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RESOURCE ARTICLE

DNA barcodes highlight genetic diversity patterns in rodents from lowland desert and Andean areas in Argentina

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Abstract

Rodents are an important component of South America fauna. Their high diversity has motivated researchers to continually review their taxonomy, genetic diversity, species limits, and phylogenetic relationships. Here, we applied DNA-barcodes for assessing the taxonomic and genetic diversity in the two major lineages of South American rodents: caviomorphs and sigmodontines. We analysed 335 COI barcodes in 34 morphologically determined species from 39 localities along central Andes and arid lands of Argentina. Neighbour-joining and maximum likelihood reconstruction provided clear separation between species. The Barcode Index number and Bayesian Poisson tree processes were used to confirm concordance between sequence clusters and species designations by taxonomy. We found deep divergence within the *Phyllotis xanthopygus* species complex, with distances up to 13.0% between geographically separated lineages. Minor divergences (3.30% and 2.52%) were found within *Abrothrix hirta*, and *Tympanoctomys barrerae*, respectively, with differentiation in their genetic lineages. Also, we documented geographically separated clusters for *Akodon spegazzinii* and *A. oenos* with up to 2.3% divergence, but clustering methods failed to distinguish them as different species. Sequence results show a clear barcode gap with a mean intraspecific divergence (0.56%) versus a minimum nearest-neighbour distance averaging (10.1%). Distances between congeneric species varied from 4.1 to 14%, with the exception of two related forms within *Euneomys* and the sister species *Akodon spegazzinii* and *A. oenos*. This study constitutes a substantial contribution to the global barcode reference library. It provides insights into the complex phylogeographic patterns and speciation scenarios in rodents, while highlighting areas that require in-depth taxonomic and integrative research.

KEYWORDS
Caviomorpha, COI, DNA barcoding, Sigmodontinae, South America, species identification

1 | INTRODUCTION

Rodentia is the largest order of mammals, with over 2600 species representing approximately 40% of the living mammal species (Burgin et al., 2018; D'Elía et al., 2019). Its great taxonomic and phenotypic diversity place them as a very distinctive placental order, and one of the most successful radiations in mammal evolution (Fabre et al., 2012; Parada et al., 2013; Upham & Patterson, 2012).

In South America, the rodent fauna comprises two main groups: the caviomorphs and the sigmodontines, which differ markedly in the pathways and timelines of their colonization to the continent (Parada et al., 2013; Rowe et al., 2010). The New World Hystricognath or Caviomorph rodents (guinea pigs, new world porcupines, chinchillas, degus, and allies) are the most divergent lineage of New World rodents. According to relaxed molecular clock estimates, they colonized South America between 45.4 and 36.7 million years ago (Ma) via trans-Atlantic migration from Africa (Poux et al., 2006); however, the oldest known fossil records are from the Middle Eocene of Peru (Antoine et al., 2011). The mostly autochthonous adaptive radiation of caviomorphs within South America has resulted in c. 246 living species, arranged in four superfamilies, 13 families, and 56 genera, inhabiting a wide spectrum of landscapes, elevations, and habitats (Fabre et al., 2012; Ojeda et al., 2015, 2016; Upham & Patterson, 2012). By contrast, sigmodontine rodents (cotton and rice rats, grass, leaf-eared and vesper mice, and allies) colonized and radiated in South America more recently, during the Late Miocene before the completion of the Isthmus of Panama (3 Ma) (Parada et al., 2013, 2015). The oldest record of the South American sigmodontines come from the late Miocene in Argentina (Nasif et al., 2009; Prevosti & Pardiñas, 2009; Verzi & Montalvo, 2008). Despite having more recent radiation history, the subfamily is highly speciose, with 87 living genera and more than 400 extant species, and is one of the most taxonomically complex groups of New World mammals (D'Elía & Pardiñas, 2015). Like caviomorphs, sigmodontines inhabit a diverse range of ecosystems and habitats, from sea level to nearly 6700 m, including deserts, salts flats, tropical and temperate forests, scrublands, savannas, steppes, and high elevation grasslands (D'Elía & Pardiñas, 2015). Both rodent lineages are important components of mammal communities across the continent.

The taxonomy, distribution, and phylogeny of this remarkable neotropical rodent diversity is a matter of continuous debate and revision. New taxonomic discoveries also continue with new genera and species being constantly described (D'Elía et al., 2019). It will probably take several years to approach completion of the taxonomic inventory of extant rodent diversity in South America (D'Elía et al., 2019; Fabre et al., 2015; Lessa et al., 2014). Much of this diversity is cryptic (i.e., impossible to assess using morphological criteria alone); however, accurate species identification is essential in taxonomic studies and beyond (e.g., biogeography, community ecology, conservation, epizootology, ecological monitoring, etc). This has led to a recent increase in the use of molecular data (mitochondrial and nuclear genes) to assist morphology in delimiting species. DNA-based taxon delimitation significantly clarified species boundaries, resulting either in the validation or synonymization of several nominal forms (e.gD'Elía et al., 2008, 2015; Jayat et al., 2016; Teta et al., 2017, 2020), and has been crucial to accelerate and improve integrative taxonomic work (Padial et al., 2010). In particular, mitochondrial genes have been used to help resolve or assess the alpha-taxonomic structure of mammals, due to the relative ease of amplification and sequence alignment, combined with adequate rate of divergence to provide species-level resolution in short (<1000 bp) fragments. The mitochondrial cytochrome *b* (cyt *b*) gene has been traditionally used in mammalian species discrimination (Bradley & Baker, 2001; Tobe et al., 2010). More recently, a short standardized (~650 bp) DNA fragment from the mitochondrial 5' end of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene has been adopted as the standard marker for DNA-based species diagnosis in metazoan species (Hebert et al., 2003; Hebert et al., 2003). This initiative, worldwide known as "DNA barcoding", has emerged as a widely accepted and powerful tool for specimen identification, because of its enhanced focus on standardization and data validation, and for generating hypotheses of new species (Goldstein & DeSalle, 2010; Teletchea, 2010).

The DNA barcoding has gained recognition due to the utilization of standardized analytical methodologies across large taxonomic entities, and as a result of developing the standard data repository and analytical platform – the Barcode of Life Data system (BOLD) (Ratnasingham & Hebert, 2007). This approach assumes that intraspecific variation in COI is usually lower than interspecific differences, and that both ranges do not overlap (Hebert, Cywinska, et al., 2003; Meyer & Paulay, 2005). DNA barcode studies using COI in several animal groups have been shown to be a powerful tool for species identification (e.g., Lepidoptera [Hajibabaei et al., 2006]; amphibians [Smith et al., 2008]; fishes [Díaz et al., 2016; Ward et al., 2005], and lizards [Corbalán et al., 2016]).

So far, a limited number of studies have investigated COI sequence divergence in South American rodents and these studies have been confined to the Neotropics (Andrade et al., 2021; Borisenko et al., 2008; Da Cruz et al., 2019; Müller et al., 2013; Pinto et al., 2018). Here, we assess the performance of DNA barcodes in discriminating among recognized species, assessing the patterns of their genetic diversity and taxonomy in the major lineages of South American caviomorph and sigmodontine rodents, from dry and humid Andes regions and the arid lands of west-central Argentina. We also provide the first DNA barcode reference library for these groups of New World mammals.

2 | MATERIALS AND METHODS

2.1 | Specimens examined and studied area

We examined 335 specimens (297 sigmodontines; 38 caviomorphs), representing 34 species (26 sigmodontines; 8 caviomorphs) (Table S1) from 39 localities along the central Andes, the lowland Monte Desert, and the Patagonian steppes, in west-central Argentina (Figure 1, Data S1). Some specimens were capture and handled according to the recommendations of the American Society of Mammalogists for the use of wild mammals in research (Sikes & The Animal Care & Use Committee of the American Society of Mammalogists, 2016). These specimens were collected under scientific collection permits from: Dirección de Recursos Renovables, Mendoza Province (No 461–1–04–03873); Secretaría de Ambiente y Desarrollo sustentable, San Juan Province (No 1300–655–17); Ministerio de Medio Ambiente, San Luis Province (Res. No 164–2013); Áreas

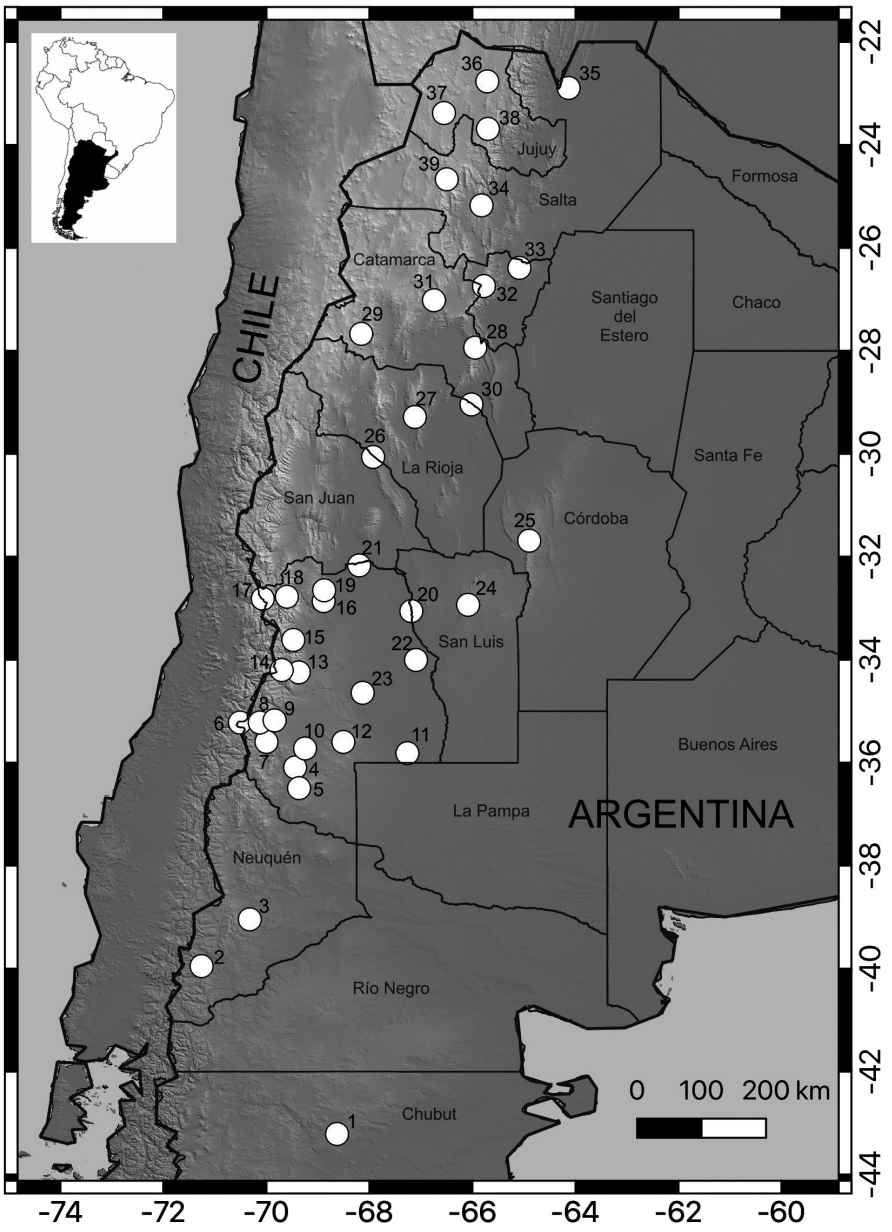

FIGURE 1 Map of collection sites for specimens examined in this study, along the central Andes, lowland Monte Desert, and Patagonia, Argentina. For localities references see Table S2

Naturales Protegidas Neuquén Province (No 7103-000449/17). Also, we used tissue samples ethanol-preserved (96%) of vouchers specimens housed in the following mammal collections: Colección Nacional de Mastozoología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-Ma), Ciudad Autónoma de Buenos Aires; Colección de Mamíferos Lillo (CML), Universidad Nacional de Tucumán, Tucumán; Colección de Mamíferos del Instituto Argentino de Investigaciones de Zonas Áridas (CMI), Mendoza; and Colección de Mamíferos del Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de La Rioja (CRILAR), La Rioja. Tissue samples used in this study are housed in the Mammal collection of Instituto Argentino de Investigaciones de Zonas Áridas (CMI)-(IADIZA), CCT Mendoza CONICET, Argentina. We included one sequence of *Peromyscus maniculatus* (Neotominae) and one sequence of *Thryonomys swinderianus* (Thryonomyidae) from published data as outgroups for sigmodontine and caviomorph rodents, respectively.

2.2 | DNA extraction, PCR amplification and sequencing

The 657 bp 5' segment of the cytochrome *c* oxidase subunit I (COI) gene (DNA barcode region) was recovered from 335 specimens using standard high-throughput barcoding protocols (Ivanova et al., 2012). Whole genomic DNA from small (approximately 1–2 mm^3) pieces of ethanol-preserved (96%) muscle or liver was extracted on the Biomek FX liquid handling station using 1.0 μm PALL glass fibre media filter plates following the protocol of Ivanova et al. (2006). PCR amplification using M13-tailed primer cocktails C_FishF1t1/C_FishR1t1 (Ivanova et al., 2007) was done as described in Clare et al. (2007) and Borisenko et al. (2008). The 12.5 μl PCR reaction mixes included 6.25 μl of 10% trehalose, 2 μl of ultrapure water, 1.25 μl of 10x PCR buffer, 0.625 μl of $MgCl_2$ (50 mM), 0.125 μl of each primer (0.01 mM), 0.0625 μl of dNTP mix (10 mM),

TABLE 1 K2P genetic divergence values within different taxonomic levels from 297 sigmodontinae specimens

		K2P genetic divergence (%)			
	Comparisons	Minimum	Mean	Maximum	SE
Within species	4493	0.00	1.43	1.68 3.30[a] 13.20[b]	0.00
Within genus	3435	1.08[c]	9.14	14.63	0.00
Within subfamily	36028	14.66	19.85	24.52	0.00

Abbreviation: SE, standard error.

[a]Including *Abrothrix hirta* (see the text, Case 1).

[b]Including the *Phyllotis xanthopygus* species complex as a unique species (see the text, Case 5).

[c]This low value within the genus is due to the case of *Euneomys* and the sister species *Akodon spegazzinii* and *A. oenos* (see the text, Cases 3 and 4).

TABLE 2 K2P genetic divergence values within different taxonomic levels from 38 caviomorph specimens

		K2P genetic divergence (%)			
	Comparisons	Minimum	Mean	Maximum	SE
Within species	170	0.00	0.85	2.52	0.00
Within genus	64	4.78	6.53	9.10	0.02
Within family	56	8.97	10.07	11.36	0.01

Abbreviation: SE, standard error.

0.3125 U of Taq polymerase (Invitrogen), and 2.0 μl of DNA. PCR products were visualized on 2% precast 96-well agarose gels (E-Gels, Invitrogen). Products were labelled by using the BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems, Inc.) as described in Ivanova et al. (2012) and sequenced bidirectionally using an ABI 3730XL capillary sequencer following the manufacturer's instructions. Bidirectional reads were assembled and manually edited in CodonCode Aligner software v. 3.5.2 (CodonCode Corp.). DNA barcode data (COI sequences, chromatogram trace files, and collateral specimen information) are stored in the BOLD System (Ratnasingham & Hebert, 2007) at http://www.barcodinglife.org in the public Data set DS-AODAR21. Sequences have also been deposited in NCBI GenBank. For GenBank accession numbers see Data S1.

2.3 | Sequence alignment and data analysis

Electropherograms were scored using PROSEQ version 2.91 (Filatov, 2002) and aligned using the default parameters of CLUSTAL X (Thompson et al., 1997). Sequence divergences were calculated in the BOLD Workbench and MEGA6 (Tamura et al., 2013) using the Kimura-two- parameter K2P nucleotide substitution model (Kimura, 1980). The K2P model is an appropriate metric to employ when genetic distances are low (Nei & Kumar, 2000) and is one of the simplest and most common models used for describing differentiation among species using COI (Hebert, Ratnasingham, et al., 2003). Because the K2P model is widely employed within.

the DNA barcoding literature, its use allows direct comparison with past barcoding studies.

Comparisons at the species level of the maximum intraspecific genetic distance with the minimum distance to the nearest neighbour were performed using BOLD's Barcoding gap analysis tool.

A neighbour-joining (NJ) tree based on K2P genetic distance were created to provide a graphic representation for the patterning of distance between species using the MEGA X software (Kumar et al., 2018). Node robustness was inferred with 1000 bootstrap replicates. We also performed a maximum likelihood (ML) analysis under the best-fit model of evolution obtained with jModelTest 2.1.4 (Darriba et al., 2012) in PhyML (Guindon et al., 2010) with 1000 bootstrap repetitions. For sigmodontines, the selected model under the Akaike Information Criterion (Akaike, 1974) was (GTR+I+G) with base frequencies A = 0.3484, C = 0.2769, G = 0.0839, T = 0.2909. The proportion of invariable sites was I = 0.5900, and the gamma distribution shape parameter was G = 1.2680. For caviomorphs the selected model under the Akaike information criterion (Akaike, 1974) was (HKY+G) with base frequencies A = 0.3386, C = 0.2311, G = 0.1044, T = 0.3259, and the gamma distribution shape parameter was G = 0.0860. Finally, we used barcode index number (BIN) system (Ratnasingham & Hebert, 2013) and the Bayesian Poisson tree processes (bPTP) (Zhang et al., 2013) as clustering methods to confirm the concordance between sequence clusters and species designations inferred by taxonomy.

3 | RESULTS

COI amplified DNA fragments (~657 bp) were obtained from 297 sigmodontine and 38 caviomorph specimens. No stop codons, insertions, or deletions were found in any of the amplified sequences, suggesting that all of them constitute functional mitochondrial

COI sequences. Genetic distances increased from lower to higher taxonomic levels. For sigmodontines, the mean K2P genetic distance between specimens within species was 1.43%, within genera (between species of the same genus) 9.14%, and within subfamily (between genera) 19.85% (Table 1). The intraspecific mean genetic distance range from of 0.0% to 1.68%, except for the leaf-eared mouse of the *Phyllotis xanthopygus* species complex, where the mean genetic distance was 4.72% and the maximum genetic distance was 13.20%; and for the long-haired grass mouse, *Abrothrix hirta* where the mean genetic distance was 1.68% and the maximum genetic distance was 3.30%. For caviomorphs, the mean K2P genetic distance between specimens of the same species was 0.85%, between species of the same genus was 6.53% (within genera), and within family (between genera) 10.07% (Table 2). The intraspecific mean genetic distance ranges from 0.0% to 1%, except for the red viscacha rat, *Tympanoctomys barrerae*, where the mean genetic distance was 0.93% and the maximum genetic distance was 2.52%. Taking the whole data set, the mean intraspecific divergences averaged 0.56%, while mean distance to the NN taxon was 10-fold higher, averaging 10.1%. Consequently, there was a clear barcode gap for most species (Figure S1).

For both taxon data sets, the NJ tree (not shown) and ML phylogenetic tree provided a similar result and a clear separation between all currently recognized species, with >95% bootstrap support. No cases of sequence overlap between species were observed (Figure 2). In order to confirm the concordance between sequence clusters and species designation by taxonomy, the data sets were analysed by the BIN clustering method and the bPTP (Figure S2). Within sigmodontines, sequences were assigned to 26 BINs, and the bPTP species delimitation analyses indicate 26 lineages (species), which correspond to 26 species previously identified by taxonomy. Within caviomorphs, sequences were assigned to nine BINs, and the bPTP species delimitation analyses indicate nine lineages (species), which correspond to eight species identified by taxonomy.

Below we show the results and discuss four cases that deserve special attention.

4 | DISCUSSION

4.1 | Clusters with moderate divergence within one taxonomically identified species

4.1.1 | Case 1: *Abrothrix hirta*

The long-haired grass mouse (*Abrothrix hirta*) is one of the most widely distributed and abundant Abrotrichini of southern South America. This species is distributed from south-central Chile and south-western Mendoza province in west-central Argentina, to the island of Tierra del Fuego (Teta & Pardiñas, 2014). It is a ubiquitous mouse, mostly associated with the Andean forests of *Nothofagus* (Nothofagaceae), and forest-steppe ecotones, but also occurs in shrublands, grasslands, and rocky exposures associated with moderate vegetation, from sea level to elevations of 3500 metres above sea level (m.a.s.l.) (Pardiñas, 2017; Teta & Pardiñas, 2014). Until recently, this species was included within *A. longipilis*, which is now thought to be restricted to central Chile (Teta & Pardiñas, 2014). Phylogenetic analysis of cyt *b* sequences depicts a topology for this taxon with seven strongly supported major clades, structured geographically from north to south, and with significant genetic differentiation (Sierra-Cisternas, 2010). More recently, using transcriptome-derived SNP loci, Valdez and D'Elía (2021) identified four major and allopatric intraspecific lineages within *A. hirta*. Historically, several subspecies were recognized under this taxon (e.g., *castaneus*, *francei*, *moerens*, *nubila*, *suffusa*), although molecular and morphological data sets provided conflictive evidence to their recognition (see Teta & Pardiñas, 2014).

In this study, based on 15 individuals of *A. hirta* from two localities of west central and south Argentina, we retrieved two main clusters, each of them with different BINs for one taxonomically identified species (Figure 3). Clade I (BOLD: AAC8259) contains individuals from a southern locality in Neuquén Province, while clade II (BOLD: AAD2401) includes specimens from a west-central locality in Mendoza Province. The genetic distance between these two clades was 3.1%, reflecting a moderate divergence in this species that corresponded to geographically separated lineages. These two clades were also recovered in other phylogenetic analysis using cyt *b* sequences (Sierra-Cisternas, 2010). Qualitative and quantitative morphological studies on populations of this species, including the clades recognized here, show a moderate correspondence between phylogeographic patterns and the geographic variation in morphological traits (Teta & Pardiñas, 2014). Our results underlined the need to conduct studies on integrative taxonomy of *A. hirta* combining different lines of evidence (e.g., morphological, genetic, biogeographic).

4.1.2 | Case 2: *Tympanoctomys barrerae*

The red viscacha rat (*Tympanoctomys barrerae*) is an octodontid rodent endemic to the arid west-central and southern regions of Argentina. It is a solitary species that lives in complex burrow systems, built in soft soils associated with salt basins and sand dunes, in lowland habitats of the Monte and Patagonia deserts. Previous studies conducted on phylogeography and genetic variation detected distinctiveness of some populations, showing moderate to high genetic differentiation across the species range (Gallardo et al., 2013; Ojeda, 2010). In our study, based on 11 individuals from populations located in the north and centre of its distribution, we recovered two discrete clades with different BINs. One group contains individuals from northern populations (BOLD: AAC1366) and the other group recovered individuals from central populations (BOLD: ABX4892) (Figure 4). Even though genetic distance between them (2.3%) is not as large as to differentiate species, the assignment of different BINs numbers suggests genetic distinctiveness between these clades. A similar pattern was observed in previous studies based on Control Region sequences where central populations show high genetic

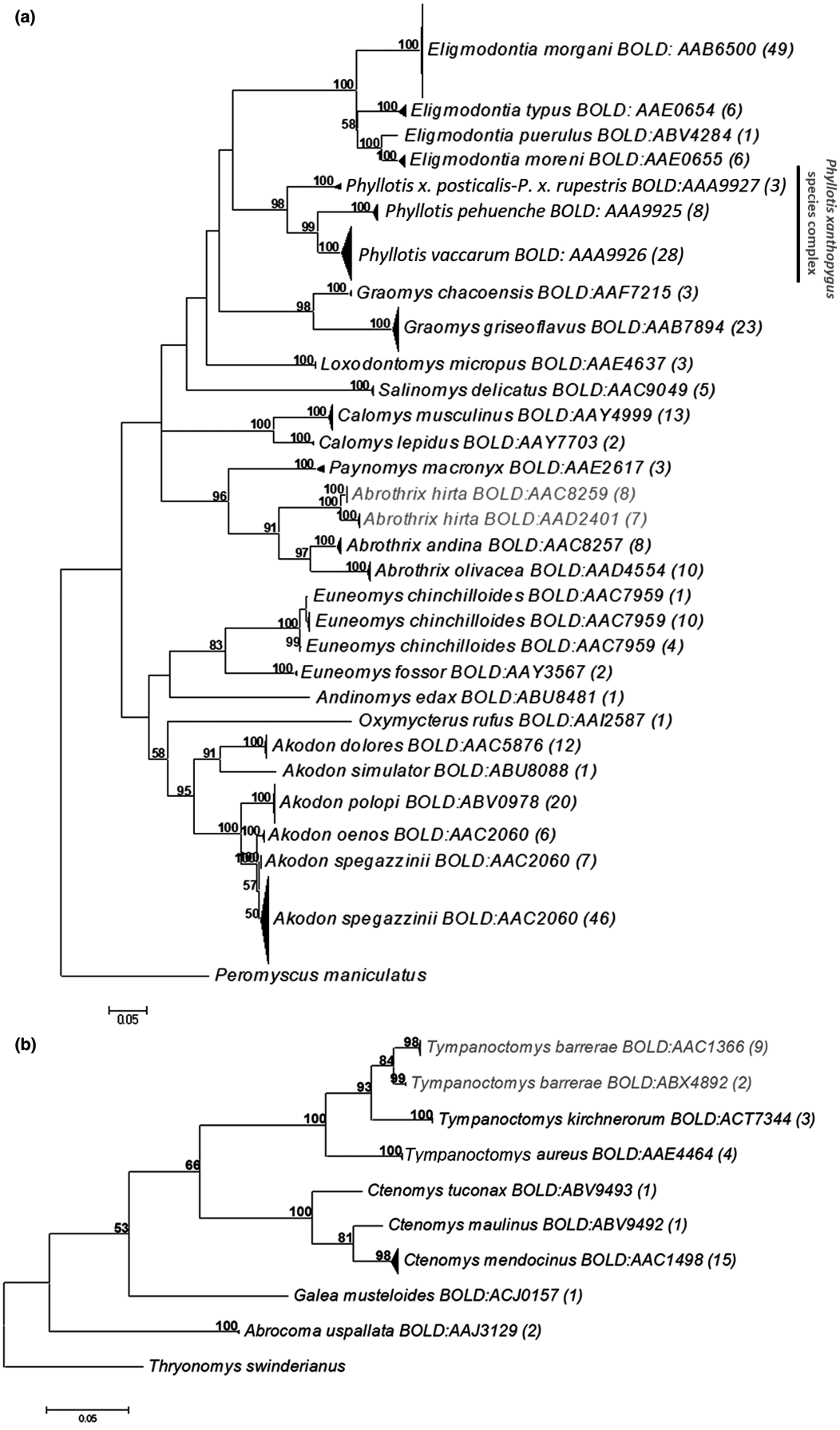

FIGURE 2 Phylogenetic consensus trees obtained with the maximum likelihood analysis of 657 bp segment of the cytochrome *c* oxidase subunit I (COI) gene. (a) Tree obtained for 297 COI sequences of 26 morphologically identified sigmodontine rodents. (b) Tree obtained for 38 COI sequences of eight morphologically identified caviomorph rodents. The number of specimens analysed for each species are shown between brackets. The grey fonts show branches of species with moderate intraspecific genetic divergence and where the BIN clustering method highlights more than one genetic lineage. Numbers above the branches indicate bootstrap supports

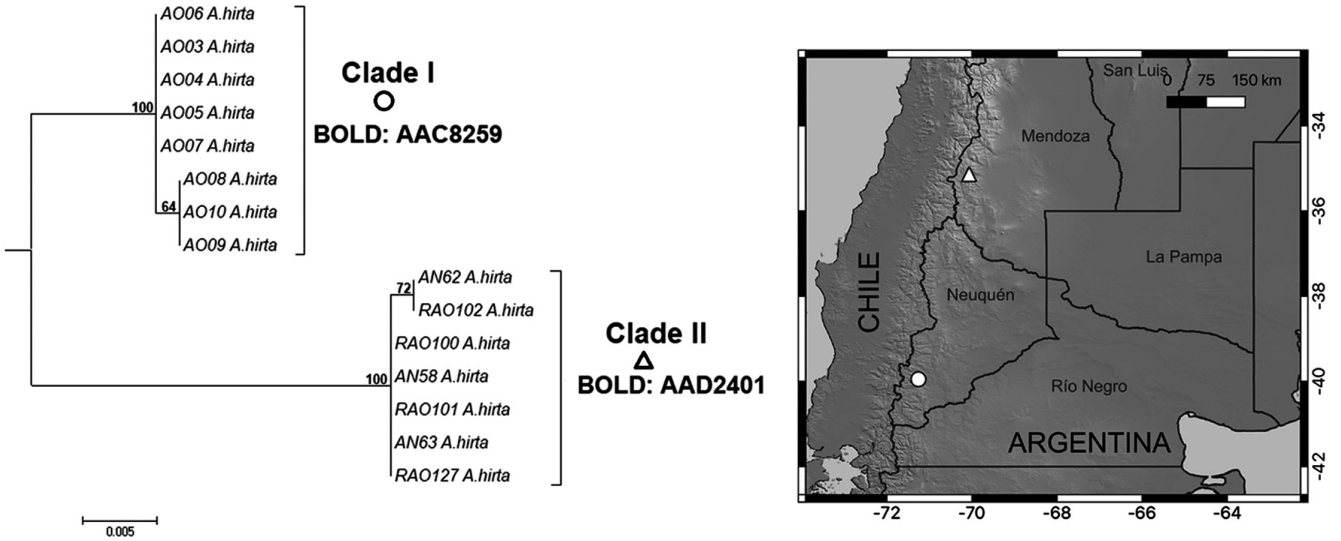

FIGURE 3 Phylogenetic tree and map of distribution corresponding to the Case 1, *Abrothrix hirta*: Clusters with deep divergence within one taxonomically identified species. Clade I recovered specimens from Neuquén Province (Lanín National Park locality) and clade II recovered specimens from Mendoza Province (Las Leñas locality). Numbers above the branches indicate bootstrap supports. Different symbols (circles and triangles) are used to indicate the geographical origin of the specimens

differentiation, unique haplotype frequency and composition, and absence of gene flow regarding populations of northern and southern distribution (Ojeda, 2010).

Characteristics like its narrow geographic range, patchy distribution, diet, habitat specialization, and low population density, led to *T. barrerae* being classified as a near threatened species (Ojeda & Tarquino-Carbonell, 2019; Roach, 2016). Additionally, a recent study predicts that under a future global climate change scenario (e.g., temperature and precipitation variables) populations of *T. barrerae*, particularly those in the central part of its distribution, will become more restricted and there will be a discontinuity between northern and southern populations (Tarquino-Carbonell et al., 2020). This evidence could have important implications for conservation and management of the red viscacha rat, suggesting that populations which occur in the central part of the geographic range, could be good candidates as management units (MU) for its conservation (Moritz, 1994).

4.2 | Clusters with low divergence for two related forms within a genus

4.2.1 | Case 3: *Akodon spegazzinii* – *A. oenos*

The spegazzini's grass mouse (*Akodon spegazzinii*) is widely distributed along the eastern slopes of the Andes in northwestern Argentina, occurring in grassland forest habitats from 400 to 3500 m.a.s.l. (Jayat et al., 2010; Pardiñas et al., 2011). Its sister species, the Wine's grass mouse (*Akodon oenos*), is distributed to the south of the previous species, in grasslands and shrublands of the eastern Andean slopes of central-western Argentina (Jayat et al., 2020). In a recent contribution, based on qualitative and quantitative morphological traits, Jayat et al. (2020) supported the distinction at the species level of *A. oenos*, challenging a previous contribution of Pardiñas et al. (2011) in which both taxa were considered as synonyms. Studies including specimens of *A. spegazzinii* collected in northern localities (Catamarca, Salta, and Tucuman Provinces) show high haplotype diversity, a genetic distance around 1.2% among them, and nongeographic structure (Jayat et al., 2010).

In this study, we analysed specimens from *A. spegazzini* ($n = 53$) and *A. oenos* ($n = 6$), from different localities along an extended latitudinal gradient in Argentina (from Salta to Mendoza Province). We detect three separate clusters with shallow genetic divergences (between 1.2% and 2.2%) that were assigned to the same BIN (Figure 5). One major and diverse clade includes individuals of *A. spegazzinii* from Salta, Tucuman, and Catamarca provinces (northwestern Argentina) (clade I), the second clade includes individuals of *A. spegazzinii* from La Rioja province (clade II), and a third group includes individuals of *A. oenos* from Mendoza province (clade III). Our analysis, according to BIN and bPTP, do not distinguish *A. spegazzini* from *A. oenos* as found by Jayat et al.

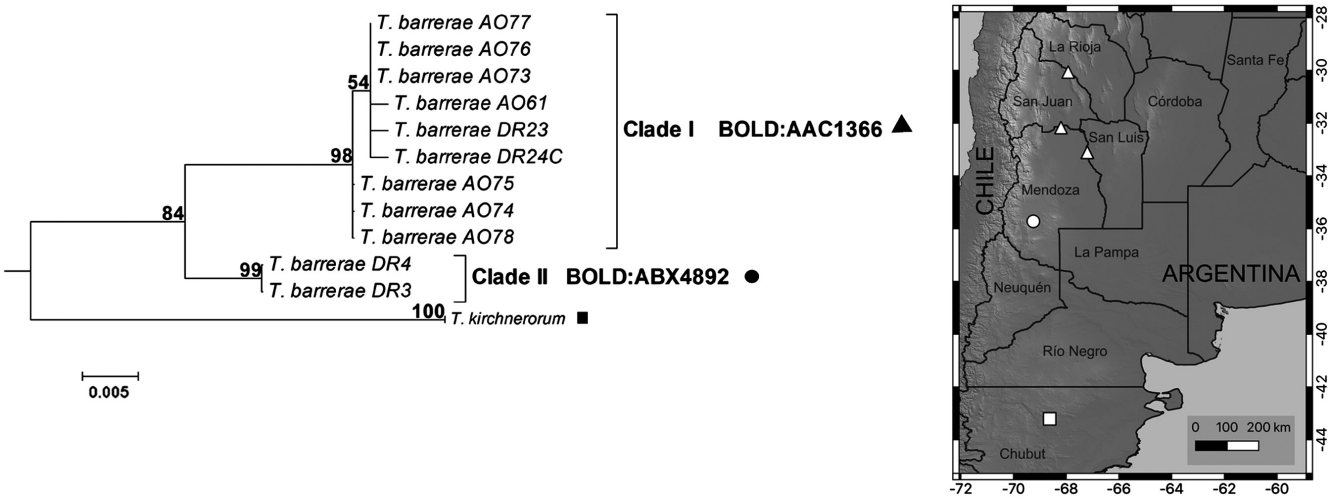

FIGURE 4 Phylogenetic tree and map of distribution corresponding to the Case 2, *Tympanoctomys barrerae*: Clusters with deep divergence within one taxonomically identified species. Clade I recovered individuals from the northern part of the species distribution (northern Mendoza Province). Clade II recovered individuals from the central part of the species distribution (southern Mendoza Province). Numbers above the branches indicate bootstrap supports

(2020) based on its morphological traits. However, the clade III of Mendoza Province was recovered as a monophyletic group separated from *A. spegazzini* specimens. Similar results to those obtained here with COI gene were reached by Jayat et al. (2020), in their phylogenetic analysis based on an 801 bp fragment of the cyt *b* gene. The authors interpreted these results as compatible with a scenario of recent peripatric speciation event, in the context of ecological differentiation. In fact, the genealogical relationship among populations of these sister species represents a good phylogeographic model in exploring hypotheses related to the influence of the environment in the speciation process. The COI tree (and those obtained with the cyt *b*) shows populations geographically more structured towards the south (clades II–III), associated with the patchy distribution of grassy environments, the preferred microhabitat of species of *Akodon* of the *boliviensis* group in arid and semiarid regions. In northwestern Argentina *A. spegazzinii* occupies humid forests and high-altitude grasslands, on the eastern side of pre-Andean ranges, whereas on the western slopes occupies xeric and grassy microhabitats along watercourses (Jayat et al., 2010). Instead, to the south, *A. spegazzinii* (in La Rioja Province) and *A. oenos* (in Mendoza and San Juan provinces) which occupy only xeric habitats. In fact, *A. oenos* is mostly restricted to isolated grasslands on Andean regions or grassy microhabitats on low-elevation halophytic desert scrub, at the base of the Andean and pre-Andean ranges (Jayat et al., 2008, 2010, 2020). This distribution pattern, with more continuous populations in the northern part of the distribution, and more isolated populations to the south, could explain the absence of geographic structure in northwestern populations, the genetically structured populations in the southern part for *A. spegazzinii*, and the peripatric speciation of *A. oenos* in highly isolated areas of the south in San Juan and Mendoza provinces.

4.2.2 | Case 4: *Euneomys chinchilloides*

Chinchilla rats of the genus *Euneomys* are distributed along the Central Andes and in Patagonian steppes, between 0–3000 m.a.s.l., from about 33°S in Argentina and Chile southward to the Cabo de Hornos (Osgood, 1943). Although some authors recognized four living species within this genus (i.e., *E. chinchilloides*, *E. fossor*, *E. mordax*, and *E. petersoni*; see Braun & Pardiñas, 2015), the available molecular and morphological data allow us to recognize only two, for which the names of *E. chinchilloides* (i.e., including *E. petersoni*) and *E. fossor* (i.e., including *E. mordax*) are available (Lessa et al., 2010; Teta et al., 2021).

In the central Andes of Argentina (Mendoza Province), Ojeda et al. (2015) studied those populations referred to the Patagonian chinchilla rat, *E. chinchilloides*. These authors obtained two different clusters with two different chromosome complements (2n = 34 and 2n = 36), allopatric distribution, and some differences in skull morphology (Ojeda et al., 2015). Here, we recovered two monophyletic clades for these populations, one composed by specimens with diploid number 2n = 34 and fundamental number of autosomal arms FNa = 62–64, and another including specimens with 2n = 36/FNa = 64–66 (Figure 6). Despite the fact the two groups show a shallow molecular divergence (1.1%) and share the same BIN, both lineages show reciprocal monophyly for the studied gene (Ojeda et al., 2015; this study). At a cytogenetic level, the modification on fundamental and diploid numbers between these two karyomorphs can be the result of a tandem chromosome fusion, which is a kind of rearrangement that probably led to reproductive isolation between the differentiated populations (Ojeda et al., 2015). Furthermore, differences in their skull morphology support the idea that these groups can have relatively high levels of isolation (Ojeda et al., 2015). Further investigations are needed to elucidate the taxonomy and geographic distribution of *Euneomys*, especially within the current concept of *E. chinchilloides*.

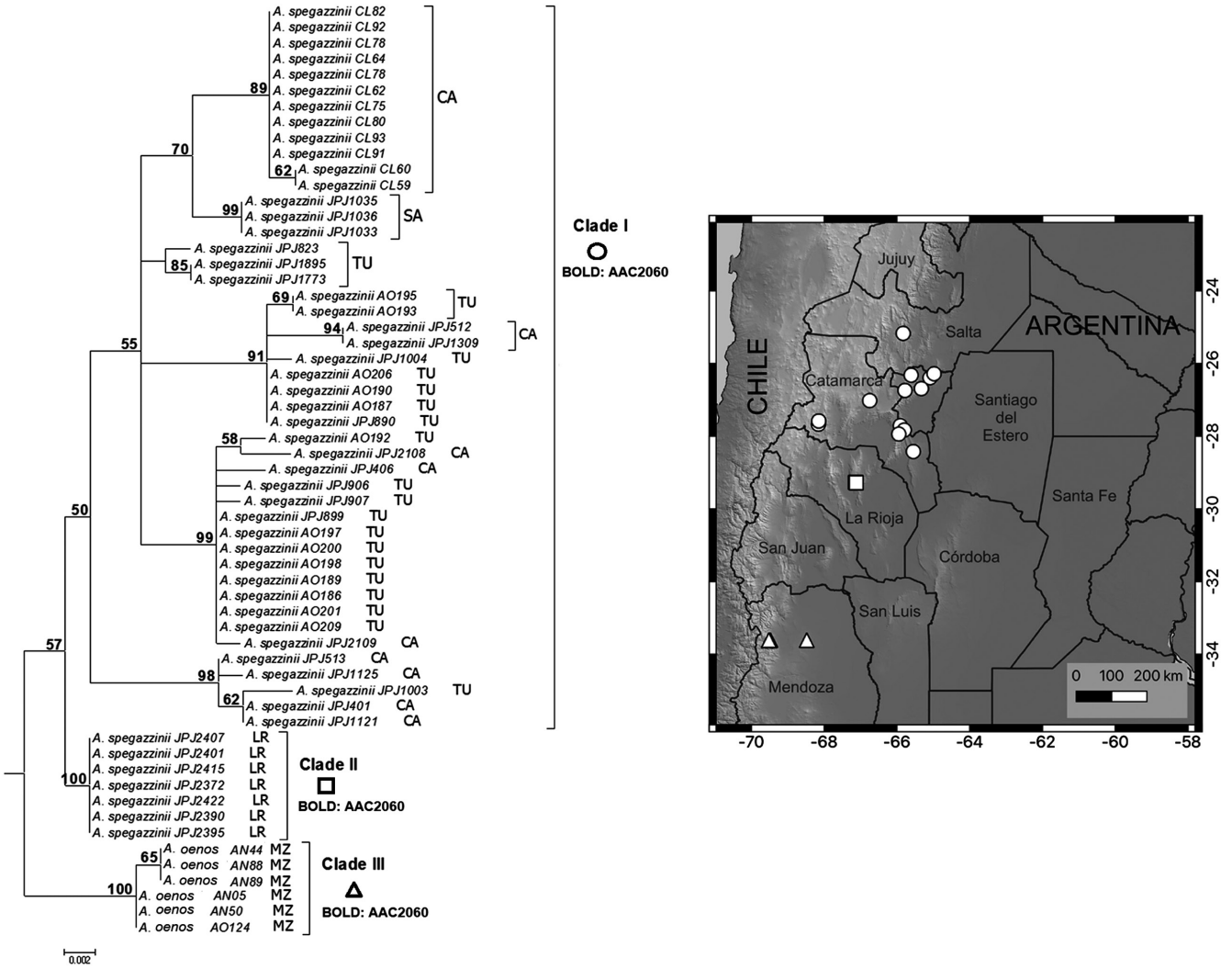

FIGURE 5 Phylogenetic tree and map of distribution corresponding to the Case 3, *Akodon spegazzinii* and *A. oenos*: Clusters with low divergence for two related forms within a genus. Clade I recovered specimens of *A. spegazzinii* from northwestern Argentina (Salta [SA], Tucuman [TU], and Catamarca [CA] Provinces). Clade II recovered specimens from La Rioja (LR) Province. Clade III recovered specimens morphologically referring to *A. oenos* from Mendoza (MZ) Province. The three clades share the same BIN (BOLD AAC2060). Numbers above the branches indicate bootstrap supports

4.3 | Clusters with deep divergence for three related forms within a genus

4.3.1 | Case 5: *Phyllotis xanthopygus* species complex

The leaf eared mice of the genus *Phyllotis* includes 26 species of small to medium-sized sigmodontine rodents widely distributed in arid and semi-arid regions of South America. Within this genus, the *P. xanthopygus* species complex has a broad distribution through Peru, Bolivia, Chile, and Argentina, occurring in an extensive elevation gradient, from high elevations in the central Andes (6739 m.a.s.l) to sea level (Storz et al., 2020), and over a variety of open rocky habitats, from grasslands to deserts in Andean and extra Andean regions (Kramer et al., 1999; Steppan & Ramirez-Baca, 2015). This wide distributional range provides an excellent natural experiment for exploring phylogeographic and speciation events (Albright, 2004). This species complex includes at least eight (perhaps 10) cryptic species, including: *P. bonariensis*, *P. caprinus*, *P. limatus*, *P. pehuenche*, *P. vaccarum*, *P. xanthopygus*, a group informally referred as *P. rupestris-posticalis*, and an unnamed species from central Argentina (see Albright, 2004; Jayat et al., 2021; Ojeda et al., 2021; Riverón, 2011; Steppan et al., 2007; Teta et al., 2018).

In this study, based on 39 individuals of the *P. xanthopygus* species complex (from different localities along a latitudinal gradient between 23°S and 36°S), we retrieved three main clusters, each of them with different BINs (Figure 7). Clade I (BOLD: AAA9927) contains individuals from a northern locality in Jujuy Province; clade II (BOLD: AAA9925) includes specimens from Las Leñas, a southern locality in Mendoza Province; and clade III (BOLD: AAA9926) contains individuals from Andean localities in northern and central Mendoza Province, extra-Andean localities of southern Mendoza, and individuals from Catamarca Province in northwestern Argentina.

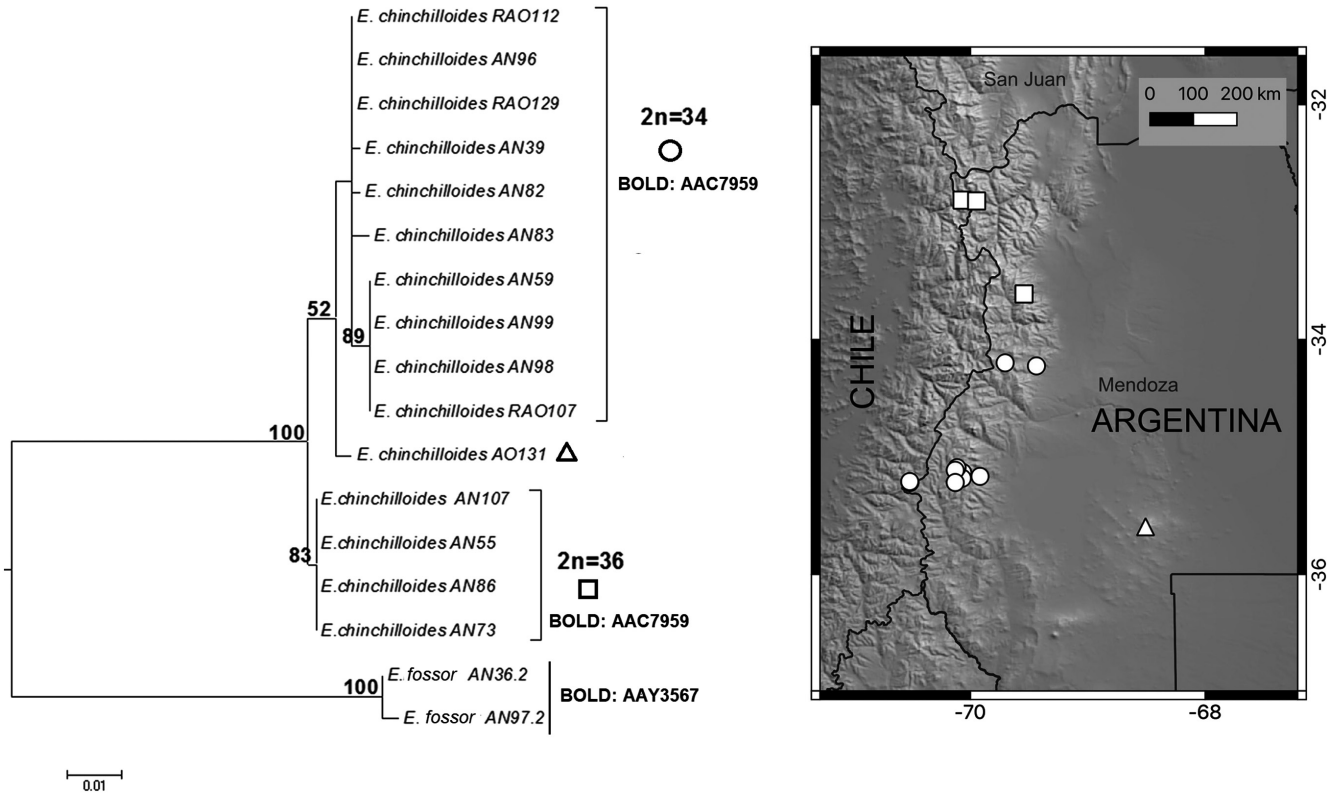

FIGURE 6 Phylogenetic tree and map of distribution corresponding to the case 4, *Euneomys chinchilloides*: Clusters with low divergence for two related forms within a genus. Clade I includes individuals with (2n = 34). Clade II includes individuals with (2n = 36). Both clades share the same BIN (BOLD: AAC7959). Numbers above the branches indicate bootstrap supports

Clade III was the most diverse, showing a certain geographical structure, with a maximum intraclade genetic distance of 2.5%. Between clades I–II, the genetic distances were 12.6%; between clades II–III, 8.6%; and between clades I–III, 10.6%, reflecting deep divergences (more in line with interspecific differences) that were accompanied by geographically separated lineages. Similar values of genetic distances for cyt *b* sequences were recently reported for these same clades (Ojeda et al., 2021). These results agree with earlier cytogenetic studies, which report some chromosome variability within and among these same populations (Labaroni et al., 2014). Overall, our results support the hypothesis that these three clades correspond to three different taxa at the species level (i.e., the clade informally referred as *P. posticalis-P. rupestris*, the recently described *P. pehuenche*, and *P. vaccarum*), as was treated in other recent contributions using other molecular markers (i.e., cyt *b*; Ojeda et al., 2021) and morphology (Jayat et al., 2021).

5 | CONCLUSIONS

This study is one of the more comprehensive to date and the first to explore DNA barcoding effectiveness in delimiting species within the two major radiations of Neotropical rodents, caviomorphs and sigmodontines, occurring in northwestern and central-western Argentina. Thirty-four species (16.4%) of the c. 200 rodent species described for Argentina were assessed in this work using COI barcodes, encompassing large areas of the dry and humid Andes and the arid lands of west-central Argentina (see Andrade et al., 2021 for a similar comprehensive work but restricted to humid areas of northern Argentina). Five prior studies on barcoding Neotropical rodents were conducted to: (a) evaluate the performance of DNA barcoding as a tool for fast taxonomic verification in small-mammals (Borisenko et al., 2008); (b) evaluate the sequence variability of COI and identification of Sigmodontinae species, many of them important from an epidemiological point of view (Müller et al., 2013); (c) identify species in taxonomically complex groups, such is the case of the long-tailed rice rats of the genus *Oligoryzomys* (Da Cruz et al., 2019); and (d) help in the identification and preliminary analysis of phylogenetic relationships (Andrade et al., 2021; Pinto et al., 2018).

Our results corroborate the effectiveness of DNA barcodes as a valuable resource for preliminary species delimitations, molecular diagnosis, and provisional assessment of taxonomic diversity, with a promising application in animal conservation (Hebert & Gregory, 2005; Kress et al., 2015). Furthermore, DNA barcoding represents an excellent tool to explore evolutionary processes and patterns, highlighting those cases that deserve further investigations. The pattern of genetic divergence found in COI sequences were similar to those found using different molecular markers (e.g., cyt *b*, *D-loop*) and reflects, in some cases, the presence of a complex of cryptic species or independent lineages not easily identifiable with traditional

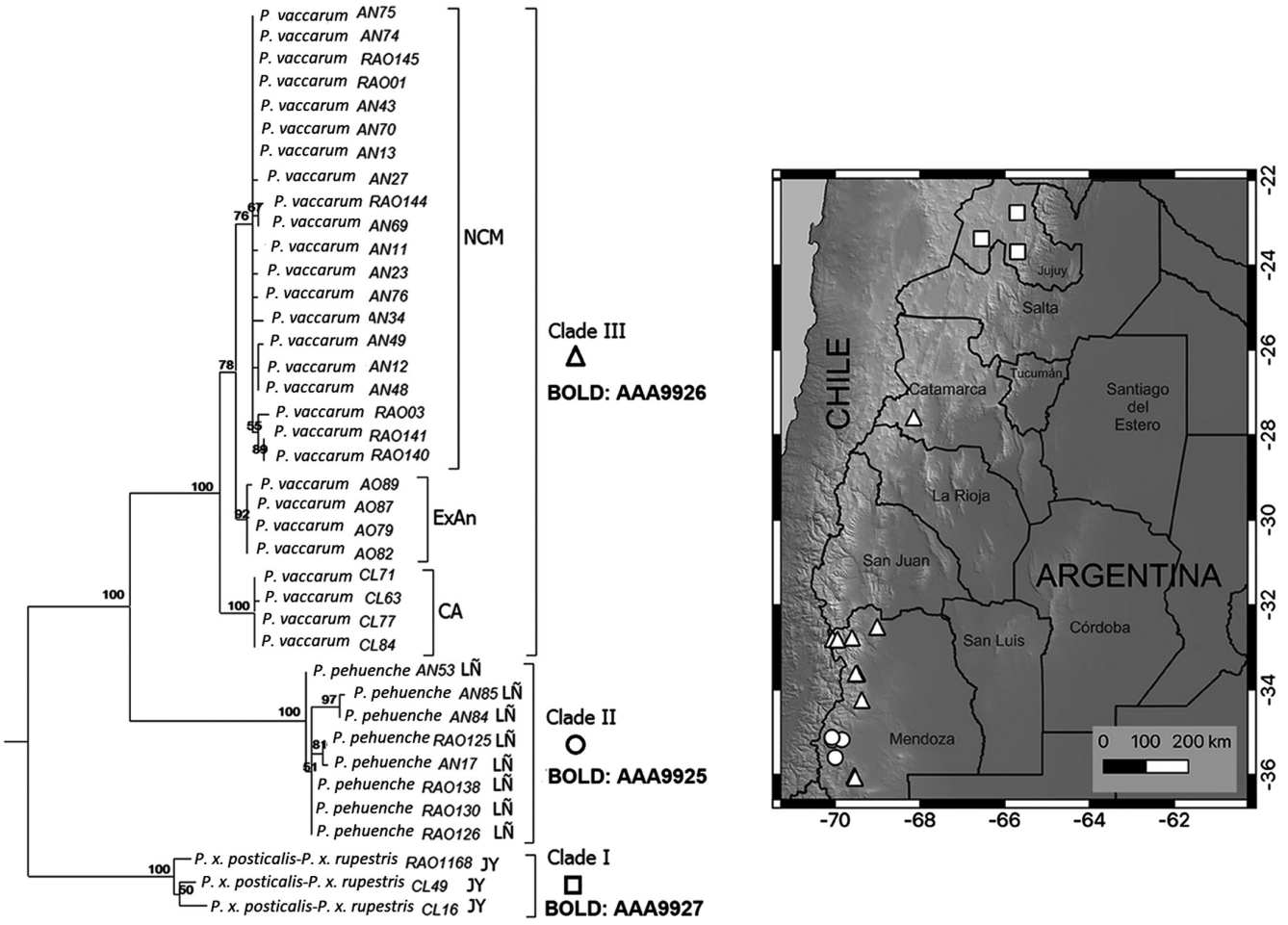

FIGURE 7 Phylogenetic tree and map of distribution corresponding to the case 5, *Phyllotis xanthopygus* species complex: Clusters with deep divergence for three related forms within a genus. Clade I recovered individuals assigned to *Phyllotis x. posticalis-P. x. rupestris* from Jujuy province (JY), clade II recovered individuals assigned to *P. pehuenche* from Southern Mendoza Province (Las Leñas locality [LÑ]) and clade III recovered individuals assigned to *P. vaccarum* from Mendoza and Catamarca provinces (north central Mendoza [NCM]), south Mendoza Extra Andean region [ExAn] and Catamarca [CA]). Numbers above the branches indicate bootstrap supports

morphological approaches. Also, the genetic distinctiveness found in some populations could have important implications for the conservation and management of threatened species. Further studies, using an integrative framework, will be required to clarify the taxonomic status of some species and populations. This approach will also be important to understand the evolutionary processes driving its great molecular diversity and low morphological differentiation found in some cases studied here.

DNA barcodes provide insights into the complex phylogeographic patterns and speciation processes in rodents of Andean and lowland arid lands, and corroborates the need to use additional molecular markers, combined with morphological and cytogenetic analyses, to clarify the patterns of geographic and genetic distribution. In the same sense, the DNA barcode approach highlights areas requiring in depth integrative taxonomic studies, not only to improve identification procedures and delimitation of taxonomic units, but also for a better and more comprehensive understanding of the dynamics of population differentiation. We second Dayrat's proposal (Dayrat, 2005), regarding the need to integrate traditional taxonomy and DNA barcoding (among other disciplines) into an "integrative taxonomy," which aims to combine different sources of evidence (i.e., morphological, molecular, chromosomal, ecological, etc.) to recognize and delimit species.

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AUTHOR CONTRIBUTIONS

Agustina A. Ojeda and Alex Borisenko initiated the project and designed and led research and analyses. Agustina A. Ojeda, Agustina Novillo, Cecilia Lanzone, J. Pablo Jayat, Pablo Teta, Ricardo A. Ojeda, and Alex Borisenko cowrote the manuscript. All authors conducted the fieldwork to obtain and process the tissue samples. Agustina A. Ojeda, Agustina Novillo, Cecilia Lanzone, J. Pablo Jayat, Pablo Teta, Ricardo A. Ojeda, and Alex Borisenko, contributed to interpretation of results and preparation of the manuscript.

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

All DNA barcode data (COI sequences, chromatogram trace files, and collateral specimen information) have been stored in BOLD at http://www.barcodinglife.org in the public Data set (DS-AODAR21). Sequences are also deposited in NCBI GenBank. For GB accession numbers see Table S1.

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