Morphologically, *A. l. longipilis* is described and differentiated from other subspecies as a ''rather large, heavy-bodied mouse with small, thinly haired ears, long, loose pelage, color mainly light brownish rather coarsely mixed with grayish sides … the sides only slightly or not at all more grayish than the back, under parts wholly gray; feet and tail dark'' Osgood (1943:184). The main features that differentiate the southern subspecies from *A. l. longipilis* are the more distinct dorsal and ventral coloration of the pelage, the predominantly bicolored tail, a narrower braincase, and a smaller size (Mann 1978; Osgood 1943). Thus, the phylogenetic information from mitochondrial and nuclear markers, along with external morphology, provides strong evidence to consider the northernmost form as species rather than a subspecies. Then the name *Abrothrix longipilis* (sensu stricto) should be restricted to the form captured by Darwin and described by Waterhouse (1837). The oldest available name for southern populations is *Abrothrix hirta* (Thomas, 1895). Thomas (1929) proposed that southern populations should be named *A. hirta hirta*, *A. hirta moerens*, *A. hirta suffusa*, and *A. hirta nubila*. Finally, within the *A. l. longipilis* clade, we observed a subdivision between specimens from the Coquimbo region (Fray Jorge and La Serena) and those of Santo Domingo in the coast of Valparaíso region. This split suggests 2 geographic races or subspecies within this taxon, 1 in the northern (Coquimbo and Valparaíso) and another in the southern (Santiago) range of *A. longipilis*. Further cranial and internal morphological studies will be necessary to assess our systematic proposition.

The structured phylogenetic pattern found in *A. longipilis* (sensu lato) along its geographic range contrasts with that found in another abundant sigmodontine in Chile, *Oligoryzomys longicaudatus*. The latter species shows low levels of morphologic and genetic variability along almost the same range as *A. longipilis*. No subspecies of *O. longicaudatus* are recognized, suggesting a strong gene flow among populations (Gallardo and Palma 1990; Palma et al. 2005). *A. longipilis* (sensu lato) is less vagile and has a smaller home range (Murúa and González 1986; Murúa et al. 1986) than *O. longicaudatus*, which might explain its more structured geographic pattern.

Abrothrix l. hirta appeared as polyphyletic with mitochondrial and concatenated sequences. The specimens represented here are from the Andes area, and we hypothesize that they represent 2 lineages isolated by Pleistocene glaciation events. During the LGM a large part of Patagonia and temperate forests in the south were covered by ice, and these masses advanced toward the north along the Cordillera de los Andes (Heusser et al. 1999; Holling and Schilling 1981). In central Chile ice masses descended to around 1,000–1,300 m (Clapperton 1990, 1994; Rabassa and Clapperton 1990). This geologic event could have isolated populations of *A. l. hirta* (represented here by the population of Quilleco; 37°S) and triggered the differentiation of 2 lineages, an eastern lineage more closely related to *suffusa* and *nubila* and a western lineage (indicated by an asterisk [*] in Fig. 2). This western

lineage was recovered in a clade together with representatives from Gorbea and coastal forms from Tomé (Fig. 3). Both lineages might have entered into secondary contact after genetic divergence in glacial retreats. We recognize as *A. l. hirta* those specimens that in our phylogeny were recovered closely related to the clade that joined *suffusa* and *nubila*, because Osgood (1943:191) stated that ''*hirta* is most similar to *suffusa*.'' Regardless, additional localities in Argentina and Chile must be explored to delimit the geographic distribution of *hirta*.

The clade that we propose as *A. l. moerens* is represented in our analysis by specimens from the eastern Chilean localities of La Picada, Quetrupillan, and Riñihue in the southern portion of the country (about 40°S). Following Osgood (1943:190), this subspecies ''may not occur in Chile … but is found very near the border'' (with Argentina), and Mann (1978) did not include it in his list of subspecies of *A. longipilis* for Chile. However, the specimens that we hypothesize as *A. l. moerens* occur in pre-Andean areas in the southern Temperate Forests only a few kilometers west of Nahuelhuapi Lake (type locality in Argentina). Osgood (1943) stated that morphological features of *moerens* (e.g., body size and cranial morphology) resemble those of *hirta* and *suffusa* rather than any other Chilean subspecies. The *Cytb* phylogeny grouped what we considered *A. l. moerens* close to *hirta* and *suffusa* as Osgood (1943) proposed. *A. l. apta* also occupies the southern portion of Chile, from Bío-Bío southward to the Lakes region (Llanquihue, Chiloé), although ranging in coastal and central valley areas. According to original descriptions, *A. l. apta* is a smaller form than the septentrional subspecies *A. l. longipilis*. The *terra tipica* or ''nearly typical'' area of *A. l. apta* includes La Picada and Riñihue (Osgood 1943), where we found *A. l. moerens*. Osgood (1943) treats the majority of populations of southern Chile as *A. l. apta* with skull morphology similar to that of *moerens*.

In the Chilean Patagonia (Aysén and Magallanes regions) 2 subspecies of *A. longipilis* (sensu lato) have been recognized according to traditional literature (Mann 1978; Osgood 1943). We detected subtle differences at the molecular level between *A. l. suffusa* and *A. l. nubila*, and the geographic differences between these 2 forms might be explained by the ecogeographic characteristics of the area. *A. l. suffusa* is restricted to the transition between Valdivian and Patagonian forests in the Aysén region (here represented mostly by specimens from Cerro Castillo), whereas *A. l. nubila* is restricted to the Patagonian steppe and forests. We have only 1 specimen from Tierra del Fuego (25 km southeast of Porvenir) that joined the *nubila* clade, showing no differentiation from mainland forms. This is an important issue given that the locality from which this sample was obtained (Tierra del Fuego) corresponds to the type locality of *A. l. francei*. Osgood (1943) proposed that *A. l. francei* could be identical with *A. l. nubila*, and differences with mainland forms could be due to preservation techniques of the type species.

Three specimens from Tomé (36°S), on the coast of the Bío-Bío region, appeared as differentiated in the phylogenetic trees, representing perhaps some relictual forms of *A. l. hirta* or *A. l. apta* as suggested by their close relationships to the latter 2 forms in the concatenated tree. The specimens from Tomé might have reached coastal areas during glacial cycles of the Pleistocene. Palynological evidence has shown that the Coastal Cordillera constituted a glacial refuge for the biota (Villagrán and Armesto 2005; Villagrán and Hinojosa 1997).

Our results allow us to confirm at the molecular level the validity of *A. sanborni* as a different species than *A. longipilis*. The range of the former is in the Temperate Forests of southern Chile, including Chiloé Island. Special attention should be focused on the taxonomic status of *A. l. longipilis*. The molecular phylogeny suggests that this taxon might constitute a different species, clearly differentiated from the southern forms occurring in Temperate and Patagonian Forests of Chile. Thus, *A. l. longipilis* would be a form restricted to central Chile (the Mediterranean ecoregion), with a larger body size than southern geographic races. A further exhaustive revision of specimens from additional localities in central Chile is necessary to clarify the taxonomic status of *A. l. longipilis*.

Resumen

Evaluamos las relaciones sistemáticas entre la mayoría de las subespecies del roedor sigmodontino de ''pelo largo'' *Abrothrix longipilis* en Chile. Para ello secuenciamos el gen citocromo-*b* completo y 634 pares de bases del intron 7 del gen β-fibrinógeno en especímenes de 17 localidades a lo largo del rango de distribución de *A. longipilis*. Los análisis filogenéticos confirman a *Abrothrix sanborni*, antes considerada una subespecie de *A. longipilis*, como especie válida y diferente de esta última. Se observó una filogenia estructurada en *A. longipilis* que muestra la mayor parte de las razas geográficas tradicionalmente reconocidas para este roedor sigmodontino. Sin embargo, se detectó una fuerte diferenciación entre *A. l. longipilis* de Chile Mediterráneo con respecto a las otras subespecies de los Bosques Temperados y Patagónicos del sur, sugiriendo que la primera podría constituir una especie diferente. Concluímos que eventos históricos (glaciaciones), así como presiones de selección local (diferentes zonas ecogeográficas), podrían explicar los actuales patrones de variación de *A. longipilis* a lo largo de su distribución geográfica.

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APPENDIX I

Specimens analyzed.

Abrothrix longipilis longipilis.—CHILE. *Coquimbo region*. (1) La Serena, Fundo El Hinojal, 30°0′0.68″S, 71°2′23.89″W: NK 106120, 106122, 106134; (2) Fray Jorge National Park, 30°38′18.27″S, 71°39′16.59″W: NK 105517, 105520, 105528. *Valparaíso region*. (3) Santo Domingo, Fundo La Ventolera, 33°44′45.15″S, 71°39′8.38″W: NK 105989, 105993, 106002, 106006, 106007, 106009, 106013, 106015, 106016, 106017. *Santiago*, *Metropolitan region*. (4) San Carlos de Apoquindo, 33°28′8.27″S, 70°29′18.53″W: NK 105006.

Abrothrix longipilis apta.—CHILE. *Bío-Bío region*. (5) Tomé, Lloicura, 36°41′21.48″S, 72°45′41.39″W: NK 120052, 120055, 120084. *Araucanía region*. (6) Gorbea, Fundo La Aguada, 39°3′36″S, 72°24′0″W: NK 120122, 120125, 120127, 120129, 120131, 120132. *Los Ríos region*. (7) Fundo La Montaña, Riñihue, 39°48′14.68″S, 72°19′15.20″W: NK 104557. *Los Lagos region*. (8) Parque Nacional Vicente Pérez Rosales, La Picada, 41°02′S, 72°30″W: ER 72, 73, 77, 81–85.

Abrothrix longipilis hirta.—CHILE. *Bío-Bío region*. (9) Hacienda San Lorenzo, Quilleco, 37°28′0.00″S, 71°57′59.99″W: NK 120140, 120141, 120145, 120147, 120148, 120149, 120152.

Abrothrix longipilis moerens.—CHILE. *Araucanía region*. (10) Fundo Hermanos García, Quetrupillán, 39°26′59.28″S, 71°47′58.19″W: NK 109061; (11) Quetrupillán, 39°25′38.1″S, 71°47′16.37″W: NK 95394.

Abrothrix longipilis suffusa.—CHILE. *Aysén region*. (12) Cerro Castillo, 46°08′32.39″S, 72°09′16.39″W: NK 160644–160646, 160648–160650; (13) Lago Cofré, Río Ibañez, 46°11′22.55″S, 72°46′32.98″W: NK 105293.

Abrothrix longipilis nubila.—CHILE. *Magallanes region*. (14) Parque Nacional Torres del Paine, 51°16′49.99″S, 73°05′31.99″W: NK 105728, 105738, 105760; (15) Fuerte Bulnes, 53°37′43.09″S, 70°55′14.49″W: NK 142552–142557, 142559–142563.

Abrothrix longipilis francei.—CHILE. *Magallanes región*. (16) Tierra del Fuego, Porvenir, 53°27′33.8″S, 70°15′36.9″W: NK 160181.

Abrothrix sanborni.—CHILE. *Los Lagos region*. (17) Parque Nacional Vicente Pérez Rosales, La Picada, 41°02″S, 72°30″W: ER 74–76; Senda Darwin, Chiloé, 41°53′01.71″S, 73°39′59.50″W: ER 48, 49, 52, 53, 55, 61–63, 67, 68.

Abrothrix lanosa.—CHILE. *Magallanes region*. Tierra del Fuego, Porvenir, 53°27′33.8″S, 70°15′36.9″W: NK 160196.

Abrothrix jelskii.—PERU. *Departament of Puno*. Carabaya Province, Hacienda Aricoma, 14°15′11.50″S, 69°45′3.09″W, 4,710 m: MUSA 2727.