Small mammals and their genetic diversity in Costa Rica in relation to altitudinal gradients

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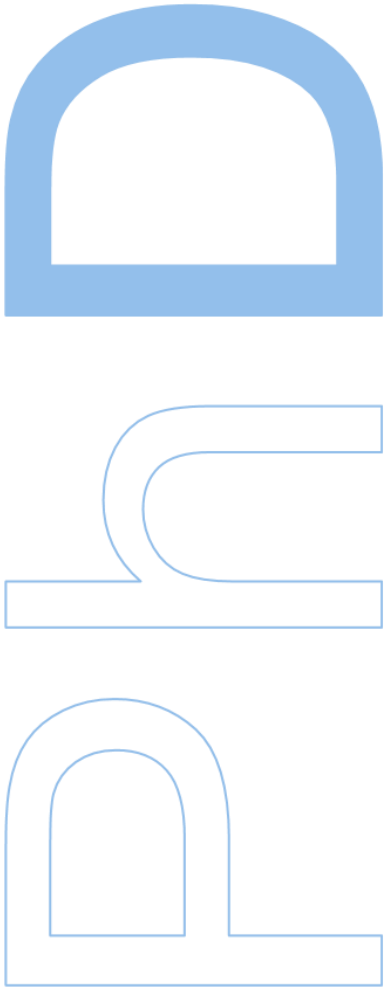

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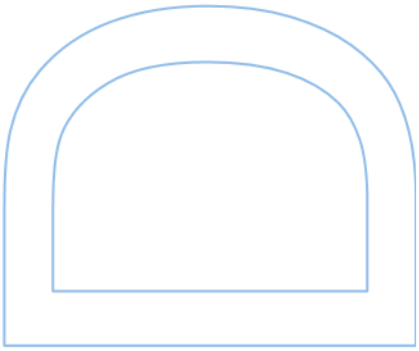

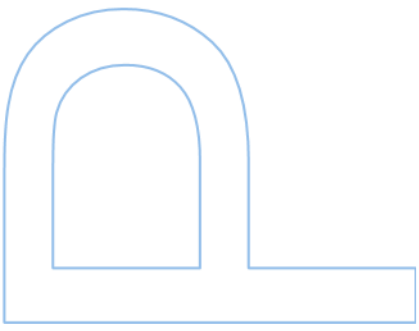

A mi Madrecita!
A mi Padre
A mis abuelos
A mis sobrinos
A mis hermanos
A Tania
A Carito
A tía Olga
A tío Luis
A mi amigo Brandon

Declaração de Honra

Eu, Alexander Enrique Gómez Lépiz, inscrito no Programa Doutoral em Biodiversidade Genética e Evolução da Faculdade de Ciências da Universidade do Porto declaro, nos termos do disposto na alínea a) do artigo 14.º do Código Ético de Conduta Académica da U.Porto, que o conteúdo da presente tese reflete as perspetivas, o trabalho de investigação e as minhas interpretações no momento da sua entrega.

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Alexander Enrique Gómez Lépiz

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Resumo

Este foi o primeiro estudo realizado na Costa Rica que estima a presença e diversidade genética da comunidade de pequenos mamíferos terrestres num gradiente altitudinal. Ao nível taxonómico, apesar da existência de registos históricos de 92 espécies de pequenos mamíferos, apenas 46 estão actualmente confirmadas, considerando-se que fatores como a evolução nas técnicas de identificação e recursos limitados para a investigação contribuem para esta incerteza taxonómica. A Costa Rica é um *hotspot* para a biodiversidade e existem evidências que indicam que a Cordilheira de Talamanca serve provavelmente como uma barreira natural, apresentando 30% de endemismo regional. A região de Talamanca, bem como outras altas montanhas da Costa Rica, podem ter servido de refúgio para pequenos mamíferos durante a Idade do Gelo, revelando uma elevada diversidade de espécies, muitas vezes críptica. No entanto, a escassez de informação genética de referência limita a identificação das espécies e pode levar a uma subestimativa do nível de endemismo. Neste trabalho foi compilada uma base de dados aberta de 2.324 registos para analisar a distribuição altitudinal de 46 espécies de pequenos mamíferos na Costa Rica. Estes registos incluem dados inéditos de trabalho de campo, bem como dados existentes em base de dados de museus nacionais e do *Global Biodiversity Information* Facility (GBIF). Os resultados obtidos demonstram a ocorrência de uma distribuição de espécies não uniforme, com maior riqueza especifica em altitudes intermedias, mas observando-se um nível de endemismo pronunciado em áreas de elevada altitude. Dos vários fatores que foram considerados na análise de dados a biotemperatura revelou diferenças significativas entre altitudes extremas e intermedias, destacando-se uma maior diversidade de espécies nas altitude intermédias. Amostras recolhidas em regimes climáticos distintos revelaram também uma elevada diversidade genética. A distribuição das espécies foi analisada considerando o sistema *Holdridge,* que classifica as zonas de vida na Costa Rica com base na altitude, biotemperatura, precipitação e evapotranspiração. Zonas intermedias como *Wet Forest, Lower Premontane* e *Wet Forest Premontane* suportam o maior número de espécies de pequenos mamíferos. Zonas extremas como a Floresta Tropical Subalpina e a Floresta Tropical Seca são consideradas inóspitas. O estudo das zonas de vida sugere picos de diversidade em altitudes médias, mas também sugere uma alta diversidade em terras baixas, que se pode justificar pela existência de sobreamostragem e elevada acessibilidade, mas com alta influência de fatores como

padrões de precipitação e elevação (aumentando e diminuindo a partir de altitudes médias). Nesse sentido, fica evidente que espécies com uma distribuição geográfica mais ampla dominam na categoria *Pre-montanha*, afetando a composição de espécies em altitudes mais altas e em terras baixas. Este padrão está de acordó com a teoria do "Efeito de Domínio Médio" e pode ser justificado pelos níveis intermedios de precipitação que estão associados à maior riqueza de espécies, enquanto a diminuição da precipitação impacta negativamente a riqueza e a abundância. Algumas espécies estão restritas a altitudes específicas, mostrando adaptação às condições locais e sugerindo que pequenos mamíferos terrestres são bons indicadores das alterações de habitat e desempenham importantes papéis ecológicos. Por isso, este estudo sugere que os pequenos mamíferos podem contribuir para acelerar a recuperação de áreas desmatadas e estabilizar a dinâmica natural das florestas. A Região do *Valle del Silencio* foi um caso de estudo sobre esta dinâmica natural, destacando as espécies dominantes como *Reithrodontomys creper* e *Peromyscus nudipes*. A coexistência dessas espécies pode ser devida a uma atividade humana mínima, mas o papel de outras espécies e suas contribuições para a comunidade devem ser melhor exploradas para se compreender a dinâmica do ecossistema. Mudanças nas condições ambientais podem afetar o equilíbrio e a estrutura da comunidade dos pequenos mamíferos. Outro fator chave que deve ser considerado é a existência de microhabitats no nível do solo, como nas florestas de carvalhos de montanha. Apesar de não existir informação suficiente sobre esses microhabitats, estes oferecem alimento, proteção e cobertura, são cruciais para a sobrevivênciae, sendo a sua preservação crucial para a conservação de pequenos mamíferos terrestres a longo prazo. A dinâmica da temperatura ao nível do solo influencia a utilização do microhabitat, por exemplo, com espécies sensíveis como as pertencentes ao genéro *Peromyscus*, que preferem microclimas adequados para mitigar as flutuações de temperatura nas terras altas. Investigar as interações entre espécies como *Reithrodontomys* spp., *Peromyscus* spp. e *Scotinomys* spp. em regiões de elevada altitude é essencial para compreender as suas funções ecológicas e para a sua conservação. A classificação taxonómica adequada, especialmente para *Reithrodontomys* spp., permitirá adequar estratégias de conservação de forma mais eficaz. Os métodos moleculares podem resolver a taxonomia de espécies crípticas, bem como fornecer informações sobre diversidade genética, estrutura populacional e parâmetros necessários para fomentar a conservação das espécies. A integração de abordagens moleculares aumenta a compreensão e facilita os esforços de conservação, especialmente de espécies crípticas. O presente estudo contribui para as áreas de

taxonomia e genética da conservação de pequenos mamíferos na Costa Rica, através da determinação de linhagens mitocondriais divergentes e da diversidade genética dentro dos géneros, que sugerem a ocorrência de especiação críptica. O DNA barcoding usando o gene mitocondrial citrocromo *b* provou ser útil, mas mais estudos genéticos são necessários para confirmar e entender essa diversidade genética. Estudos anteriores na Costa Rica e na América Central também exploraram a diversidade genética em pequenos mamíferos, enfatizando a importância da continuidade da pesquisa na região. Este estudo revela potenciais formas crípticas dentro de várias espécies de pequenos mamíferos na Costa Rica. Os resultados obtidos sugerem que o género *Philander* pode apresentar uma segunda espécie ou subdivisão genética dentro da atual especie *Philander melanurus*, enquanto *Heteromys* sp. apresenta dois clados distintos. A análise filogenética de *Peromyscus* apresenta desafios na identificação das espécies, com classificações divergentes entre *P. nudipes*, *P. nicaraguae* e *P. mexicanus*. Por outro lado, as sequências de *Melanomys caliginosus* são mais próximas de *M. chrysomelas* do que *M. caliginosus*. A análise das espécies do genéro *Reithrodontomys* sugere uma eventual existência de novas formas genéticas importantes ou mesmo novas espécies. A integração de dados moleculares, morfológicos e ecológicos é crucial para entender esta diversidade e especiação críptica que foi detectada nestes géneros de pequenos mamíferos da Costa Rica. No entanto, esforços futuros são necessários para melhorar a classificação taxonómica e a eficiência das medidas de conservação. As políticas rígidas de uso da floresta em áreas protegidas criaram condições favoráveis em geral para pequenos mamíferos terrestres. Os pequenos mamiíeros podem ser muito sensíveis ao clima atual, com algunas espécies experimentando respostas negativas e outros mostrando respostas positivas que alteram o equilíbrio natural dos ecossistemas. Por este motivo, é fundamental implementar processos de investigação mais aprofundados neste grupo de mamíferos que justifiquem medidas de conservação destas espécies e do seu habitat.

Palavras-chave: Pequenos mamíferos, Costa Rica, endemismos, espécies crípticas, genética da conservação, gradiente altitudinal, biologia da conservação

Abstract

This study was the first in Costa Rica to examine the terrestrial small mammal community across altitudinal ranges, by estimating presence and genetic traits. Taxonomically, despite the existence of historical records for a total of 92 species, only 46 are currently considered as occurring in Costa Rica. Factors like the change over time of identification techniques have contributed to this taxonomic change. Costa Rica displays high biodiversity and it is acknowledged that the Cordillera de Talamanca serves as a natural barrier and promotes speciation and adaption, showcasing 30% regional endemism. The Talamanca region, along other high mountains in Costa Rica, may have served as refugia for small mammals during the Ice Ages revealing often hidden diversity among analysed species. Limited genetic reference data hampers accurate species identification and may underestimate endemism. This study compiled 2324 records to analyze the altitudinal distribution of 46 small mammal species in Costa Rica, considering new data obtained from fieldwork, as a complement to that existing in open access databases from national museums, and the Global Biodiversity Information Facility (GBIF). The species exhibited a non-uniform distribution, with higher species richness at intermediate elevations, but also a pronounced endemism in highland areas. Several environmental variables were considered in the analysis, and biotemperature revealed significant differences between extreme and intermediate altitudes, highlighting the latter's greater species diversity. Genetic divergence was observed in samples collected from distinct climatic regimes. The Holdridge system, that classifies life zones in Costa Rica based on altitude, biotemperature, precipitation, and evapotranspiration, was used to better understand the patterns of small mammals species distribution. Intermediate zones like Wet Forest Lower Premontane and Wet Forest Premontane support the highest number of small mammal species. Extreme zones like Rain Forest Subalpine and Dry Forest Tropical are considered inhospitable. The results suggest the occurrence of species diversity peaks at mid elevations but also notes high diversity in lowlands, probably due to oversampling and accessibility with high influence of factors like precipitation patterns and elevation (increasing and decreasing from mid elevations). In this sense, it is evident that species with larger geographical ranges dominate in the Premontane category, affecting species composition at higher elevations and in lowlands which aligns with the Mid-Domain Effect (MDE) theory. A factor that could explain this

effect is the intermediate precipitation levels which is associated with higher species richness, while decreased precipitation negatively impacts richness and abundance. Some species are restricted to specific elevations, suggesting local adaptation to specific conditions and that terrestrial small mammals are good indicators of habitat modifications and play important ecological roles. This is the reason why this study argues that small mammals can contribute to accelerate the recovery of deforested areas and stabilize the natural dynamics of forests. The Valle del Silencio was a case study that illustrates this natural dynamic. The coexistence in this region of dominant species like *Reithrodontomys creper* and *Peromyscus nudipes*, may be due to minimal human activity, but the role of other species and their contributions to the community need further exploration to understand the ecosystem dynamics. Changes in environmental conditions could affect the balance and structure of the community, another key factor that should be considered is ground-level microhabitats as in this montagne oak forest. These microhabitats offer food, protection, and cover, crucial for survival. Thus, preserving these microhabitats is crucial for long-term ecosystem conservation of terrestrial small mammals. Temperature dynamics at ground level influence microhabitat utilization, with sensitive species like *Peromyscus* spp., preferring suitable microclimates to mitigate highland temperature fluctuations. Investigating interactions among species like *Reithrodontomys* spp., *Peromyscus* spp., and *Scotinomys* spp. in high-altitude regions is essential for understanding their ecological role for conservation. Moreover, proper taxonomic classification, especially for *Reithrodontomys* spp., would allow effective conservation strategies. Molecular techniques can significantly contribute to resolve species taxonomy, as well as providing insights into genetic diversity, population structure, and thus supporting conservation efforts. Integrating molecular approaches enhances understanding and facilitates conservation efforts for cryptic species. The present study contributes to the fields of taxonomy and conservation genetics of small mammals in Costa Rica. It enhances understanding of biodiversity and evolutionary dynamics, aiding conservation efforts. The observation in this study of divergent mitochondrial lineages and genetic diversity within genera suggest the presence of cryptic speciation. Phylogenetic analysis using the mitochondrial cytochrome *b* gene proved to be useful in distinguishing the different small mammals species in Costa Rica, but further genetic studies are needed to confirm and understand the high diversity detected. Results suggest that the genus *Philander* may have a second species or genetic subdivision within *Philander melanurus*, while *Heteromys* sp. exhibits two distinct clades. *Peromyscus* poses challenges in species identification, with conflicting

classifications between *P. nudipes*, *P. nicaraguae*, and *P. mexicanus*. Moreover, *Melanomys caliginosus* sequences are more closely related to *M. chrysomelas* than previously known. Phylogenetic analysis among species from the genus *Reithrodontomys* suggests new major genetic forms or potentially new species. Integrating molecular, morphological, and ecological data is crucial for understanding cryptic diversity and speciation, and should be implemented in future works. Thus, further research is needed to improve taxonomic classification of these small mammal species. Comparing data with museum specimens and optimizing genetic sequencing methods are important for resolving taxonomic uncertainties and for understanding species evolution and biodiversity. Strict forest use policies in protected areas have potentially created favorable conditions in general for terrestrial small mammals. Those animals might be very sensitive to the current climate with some experiencing negative responses and others showing positive responses that alter the natural balance of ecosystems. For this reason, it is strictly necessary to implement deeper research processes in this group that justify measures for the conservation of these species and their habitat.

Keywords: Terrestrial small mammals, Costa Rica, endemism, cryptic species, conservation genetics, conservation biology, local adaptation, altitudinal gradients, climate change, phylogenetic analysis

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Table 3.5. Estimates of diversity between the clades detected within the genus *Scotinomys* based in *cytb* sequences (1140 bp). The average distance between elements of the two groups (Dxy) is shown in the upper triangle and the average distance between elements of the two groups, corrected for the within group distances (Da) in the lower triangle.

Table 3.6. Estimates of diversity between clades detected within the genus *Scotinomys* based in *COI* sequences (653 bp). The average distance between elements of the two groups (Dxy) is shown in the upper triangle and the average distance between elements of the two groups, corrected for the within group distances (Da) in the lower triangle.

Table 3.7. Estimates of diversity between the clades detected within the genus *Scotinomys* based in *RAG1* sequences (1158 bp). The average distance between elements of the two groups (Dxy) is shown in the upper triangle and the average distance between elements of the two groups, corrected for the within group distances (Da) in the lower triangle.

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feeds the data to a computed that assembles the sequence (not shown). The sequence reads from the gel will be complementary to the sequence of the original DNA molecule. (B) A tiny part of the data from such an automated sequencing run. Each colored peak represents a nucleotide in the DNA sequence. (Adapted from: Alberts et al. 2019.).

Figure 2.1. Twelve World Life Zones (Holdridge, 1967) identified based on records for terrestrial small mammals of Costa Rica. Hexagons reflect each life zone defined in the World Life Zones classification: rp-SA: Rain Forest (Paramo) Subalpine, wf-M: Wet Forest Montane, rf-M: Rain Forest Montane, mf-LM: Moist Forest Low Montane, wf-LM: Wet Forest Lower Montane, rf-LM: Rain Forest Lower Montane, mf-P: Moist Forest Premontane, wf-P: Wet Forest Premontane, rf-P: Rain Forest Premontane, mf-T: Moist Forest Tropical, wf-T: Wet Forest Tropical, df-T: Dry Forest Tropical. This classification is based on meteorological records: evapotranspiration – inclined axis (which is a projection of the potential evapotranspiration ratio), precipitation – x axis (estimation of annual average precipitation in millimeters), and biotemperature – y right axis (measurement of the heat which is effective in plant growth in a range between 0 °C and 30 °C). Altitudinal belts were defined by an elevational zonation based on average rate of change of 6°C per 1000 meters (y right axis divisions). Classification of latitudinal regions (y left axis divisions) was based on the International Institute for Applied Systems Analysis. Inside each hexagon is the number of species and the quantity of records, thus the numbers of exclusive species by life zone are indicated within parentheses.

Figure 2.2. Distribution of records along the elevation gradient for each terrestrial small mammal species in Costa Rica. The elevation range was defined by the total of altitudinal records between zero meters above sea level (m.a.s.l) to 3654 meters. Box and whisker plots represent the quartiles from 25th to 75th and from 9th to 91th percentiles, respectively. Data outside of this range were considered atypical records (●). The horizontal line inside each box indicates the median. The dashed lines represent the borders of the intermediate area between 1218-2436 m.a.s.l for all altitudinal range of records.

Figure 2.3. Non-Metric Multidimensional Scaling Analysis (NMDS) based on distributions of terrestrial small mammal species in Costa Rica, according to Holdridge's Life Zones. Life zone codes: rp-SA: Rain Forest (Paramo) Subalpine, wf-M: Wet Forest Montane, rf-M: Rain Forest Montane, mf-LM: Moist Forest Low Montane, wf-LM: Wet Forest Lower Montane, rf-LM: Rain Forest Lower Montane, mf-P: Moist Forest Premontane, wf-P: Wet Forest Premontane, rf-P: Rain Forest Premontane, mf-T: Moist Forest Tropical, wf-T: Wet Forest Tropical, df-T: Dry forest Tropical.

Figure 2.4. Occurrences by elevation for seven species of terrestrial small mammals between two periods with different use of forest in Costa Rica (deforestation: 1960-1984 and protected areas: 1994-2018). The box-plot of elevation for each species shows the trends of records by period considering the median and percentiles (25-75% for each box). Histograms represent the randomized data distribution calculated for each species between both periods. The orange line refers to observed mean values. Differences between randomized means and observed means was indicated as p-values. Roman numerals indicated the paired graphics (boxplot and histograms) for each species.

Figure 2.5. Map showing the location of Valle del Silencio inside La Amistad International Park, Cosa Rica, Limón Province. The shaded area in yellow represent the Costa Rican region of La Amistad International Park and the red circle highlights the study area (Valle del Silencio area). Map designed by Christian G. Herrera (christian.herrera@agbcr.org) using QGis 3.4.15. Elevation was defined as meters above sea level: m.a.s.l.

Figure 2.6. Arrangement of traps for sampling during fieldwork for the present research in Valle del Silencio. Four transects were oriented in the compass directions from the ranger station. Each trap was placed every 10 m for a total of 30 traps by transect. Three procedures were adopted for each animal captured: a. a microchip was implanted for subsequent identification in capture-mark-recapture analysis, b. measurements and photographs were taken for species identification and c. tissue was collected for genetic analysis.

Figure 2.7. Materials required for the three main procedures. 1: Sherman trap, 2: pens and masking tape, 3: paper towel, 4: guides for identification and notebooks, 5: disinfectant gel, 6: electricians' gloves (to avoid bites) and latex gloves, 7: anesthetic isoflurane (100%), 8: chip reader, 9: sugar water (to hydrate the animal), 10: scissors, 11: iodine, 12: Vernier calipers, 13: face mask, 14: test tube with cotton wool (to apply isoflurane), 15: ear punch, 16: Eppendorf tubes (for tissue sample), 17: 96% ethanol (to fill the Eppendorf tube with the sample), 18: cotton wool, 19: Pesola scales, 20: transparent bags (to manipulate the captured animals), 21: injection needle with chip, 22: lighter (to sterilize equipment)., 23: clamp.

Figure 2.8. Basic procedure for trap activation and deactivation. A bait mixture was prepared to generate 10 g balls to add to the traps. Traps were open from 17:00 hrs onwards throughout the night and closed again from 07:00 hrs onwards throughout the day. Before use, each trap must be checked for its general condition and sensitivity.

Figure 2.9. Any animal captured should be checked with microchip scanner to determine if it is a recapture or a new capture. Recaptures were released immediately, and new

captures were prepared for the procedure. New animals were manipulated in a transparent plastic bag. Partial sedation was implemented with a 20 ml test tube with cotton wool and some isoflurane. The animal should be constantly hydrated with a mixture of water and sugar.

Figure 2.10. Two invasive procedures were performed: microchip implantation and ear tissue collection. The partial sedation can be confirmed by applying repeated pressure to the ears with forceps. For microchip implantation the skin on the back should be lifted, close to the middle of the nape. The animal should be scanned to confirm the implantation. Ear tissue collection requires sterilization of all equipment with a lighter, the ear should be punched to collect the tissue in the Eppendorf tube with 96% ethanol. Both wounds should be disinfected with iodine. The animal should be constantly hydrated with a mixture of water and sugar.

Figure 2.11. Final steps of the procedures were body measurements (tail, body, ear, hind foot) and photographs (head, dorsal side, ventral side, lateral side, hind foot) before releasing the animal. The animal should be scanned to confirm the functionality of the microchip. The animal was monitored until it safely returns to the forest.

Figure 2.12. Boxplot of records for capture and recapture by species.

Figure 2.13. Oak forest habitat in Valle del Silencio emphasizing the characteristic presence of a bryophyte layer. Pictures: M.Sc. Roger González Tenorio (roger.gonzalez@sinac.go.cr).

Figure 3.1. Relief map of Costa Rican provinces and within them the sampling localities for the present study (Appendix 3). LAIP: La Amistad International Park., SRNP: Santa Rosa National Park., CERS: Cuatro Esquinas Ranger Station., AFRS: Aguas Frías Ranger Station., BCNP: Braulio Carrillo National Park., MANP: Manuel Antonio National Park. Map adapted from:
https://es.m.wikipedia.org/wiki/Archivo:Costa_Rica_relief_location_map.jpg.

Figure 3.2. Highlighted branches of the cytochrome *b* phylogeny for small mammals in Costa Rica (see Appendix 2 for the complete phylogeny). Maximum likelihood tree (IQ-TREE). Branch support: ultrafast bootstrap approximation/SH-like approximate likelihood ratio test results. **A**: Branch including the cricetid rodent genera *Handleyomys*, *Melanomys*, *Nephelomys*, *Nyctomys*, *Oecomys*, *Oligoryzomys*, *Oryzomys*, *Ototylomys*, *Rheomys*, *Sigmodon*, *Sigmodontomys*, *Transandinomys*, *Tylomys*, *Zygodontomys*., **B**: Branch including the cricetid rodent genus *Peromyscus*., **C**: Branch including rodent (echimyid and heteromyid) and eulipotyphlan genera., **D**: Branch including the marsupial genera.

Figure 3.3. Cytochrome *b* phylogeny for *Reithrodontomys* in Costa Rica. Maximum likelihood tree (IQ-TREE). Branch support: ultrafast bootstrap approximation/SH-like approximate likelihood ratio test results*. SM(15 Sequences)*: *SM2781*, *SM2782*, *SM2903*, *SM3123*, *SM3367*, *SM3412*, *SM3417*, *SM3460*, *SM3462*, *SM3463*, *SM3470*, *SM3471*, *SM3488*, *SM3513*, *SM3535*.

Figure 3.4. Distribution of the genus *Scotinomys* in Mesoamerica (obtained from GBIF, 2019). The left square shows elevation (modified from Brushnell *et al.* 2020), highlighting the main barriers and regions: 1 = Chiapas Guatemalan depression., 2 = Nicaraguan lowlands depression., NCA = Northern Central American mountain system., CAN = Central American Nucleus., CT = Talamanca Mountain Range.

Figure 3.5. Distribution *of S. xerampelinus* (pink) and *S. teguina* (green) species in Cordillera de Talamanca (modified from Pino, 2015). LAIP: La Amistad International Park. BCNP: Braulio Carrillo National Park. The left square shows Cordillera de Talamanca elevation (obtained from https://www.mapsland.com/).

Figure 3.6. a) Bayesian inference tree for *cytb* (1140 bp). b) Bayesian inference tree for *COI* (653 bp). Red branches represent our samples. Posterior probabilities of all nodes are indicated. CR = Costa Rica., LAIP-PT = La Amistad International Park – Ranger Station Pittier (at medium altitude)., LAIP-VS = La Amistad International Park – Sector Valle del Silencio (at high altitude)., BCNP = Braulio Carrillo National Park. Outgroups: *Peromyscus mexicanus* and *Reithrodontomys creper*. c) *RAG1* (1158bp) median joining haplotype network of the genus *Scotinomys*.

Figure 3.7. Geographic locations of the *Scotinomys* clades identified in Mesoamerica. *S. teguina* clades are in red. Filled square = Mexican clade., Empty square = Guatemalan clade., Filled circle = Central Mesoamerican clade., Empty circle = Nicaraguan clade., Filled star = Central-northern CR clade and empty star = Southern CR clade. *S. xerampelinus* clades are in blue. Filled circle = Central CR clade and empty circle = Southern CR clade.

List of abbreviations

ADPCRC	Atlas Digital Project of Costa Rica
AIC	Akaike Information Criterion
ALOS	Advanced Land Observing Satellite
BCNP	Braulio Carrillo National Park
GBIF	Global Biodiversity Information Facility
CATIE	Tropical Agricultural Research and Higher Education Center
Cibio-InBio	Research Centre in Biodiversity and Genetic Resources / Research Network in Biodiversity and Evolutionary Biology.
CONAGEBIO	National Commission for Biodiversity Management.
COI	cytochrome oxidase subunit 1
CJS	Comark-Jolly-Seber
CTM	Centre for Molecular Analysis
Cyt*b*	cytochrome *b*
DNA	Deoxyribonucleic acid
GABI	Great American Biotic Interchange
IUCN	International Union for Conservation of Nature
LCA	Lower Central American
MDE	Mid-Domain Effect theory
m.a.s.l.	meters above of sea level
m.y.	millions of years ago
NGS	Next-generation sequences
NMDS	nonmetric multidimensional scaling

PCR-RFLP	polymerase chain reaction-restriction fragment length polymorphism
PILA	La Amistad International Park
PT	Pitier Ranger Station
QGis	Quantum GIS
RAG1 and *RAG2*	Recombination activating gene 1 and 2
RAXML	Randomized Axelerated Maximum Likelihood
SINAC	National Protected Areas System
SNPs	Single Nucleotide Polymorphisms
IUCN	International Union for Conservation of Nature
VS	Valle del Silencio
WGS84	World Geodetic System 1984
INSDC	International Nucleotide Sequence Database Collaboration

Chapter 1: General Introduction

1.1 Deforestation and recent history of conservation in Costa Rica

During the last decades Costa Rica has adopted different rigorous policies to reach a model based on sustainability. As a result, the current protected areas system has an international prestige since it protects adequately both marine and terrestrial richness. However, the execution of this visionary initiative has involved a considerable investment for this developing country in efficient administration of economic resources, target guidelines for environmental programs, systematic research programs and stringent controls that guarantee a property guard of natural heritage (Chen *et al.* 2018). It has been suggested that Costa Rica is the country with the highest density of biodiversity in the world (Fig. 1.1) (Obando 2007, Kholmann 2011).

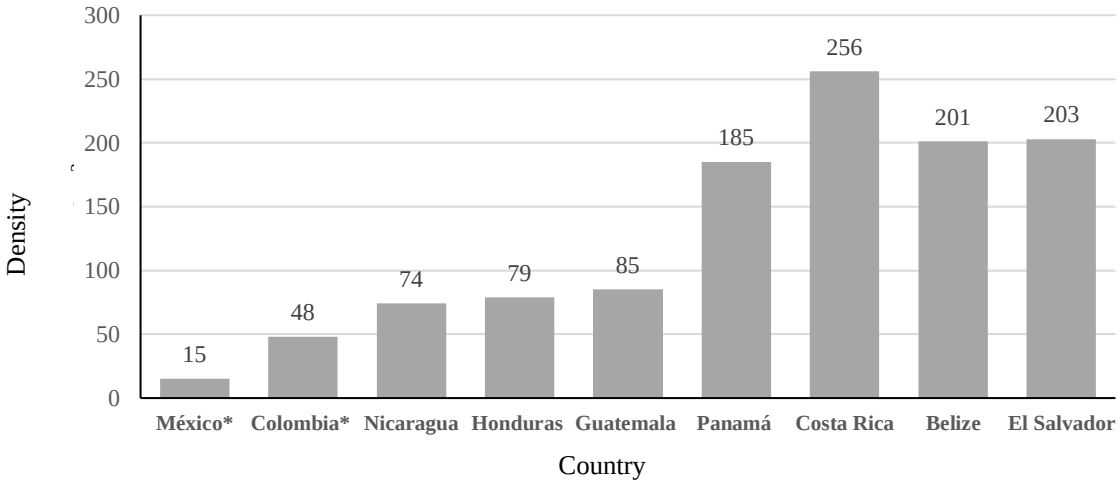
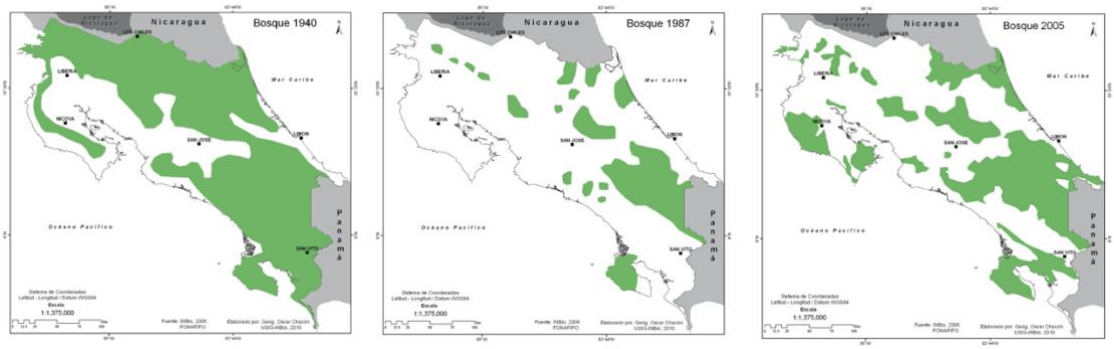


Figure 1.1. Number above the bars represent density of species of vertebrates and plants by square kilometre from countries of Central America and two megadiverse countries (*). Countries in order of size. Adapted from: Obando 2007.

Undoubtedly, the model created in Costa Rica has reversed the dark scenario in terms of conservation faced during the 1970`s and 1980`s when dramatic levels of deforestation were registered, with only around 21% of forest cover (Fig. 1.2) remaining in different isolated patches toward to the end of the 1980's (Powlen and Jones 2019). Throughout this period, Costa Rica had one of the highest rates of deforestation around the world due to an aggressive scheme of development which had focused on replacing the original forest by extensive grass planting to feed cattle (Sander and Joyce 1988).



(a): 75% forest covert (b): 21% forest covert (c): 51% forest covert

Figure 1.2. Three different periods of forest cover in Costa Rica. Costa Rica cover during 1940 (a)= 75%, 1987 (b)= 21% and 2005 (c)= 51%. Adapted from https://sites.google.com/a/inbio.ac.cr/geomatica/gallery/mapas (Consulted date: 2020, January 27 th).

The effect of that accelerated forest expansion generated a complex matrix with a deep fragmentation effect where some remote patches from the original forest was left as a result of several situations, for instance, previous protected areas (volcanoes and areas around them by an ancient law) or very inaccessible zones and places uninteresting for the prevailing model of development (terrain with steep slopes). Strikingly, those remaining blocks of forest nowadays constitute the basis of the current prominent system of protected areas. However, these protected areas may lack sufficient area or connectivity to support some species, particularly those with low population densities and/or large home range requirements (Moran *et al.* 2019).

At the same time, some initiatives reversed that aggressive policy of deforestation. That is how during 1963 the National Absolute Reserve Cabo Blanco was officially created as the first protected area in Central America focused on conservation (Executive Decree 1963). This reserve was created due to the visionary perspective of two foreigners that lived around Cabo Blanco, namely the Swedish Nicolas Wessberg, and his Danish wife Karen Mogensen (Fig. 1.3) (Wessberg 2014). This couple was especially sensitive to the loss of natural forest and the associated biological richness.

This scenery inspired the couple to obtain international funds to buy some remaining patches of forest in Cabo Blanco and protect its biodiversity. The initiative encouraged other proposals from national authorities, researchers and other interested foreigners (Ugalde 2016). After that, the government assumed the initiative as an official project to establish a National Park System in 1970, which was the basis for the current National Protected Areas System (SINAC) (Brenes and Soto 2017). In fact, this event

allowed the gradual decrease in the rate of loss of biodiversity and constitutes the basis for the establishment of the conservation movement that was officially implemented in 1994, with the adhesion of Costa Rica to *The 1992 Convention of Biological Diversity* and additionally in 1998 a Law of Biodiversity was approved.

Figure 1.3. Alvaro Ugalde and Karen Mogensen were two of the pioneers of the current protected areas system of Costa Rica. Figure from Ugalde (2016).

In this regard, Costa Rica had encouragement by international organizations that recognized both efforts, the implementation of guidelines for sustainable development based on ecotourism and the protection of its biological richness (Wainwright 2007). An optimist panorama has been associated with the biodiversity in this country over recent decades, perhaps due the transition to policies more sensitive in terms of conservation that supported good results. The recovered forest currently represents around 56% of the territory and more than 25% of those areas are protected by different management categories (CONAGEBIO 2015).

The next step seems to be the validation of these areas in terms of supporting healthy and highly divergent wildlife populations that naturally inhabit the areas, and the gradual recovery of the ecosystems. Indeed, populations of jaguars as top of predators in the northwest of Costa Rica constitute a specific case (Fig. 1.4). Populations of jaguars would be unable to survive if they only inhabit the current patches of forest - it is totally necessary to apply specific policies that guarantee the connection between patches to guarantee enough animals for stable populations (Moran *et al.* 2019).

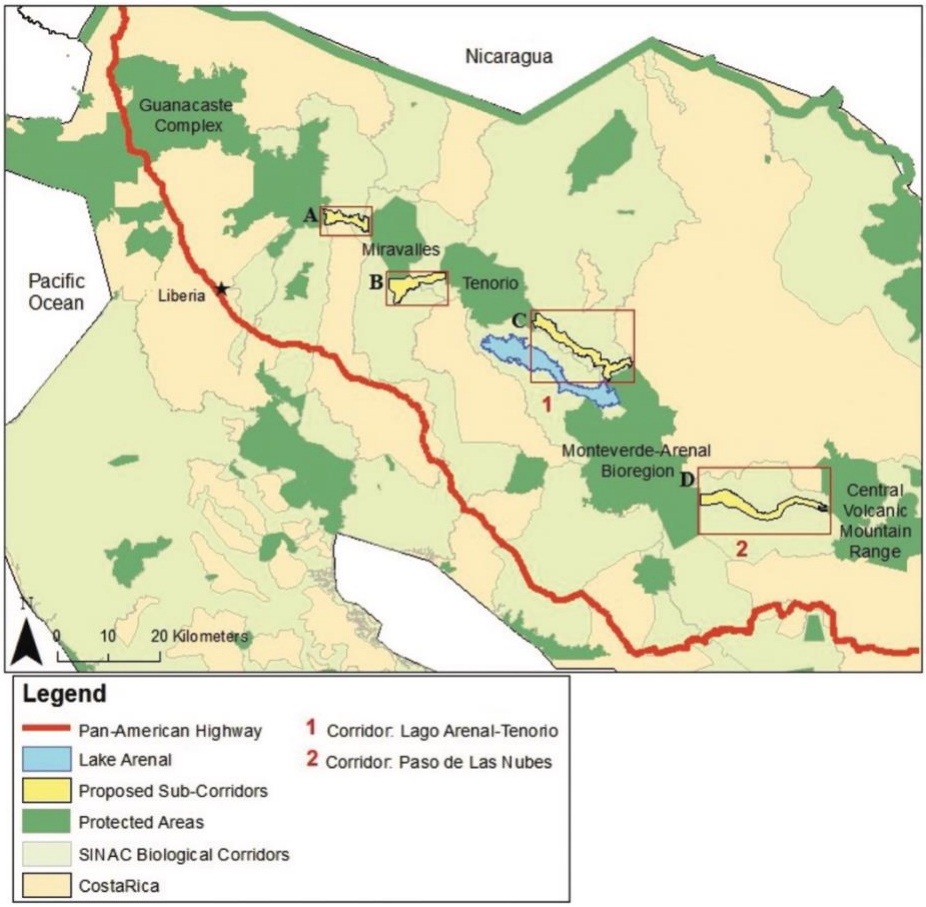


Figure 1.4. Proposed biological sub-corridors within the protected land and Costa Rican government designated biological corridors in north-western Costa Rica. **A** Guanacaste – Miravalles **B** Miravalles – Tenorio **C** Tenorio – Monteverde-Arenal Bioregion **D** Monteverde-Arenal Bioregion – Central Volcanic Mountain Range, 1 = Arenal – Tenorio Biological Corridor (SINAC-designated), 2 = Paso Las Nubes Biological Corridor (SINAC-designated) (Moran *et al.* 2019).

1.2 Geological and geographical complexity of Costa Rican territory as the basis for the high biodiversity

The high biodiversity of Costa Rica is related to its privileged geographical location, but also geological traits support a highly complex territory. Costa Rica has its origin in a dynamic geological process as result of interaction between three tectonic plates: Cocos Plate, Caribbean Plate and Nazca Plate. In this process, the early Caribbean Basin approximately 170 m.y. ago formed a narrow passage between the Pacific and the Proto-Caribbean (Alvarado and Guaria 2016). This conformation was the last united continental structure in the middle of North America and South America, before the current connection between both continental masses, which occurred approximately 75 m.y. ago. This union culminated with the establishment of Central

America 4-6 m.y ago when the proto-Antilles formed a land bridge between the Americas. This closure resulted of the apparent activity of the subduction of the oceanic plates and the subsequent phases of intense volcanic processes from the Mesozoic Ocean, thus forming the Lower Central American (LCA) zone to which Costa Rica belongs (Bagley and Johnson 2014).

Consequently, the persistent collision between the Cocos and Caribbean plates is intensely manifested in the Central American trench. This resulted in considerable instability at the crustal level causing accelerated tectonic activity and frequent volcanism (Stehli and Webb 1985). Both elements are considered fundamental to the current geological conformation of Central America. In this sense, the territory of Costa Rica is perhaps one of the most complex in Central America, since despite its small size and narrow configuration, it is defined by the largest number of physiographic provinces established for countries of the region (Fig. 1.5) according to the approach defined by Marshall (2007): Nicaragua depression; Sandino fore arc; Chorotega volcanic front; Chorotega fore arc; and Chorotega back arc.

The mountainous complexity of Costa Rica is currently defined by an abrupt central system of mountains with a NW-SE direction (Enquist 2002) which divides the country into two basins: Pacific and Caribbean. Additionally, altitude varies from zero to 3820 meters above sea level with the influence of multiple factors such as: wind patterns, solar radiation, sea currents. Those factors have a direct effect on the continental part due to the narrowness of the territory. For example, strong rainfall during the months of the rainy season reflects the north-south dynamic related with the Intertropical Convergence Zone (Gómez 1986). Associated with this central mountain assembly there is a secondary mountain system defined by a complex series of topographic conditions which dominates about half of the country (Alvarado and Cárdenas 2016).

Synergistic interactions between geographic and topographic factors could provide the conditions to support a high biological richness present in Costa Rica. First, as previously indicated, this territory has a privileged position in the tropics of America. Second, this small region of America is a narrow bridge of biological interaction between two big continental masses (North America and South America). Additionally, the

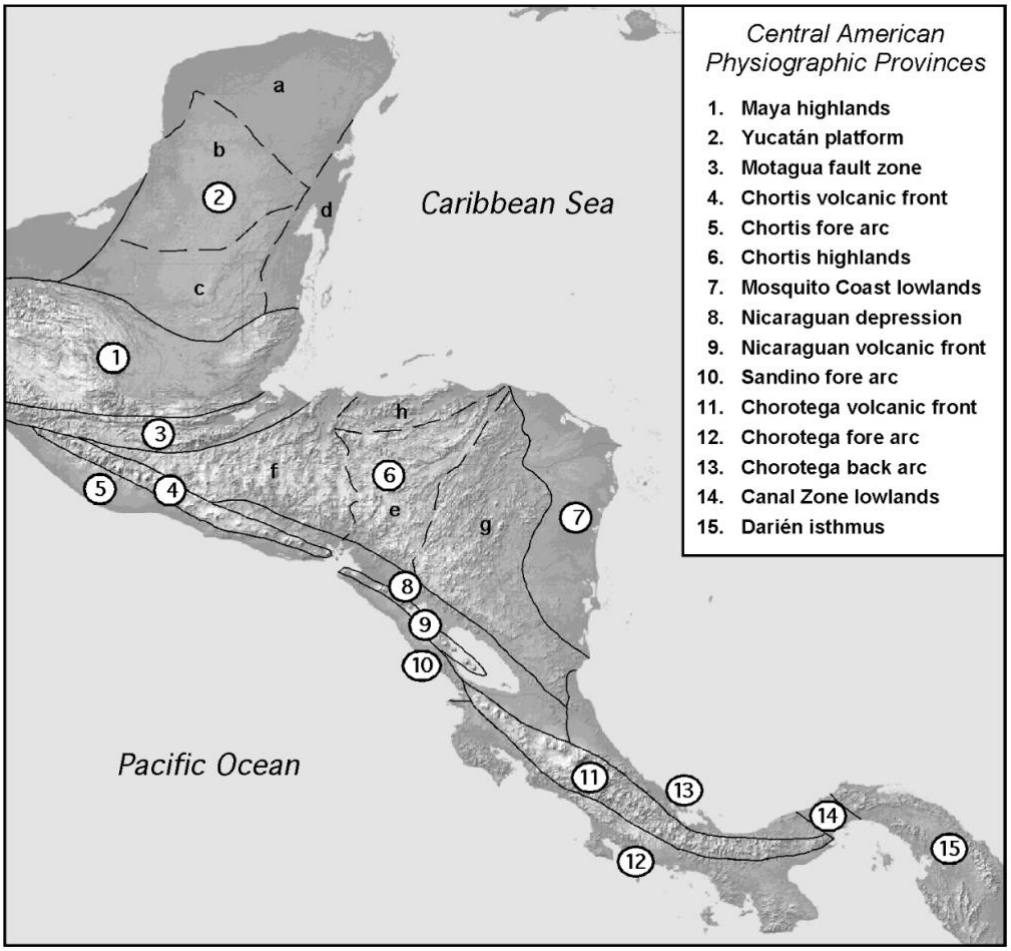


Figure 1.5. Map of Central American Physiographic Provinces (From: Marshall 2007). Map of the physiographic provinces of Central America as defined in this chapter. Solid lines indicate province boundaries. Numbers on map refer to the list of physiographic provinces to the right. Yucatán platform and Chortis highlands sub-regions: a, Northern pitted karst plain., b, Southern hilly karst plateau., c, Petén karst plateau and lowlands., d, Eastern block-faulted coastal plain., e, Central Chortis plateau., f, Western rifted highlands., g, Eastern dissected plateau., h, Honduran borderlands. Digital elevation model derived from NASA Shuttle Radar Topography Mission (SRTM) image PIA03364.

continental zone is influenced by two coasts (Pacific and Caribbean) bounded by the central mountain system, which constitutes a natural barrier along the middle of this country (Fig. 1.6) (Kappelle 2016). Therefore, these factors promote numerous and assorted microclimates which support a variety of evolutionary adaptative process.

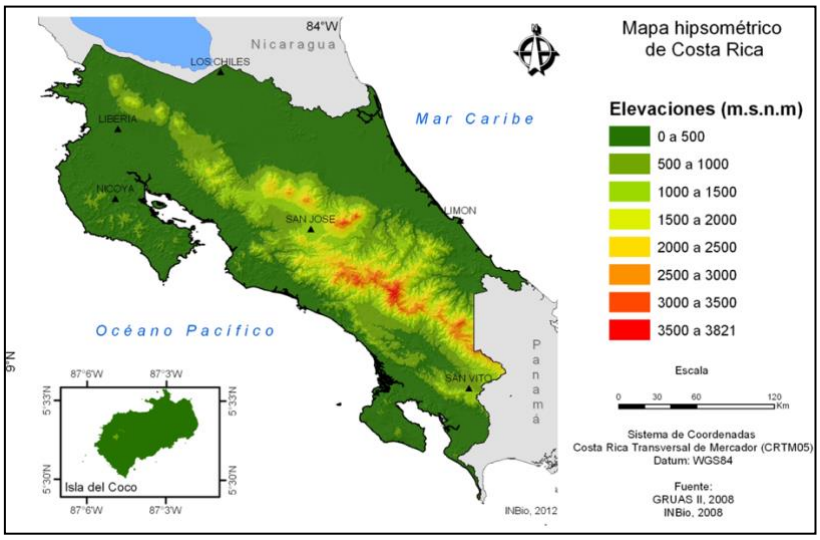


Figure 1.6. Hypsometric map of Costa Rica. https://sites.google.com/a/inbio.ac.cr/geomatica/gallery/mapas (Consulted date: 2020, January 27 th).

1.3 Geographically privileged position and natural richness

Regarding the previous section, the Mesoamerican region comprises tropical countries that share not only geographical aspects but also unique features in terms of ecological and climatic characteristics associated with high biodiversity (Suzart de Albuquerque *et al.* 2015). Mesoamerica has been considered one of the most important biodiversity hotspots of the world because its extraordinary quantity of vertebrates (around 2859 species) and the high number of endemic species (around 1159 species) (Myers *et al.* 2000).

A compilation of data for Costa Rica indicates the exceptional contribution to the region in terms of biodiversity. With only 0.03% of the continental area of the planet, the country concentrates around of 5% of the biodiversity registered for all the world (U.S. Fish & Wildlife Service 2017). Costa Rica is distinguished in Mesoamerica mainly by two extraordinary centres of high priority for conservation due to highly sensitive ecosystems and species of high fragility associated with them, Indio Maíz-Tortuguero and La Amistad International Park, both regions shared with Nicaragua and Panamá respectively.

Interestingly, as previously indicated, Costa Rica is not considered a megadiverse country, but in terms of density of biodiversity no other country matches it (Obando 2007). A key factor that could explain the high density of species is the fragmented topographic conditions along the country, which offer a complex variety of microclimates and refuges. In this sense, Rabl *et al.* (2019) found a clear small-scale topographic influence for moths in Costa Rica: moths exhibited a differentiated response

likely resulting from filtering processes due to varying ecological conditions along topographic gradients. Those authors consider that this is a crucial factor to accurately estimate biodiversity of Costa Rica.

However, there is an overall absence of knowledge for most of the taxonomic groups in terms of natural history and habitat requirements in Costa Rica. The Life Zones Systems of Holdridge (1967) has been a reference to identify different kinds of biomes in Costa Rica, based on latitude, altitude, biotemperature, precipitation, humidity and evapotranspiration. This information allows a mix of conditions to be associated with the distribution of species to specific ecological gradients. Kolhmann (2011) defined for Costa Rica 12 life zones, with 11 additional transitions zones between them according to a wide variety of species (scarab beetle, dung beetles, fishes and several plants of different families: Araceae, Arecaceae and Bromeliaceae). Moreover, this study recognized a deep differentiation with high levels of species richness associated with specific conditions in some life zones: Tropical Wet Forest; Premontane Rain Forest; Premontane Wet Forest; Tropical Wet Forest (Premontane Transition); and Premontane Wet Forest (Basal Transition).

Despite the lack of information for most taxonomic groups across these variations along the mountains and the effect of microclimates, there have been several studies relating to internal variation in species richness in Costa Rica (Janzen 1967, Holdridge 1967, Whitfield *et al.* 2007, Rabl *et al.* 2019). Several studies already recognize the importance of heterogeneity associated to microclimates in maintenance of tropical biodiversity and how it works as a refuge to face current climate change (Scheffers *et al.* 2017, Jucker *et al.* 2018, Llewellyn *et al.* 2018, Sheldon 2019, De Frenne *et al.* 2019).

The unique assemblage of the current Costa Rican territory is also evident in terms of phytogeographic units. According to the analysis by Zamora (2008), 33 phytogeographic units are present within the country based on biotic parameters, mainly defined by the floristic composition and abiotic such as: precipitation regimes., temperature variation number of dry months., topographic variation., altitude ranges., edaphic factors., among others. Consequently, this analysis emphasizes the high contrast for the country between phytogeographic units (for example in terms of the strong elevational and temperature variations) even for those that are geographically close. Therefore, this reflects the variety of unique conditions associated with complex geomorphological features allowing the development of a diverse array of vertebrate

fauna because of altered dispersal, vicariance, and extinction probabilities over time (Bagley and Johnson 2014).

1.4 Endemism and local microclimate

Environmental heterogeneity associated to topographic complexity gradients is considered a key factor in the high degree of endemism according to the approach of Noroozi *et al.* (2018). Consequently, variation of major atmospheric parameters (solar radiation, precipitation, cloudiness) generates very diverse climates in Costa Rica, and the local climate and its effects become even more diverse through the complex interaction with the microtopographic relief, surface waterways drainage, elevation, and vegetation cover (Coen 1983). In fact, the territory of Costa Rica probably deeply influences the evolution of terrestrial small mammal species according to DaSilva *et al.* (2015), as result of the three main barriers that produce high degrees of endemism: rivers., mountains., and vegetation. The current topography of Costa Rica presents multiple barriers, which have still not been properly evaluated across ecosystems (Fig. 1.7).

Interaction between the topographic conditions at the local level and the effect of the variables described above have probably created microclimatic scenarios that have allowed differential evolution of species (Stallins 2006, Urban and Daniels 2006); which to date has been poorly studied, not only from this country, but also in general in the Central American region for most taxonomic groups (Rich and Rich, 1991, Barrantes, 2009., McCain, 2005).

Kohlmann *et al.* (2010) analysed the patterns of endemism for Costa Rica and emphasized the necessity to include and compare different taxonomic groups to obtain more robust and detailed results on biodiversity-endemism patterns to prioritize adequately areas for conservation. More recently, this author with other researchers suggest that the Cordillera de Talamanca has served as a center of long-term survival and differentiation of species but also these mountains acted as a stable area during the last glaciation for species of beetles (Kohlmann *et al.* 2019). This mountain range includes the highest point in Central America, reaching 3820 m altitude and this complex has played a role as a possible built-in buffer against sudden and dramatic climate change. Hence, Kohlmann *et al.* (2019) identified the Talamanca region as an important refuge for species. The observed distribution pattern suggests that montagne species could move short distances up or down in elevation, tracking their required microclimate,

whereas lowland organisms would move greater distances north or south in order to achieve the same result.

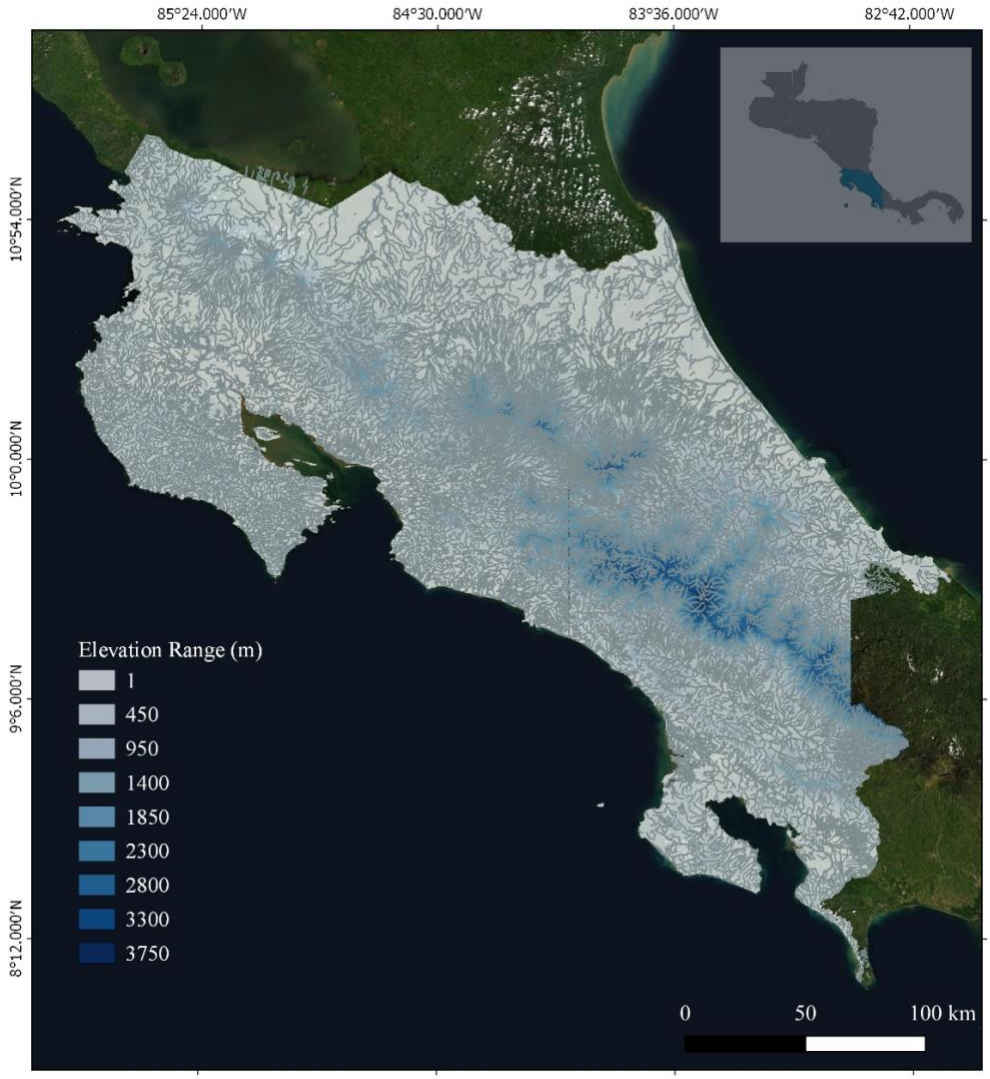


Figure 1.7. Rivers and elevation ranges for Costa Rica. Geographic coordinate system WGS84. Map by Christian G. Herrera (christian.herrera@agbcr.org) using QGis 3.4.15 with shape files Costa Rica 2014 v.1.2.

Areas of endemism are considered to have a non-random distributional congruence among different taxa (Morrone 1994). Cordillera de Talamanca can illustrate this situation; even though many species have been not properly examined yet, probably due to the inaccessibility of this mountain complex. In this sense, small mammals have been considered a strategic group to improve knowledge and conservation efforts to define zones for endemism in tropical ecosystems, as suggested by Dalapicolla *et al.* (2021). These authors used small mammal diversity as a tool to determine the degree of

protection for areas of high endemism and the historical distribution of species to identify areas of urgent protection within the biodiversity hotspot. Mammalian conservation implies safeguarding complex ecological interactions normally associated to large regions and sensitive habitat (Roberge and Angelstam 2004, Pacifici *et al.* 2020).

In the case of Costa Rica, small mammals have very limited data in all respects, including natural history, ecological interactions, behaviour patterns and other biological features. Despite their ecological relevance, there has been little effort to better understand the diversity, distribution, and evolutionary natural history of small mammals from Costa Rica (Janzen and Wilson 1991, Wainwright 2007, Reid 2009, Rodríguez-Herrera *et al.* 2014, González-Maya *et al.* 2015, Villalobos-Chaves *et al.* 2016). In Costa Rica, terrestrial small mammal richness represents around 20% of the full mammalian diversity, but information available for this group is very scarce (Rodríguez-Herrera *et al.* 2014). It is also known that the great majority of terrestrial small mammal species have a crucial role as primary prey for many vertebrate predators, but additionally they are key pollinators or seeds dispersers (Lim and Pacheco 2016). For all these reasons, conservation of these mammals is critical to maintain a balance between interactions within forests and adequate ecological processes associated with them.

Specific studies on small mammals in Costa Rica have identified species that inhabit transitions between life zones, and which occur at different altitudinal levels according to habitat productivity and climatic variations, with a peak of diversity at intermediate elevations according to the mid-domain effect (McCain 2004, 2005). Also, distributional patterns of those species and their range shifts across time has been suggested to be good predictors of the effect of current climate change (Boutin and Lane 2013, Santos *et al.* 2017). Moreover, small mammals species display characteristics in terms of morphological traits, behaviour and ecology that can be exploited to provide indicators of habitat disturbances (Rengifo *et al.* 2022).

The diversity of small mammals is currently represented by 52 species (terrestrial small mammals defined here as mammals under 500g). The list contains seven species of didelphids (marsupials), five soricids (shrews), 39 rodents and one carnivore (Table 2.1- below). Of these, 14 species (30%) are regional endemics, of which three species only occur in Costa Rica (*Heteromys desmarestianus*, *Heteromys nubicolens*, *Reithrodontomys rodriguezi*), two species occur in Costa Rica and Nicaragua (*Reithrodontomys brevirostris*, *Reithrodontomys paradoxus*), and eight species occur in Costa Rica and Panama (*Cryptotis gracilis*, *Cryptotis nigrescens*, *Nephelomys devius*,

Oligoryzomys vegetus, *Reithrodontomys creper*, *Rheomys raptor*, *Rheomys underwoodi*, *Scotinomys xerampelinus*) (Table 1.1).

1.5 Cryptic species and current taxonomy information

Detection of endemism might increase if improvement in methodological approaches could allow the detection of cryptic species, which are defined as species morphologically indistinguishable but reproductively isolated (Allendorf *et al.* 2013). During the field work conducted in this thesis, uncertainties in taxonomy for terrestrial small mammals were apparent. These uncertainties could reflect the low knowledge available in terms of genetics for these species, which undoubtedly could show high levels of cryptic speciation. Although most of the species of small mammal in Costa Rica are considered in the Least Concern category from the IUCN (Table 1.1), the review of genetic information from the GenBank indicated mostly incomplete or non-existent data for endemic species. This contrast perhaps explains the uncertain identity of several specimens during fieldwork, which would indicate a potential current high level of cryptic species without any genetic information available before this thesis.

Probably, the rest of the species (not endemic) follow the same pattern in relationship to cryptic species, since there is also a deficiency on genetic information for those. The overall absence of phylogenetic information in small mammals, was already highlighted by Reid (2009), by referring to this group as having high potential to describe new species, especially in the Neotropics, based on application of chromosomal and molecular studies. For example, Ordoñez and Bradley (2019) suggested that the complex distribution pattern of rodents in Central America can be explained by the effect of the Great American Biotic Interchange (GABI). These authors emphasise the existence of high genetic differentiation among species reflecting demographic changes during several geological events in Mesoamerica.

Table 1.1. Regional endemic terrestrial small mammals of Costa Rica. Compilation of regional endemism, IUCN category and GenBank information available.

	Regional Endemism			**IUCN Category**	**GenBank Information**
	CR	PA	NI		
Species					
Cryptotis gracilis Miller, 1911	✓	✓	-	VU	P
Cryptotis nigrescens J. A. Allen, 1895	✓	✓	-	LC	P
Heteromys nubicolens Anderson & Timm, 2006	✓	-	-	NE	P
Heteromys oresterus Harris, 1932	✓	-	-	LC	P
Nephelomys devius Bangs, 1902	✓	✓	-	LC	OG
Oligoryzomys vegetus Bangs, 1902	✓	✓	-	LC	P
Reithrodontomys brevirostris Goodwin, 1943	✓	-	✓	LC	P
Reithrodontomys creper Bangs, 1902	✓	✓	-	LC	T
Reithrodontomys musseri Gardner & Carleton, 2009	✓	-	-	NE	NI
Reithrodontomys paradoxus Jones & Genoways, 1970	✓	-	✓	DD	NI
Reithrodontomys rodriguezi Goodwin, 1943	✓	-	-	LC	NI
Rheomys raptor Goldman, 1912	✓	✓	-	LC	T
Rheomys underwoodi Thomas, 1906	✓	✓	-	LC	NI
Scotinomys xerampelinus Bangs, 1902	✓	✓	-	LC	T

Regional endemism according to geographical distribution of the IUCN Red List: Costa Rica (CR), Panama (PA) and Nicaragua (NI) (https://www.iucnredlist.org/species/19487/22354257). Three IUCN categories described by threat level for each species were: Vulnerable (VU), Least Concern (LC) and Data Deficient (DD). Species without information in the database of the IUCN were named as Not Evaluated (NE). Information from the GenBank (https://www.ncbi.nlm.nih.gov/genbank/) was indicated as following: complete *cytb* (T), partial *cytb* (P), information only available for other genes (OG), non-exist genetic information (NI).

Recent editions of the main books about the natural history of Costa Rica point out the potential for research on small mammals in the country combining available taxonomic information with genetics. Nowadays, species of terrestrial small mammal listed for Costa Rica (less than 500g) have some species with not only a lack of information about natural history or ecology but also in terms of genetics. A brief compilation of some information for the genera occurring in Costa Rica is presented below, following Wainwright (2007) and Reid (2009):

Caluromys

Caluromys is a genus with only one species, *C. derbianus*, with a wide latitudinal and altitudinal distribution in Costa Rica. The literature indicates morphological differentiation in Costa Rica that would be ideally complemented by genetic studies. Probably, this species has a key role for ecosystems because it disperses seeds, pollinates flowers and is considered a common prey species.

Marmosa

This genus is represented in Costa Rica by three species (*M. mexicanus*, *M. alstoni* and *M. zeledoni*) with very limited information available in terms of ecology, behavior, and genetics. Currently, the species are clearly differentiated mainly by distribution and morphologic traits, however historically there have been uncertainities in their classification. Genetic studies would provide valuable complementary information since there is practically no genetic information on specimens from the country.

Metachirus

Only one species is represented in this genus: *M. nudicaudatus*. This species is considered uncommon with a distribution over the whole country. No genetic studies are reported for Costa Rica, where this species is exceptionally rare, despite considered common in South America. The rarity of *M. nudicaudatus* could be an interesting aspect for genetic analysis.

Philander

Only one species is represented in this genus: *P. melanurus*. This species is considered abundant in deciduous and evergreen forest, secondary growth often found near to streams. This species is considered a good disperser of seed in secondary growth forest, which makes it an excellent organism in the regeneration of forests. Genetic information is limited in Costa Rica.

Cryptotis

This genus is represented in Costa Rica by five species (*C. gracilis*, *C. merriami*, *C. nigrescens*, *C. monteverdensis* and *C. orophila*). Currently, species are clearly differentiated mainly by distribution and morphological traits, however historically there have been some inconsistencies in the identification of species. Comprehensive genetic studies are not available, and should be intergrated with complementary information, such as morphological, ecological and behavioural.

Heteromys

This genus is one of two included in the family Heteromyidae. Until recently, three species constitute this genus in Costa Rica: *H. desmarestianus*, *H. nubicolens* and *H. oresterus*. These species differ regarding to the degree of threat. *H. desmarestianus* is common, widespread and abundant in deciduous, evergreen and secondary growth forests. This species is considered a species complex along with *H. goldmani (*Anderson and Timm 2006*)*. On the other hand, *H. nubicolens* is a species with a restricted distribution and it is difficult to distinguish from *H. desmarestianus*. *H. nubicolens* is common in mature and secondary cloud forest where the species is limited. Finally, *H. oresterus* is one of the most rare species of terrestrial small mammals in Costa Rica, due to its very restricted distribution in the highland oak forest in a small area near to El Copey de Dota.

Heteromys salvini has recently been added to this genus, a species with restricted distribution in the northwest of Costa Rica. This mouse is widespread over Mesoamerica in deciduous forest, brush, and weedy fields often near walls or rocks. This species is adapted to disperse and consume seeds. Its characteristic cheek pockets help it to transport and distributed seeds. For example, seeds of the Guanacaste tree (*Enterolobium cyclocarpum*) are more than 90% removed by this mouse, and are partially digested, which facilitates germination. This species is able to consume seeds that are toxic for other rodents. This small mammal species has multichambered tunnels, which constitute another way to dispersal seeds in areas where the number of individuals is very high.

Rheomys

There are two species in Costa Rica for this genus: *R. raptor* and *R. underwoodi*. Both are regionally endemic in Panama and Costa Rica. *R. raptor* is considered diurnal and nocturnal. *R. underwoodi* is poorly known. Both species are considered semiaquatic. Probably the difficulty in capturing and known narrow distribution of both *R. raptor* and

R. underwoodi are factors that limit our knowledge of them and for this reason they are considered rare species.

Sigmodon

This genus had some changes during recent years from molecular studies. Currently, there is considered only one species for Costa Rica (Bradley *et al.* 2008), which is *Sigmodon hirsutus*. Same authors separated specimens of *S. hirsutus* from the complex, *Sigmodon hispidus* spp. The distribution of *S. hispidus* is restricted to half of the United States and northeastern Mexico (Nuevo León and Tamaulipas). For *S. hirsutus* the distribution in Costa Rica is on both slopes, however on the Caribbean side this species is considered rare or absent.

Handleyomys

This genus is represented by only one species: *Handleyomys alfaroi*. This species is often trapped on the edge of small streams. It has a widespread distribution in Mesoamerica, but is also found in South America down to Ecuador. It is considered an uncommon species with an altitudinal distribution from the lowlands to 2500 m.a.s.l., but it is normally associated with elevations around 1500 m.a.s.l.

Melanomys

Melanomys chrysomelas is the unique species currently described for this genus previously referred to as *M. caliginosus*. This is a mostly diurnal species, solitary and mostly terrestrial. Its distribution ranges from Honduras to Colombia, the northwest of Venezuela and the southwest of Ecuador. It is associated with lowlands up to 1400 m.a.s.l. and is normally found around 700 m.a.s.l. This is considered a common to abundant species, associated to brushy secondary growth, and edges of evergreen and semideciduous forests.

Nephelomys

In Costa Rica *Nephelomys devius* is the only species of this genus, which is a regional endemic from Costa Rica and Panama. This is very similar to the other species distributed towards the extreme south of Panama: *N. albigularis*. Nevertheless, *N. devius* is a species with an elevational distribution between 1000- 3000 m.a.s.l. It is common in wet highland forest along streams. It swims well and sometimes escapes by diving into the water. There is relatively poor knowledge about this species, which is probably linked to the high elevation range of its distribution.

Oecomys

This is a genus with only one species: *Oecomys trinitatis*. The distribution of this species starts in the southwest of Costa Rica and is continuous to the southeast of Brazil, but it is also found in the Guianas and the east of Colombia to Perú, including Trinidad and Tobago. Its altitudinal range includes lowlands to 1500 m.a.s.l. The species is considered uncommon in Central America. It is semiarboreal and usually it is trapped in vines. Formerly it was considered as: *O. concolor*.

Oligoryzomys

Two species are included in this genus: *O. vegetus* and *O. fulvescens*. Both species are very similar morphologically. *O. vegetus* was formerly considered a subspecies of *O. fulvescens*. *O. vegetus* is restricted to the mountains of Costa Rica and west of Panama. Its elevational distribution goes from 850 to 3000 m.a.s.l. This species is common in secondary growth forest. In the other hand, *O. fulvescens* is distributed from México to Central America (except high mountains and wet Caribbean lowlands), and to Ecuador. Its altitudinal distribution goes from lowlands to 2000 m.a.s.l. but usually it is found below 1500 m.a.s.l. *Oligoryzomys* is easily confused with *Reithrodontomys*.

Oryzomys

Oryzomys couesi is the unique species of this genus and it has a widespread distribution from the extreme south of Texas, United States, through Mexico to central Panama, until the northwest of Colombia. It is considered a common species and widespread. This species is terrestrial and semiaquatic, but also a good climber. Usually, it is trapped above the ground. It was formely considered a subspecies of *O. palustris*.

Sigmodontomys

This is another genus with a single species: *Sigmodontomys alfari*. It is distributed from the southeast of Honduras to the northwest of Ecuador and the northwest of Venezuela. It is found from lowlands to 900 m.a.s.l. This species is locally common in wet and secondary forest. Generally it is rare in the north of Panama. This species is poorly known and is considered nocturnal. It was formerly placed in the genus *Nectomys*. The genus is currently considered to be closer to *Oryzomys*, not to *Nectomys*.

Tanyuromys

This is a recently described genus with two species (Timm *et al.* 2018): *T. aphrastus* and *T. thomasleei*. Only *T. aphrastus* is a regionally endemic species, distributed in Costa Rica and in Panama between 1200 to 2500 m.a.s.l.. This species

was formerly known as *Sigmodontomys aphrastus*. It is considered a rare species and its behaviour and ecology are unknown.

Transandinomys

Two species are represented in this genus for Costa Rica: *T. bolivaris* and *T. talamancae*. *T. bolivaris* is mainly distributed on the Caribbean slope from the east of Honduras to the north of Ecuador. It occurs from the lowlands to 1500 m.a.s.l., normally at mid-elevation between 600-900 m.a.s.l. It is a species considered uncommon. It is found in mature and evergreen forest. On the contrary, *T. talamancae* is widespread, occurring from the south of Costa Rica to Ecuador and the north of Venezuela. This species is considered common and abundant in evergreen forest between lowlands to 1000 m.a.s.l.

Zygodontomys

For this genus *Z. brevicauda* is a unique species. The species is distributed from the south of Costa Rica to the north of Brazil, the Guineas and Venezuela. Trinidad and Tobago are included in the distribution together with some small islands of Panama and Venezuela. It is considered a common species and often is abundant. It is nocturnal and terrestrial. Its diet includes seeds, fruits, and green plant material.

Nyctomys

Nyctomys sumichrasti is the only species in this genus. This mouse is arboreal and it is considered an uncommon species but is probably locally common to abundant. It is found in evergreen and semideciduous forest. It is present on both slopes, in some areas up to 1800 m.a.s.l. Its current distribution includes Jalisco and Veracruz, México, through Central America, except the Yucatan Peninsula, to the east of Panama.

Ototylomys

Ototylomys phyllotis is a species distributed from Guerrero, Chiapas, Mexico, to the south of Costa Rica. It occurs from the lowlands to 1900 m.a.s.l. This species is fairly common in a wide variety of habitats. Its diet includes fruit, seeds, and vegetable matter. It has typically scales in the tail like rings.

Tylomys

Tylomys watsoni is regional endemic from the north of Costa Rica to the east of Panama. It is absent from the northeast of Costa Rica. It is generally uncommon. This species mostly occurs in mid-elevation evergreen forest on the Caribbean slope. It is considered a semiarboreal species. Is considered rare and is poorly known throughout

its range. Two subspecies have been described in Panama: *T. w. panamensis* and *T. w. fulviventer*.

Scotinomys

Two species are recognized for this genus: *S. teguina* and *S. xerampelinus*. They are really similar species. However, *S. teguina* has a widespread distribution from Oaxaca, Mexico, through the highlands to the west of Panama at elevations from 900 to 2900 m.a.s.l. On the other hand, *S. xerampelinus* is a regional endemic species restricted to the highlands of Costa Rica and the west of Panama. The distribution in elevation ranges from 2100 to 3400 m.a.s.l. A typical characteristic of both species is the sound *they* make, and this is the reason why they are considered singing mice. Behavioural and ecological characteristics of both species are poorly known. Both species are mainly insectivorous.

Peromyscus

*The*re are two species in Costa Rica *P. nudipes* and *P. nicaraguae*, those species were previously considered one species *P. nudipes*. Based on fieldwork of the present study, this species might be considered "trap-happy" (Pacheco *et al.* 2013), according to the frequency of recaptures of the same individuals. This species is considered a regional endemic, and it is distributed from mountains of Costa Rica to the west of Panama. It is referred as a common species in suitable habitats. It is found in tall evergreen forest, riparian areas in deciduous forest, secondary growth, and shaded coffee fields.

Reithrodontomys

There are nine species currently reported for this genus in Costa Rica, according to Villalobos *et al.* (2016). Those authors included three species not listed in previous literature: *R. cherrii*, *R. garichensis*, *R. musseri*. The natural history for these three species is poorly known. The rest of the species were historically identified without difficulties. However, in this study some specimens trapped for this genus were not coincident with any species described according with literature available, especially for individuals captured in the region of Valle del Silencio in the Amistad International Park. Some species are considered uncommon or rare: *R. brevirostris, R. paradoxus* and *R. rodriguezi*. Endemism is considered high among species of this genus, and most of species are regional endemics from Costa Rica and Panama. The altitudinal distribution

is highly variable among species of this genus, however, most of the species occur at intermediate elevations of all range records posted for this genus.

Hoplomys

Hoplomys gymnurus is the unique species reported for this genus. This is one of the spiny rats in Costa Rica and it is considered uncommon to locally abundant in evergreen forest. Its distribution includes the north of Honduras to the northwest of Ecuador. Its elevational distribution goes between lowlands to 800 m.a.s.l. This species is often associated with creeks or streams, and its diet is mainly fruits, insects, and plants.

Proechimys

P. semispinosus is the only species in this genus. Its distribution is defined from the east of Honduras to the southeast of Ecuador. It occurs between the lowlands and 800m.a.s.l. It is considered a common species, often abundant in evergreen forest and deciduous forest. This is a nocturnal species and its diet comprises fruits, seeds, plants, fungi, and insects.

Neogale

N. frenata is the only carnivore listed in this research as a terrestrial small mammal. This species has a widespread distribution from the south of Canada and continues to United States, México, Central America to Venezuela including the west of the Andes to Bolivia. In Costa Rica it is rare from both the Pacific lowlands and the Caribbean lowlands. It is mostly diurnal, and fossorial with a long, narrow body that allows it to get into the burrows of its prey like mice. It is considered a common species but inconspicuous above about 1000 m.s.a.l.., in both forest and perturbed ecosystems.

The above short summary of the main genera of small terrestrial mammals occurring in Costa Rica shows the overall limited knowledge in terms of natural history, taxonomy, status, distribution, habits, and habitat. Moreover, it also illustrates the absence of information for a considerable number of species, thus limiting our knowledge and hampering conservation efforts. Also, the latest compilation of this type of information that includes Costa Rica is from Reid (2009), and it is out of date.

1.6 Genetic tools and current use for conservation in Costa Rica.

Genetic information is a key field of research for small mammals and particularly necessary for their conservation. Describing, measuring, and interpreting genetic variation has become a central component of modern wildlife population biology (Mills

2013). The common way of measuring and comparing the genetic variation within different populations is heterozygosity, which is defined as the proportion of individuals that are heterozygous, ranging between zero and one (Allendorf *et al.* 2013). Another way to assess the genetic diversity, is determining the level of polymorphic loci, and the number of alleles per locus. The number of studies on genetic diversity of terrestrial small mammals from Costa Rica, and surrounding countries, is very limited (Bradley *et al.* 2008, Rogers *et al.* 2009, Bedford and Hoekstra 2015, Timm *et al.* 2018).

There is an obvious divergence between the use of advances in technology for genetics and the information available for terrestrial small mammals, especially in relation to the high biodiversity associated to tropical countries. According to Hohenlonhe *et al.* (2021), genetic monitoring of natural populations has played an important role in conservation, including both monitoring of genetic diversity and using genetic tools to monitor other aspects such as population size or hybridization. Most of the species from Costa Rica have no genetic data available, even at the most basic levels of information, as presented in Table 1.1.2. The field of conservation genetics has matured, and it has come to play an essential role in the conservation and management of species (Allendorf 2017). However, unfortunately conservation genetics has not grown at the same rate in Costa Rica during the last decades. Nevertheless, applications of current conservation genetics tools in Costa Rica have an extraordinary potential to delimit adequately the distribution of terrestrial species of small mammals in Costa Rica to allow their conservation. Among the different available molecular markers, mitochondrial DNA (mtDNA) is referred by Mills (2013) as very useful tool for studying population biology, mainly due to three main properties: i) it is present as multiple identical copies (thousands within mammalian somatic cells); ii) it has a maternal inherence of haploid mtDNA molecules, without recombination; and iii) it is easy and not expensive to assess, often allowing the phylogenetic analysis and the reconstruction of the evolutionary history of species.

Mitochondria have their own DNA replication, transcription, and protein-synthesis machinery, which operates independently of the corresponding machinery in the rest of the cell, and they have been able to accommodate minor changes to the otherwise universal genetic code (Johnson *et al.* 2019). Hence, mitochondrial DNA provides a different perspective of the genetic structure of natural populations from nuclear DNA because of its maternal inheritance and general lack of recombination between mtDNA molecules (Allendorf 2017).

The first studies of DNA variation in natural populations examined mitochondrial mtDNA because it is a small molecule (approximately 17,000 base pairs in vertebrates and many other animals), relatively easy to isolate from genomic DNA, since it has many copies per cell (Allendorf 2013). Moritz (1994) identified the uses of mtDNA in conservation as an analysis which fall into two different areas: (i) “gene conservation”- the identification and management of genetic diversity, and (ii) “molecular ecology” - the use of mtDNA variation to guide and assist demographic studies of populations. Mitochondrial genes like cytochrome *b* (*cytb*) or cytochrome oxidase subunit 1 (*COI*) are useful to determine genetic variation in DNA from natural populations, through analysis of their sequence variation. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis and sequencing of the mitochondrial *cytb* gene can be used as a robust tool in identification of different animal species including domestic, wild and fish species (Farag *et al.* 2020). In the same way, taken together, *COI* DNA barcodes can provide a standardized way to identify molecularly different species (Pentinsaari *et al.* 2016).

In this regard, resolution of cryptic species uncertainties based on genetic tools, namely by using mtDNA, allowed an improvement in conservation policies. Allendorf (2013) highlight that the use of *COI* might help to separate species indistinguishable morphologically, since this molecular marker can identify several species, namely cryptic species that have similar phenotypes. The relevance of deepening the knowledge of cryptic species lies in the fact that when a single nominal species actually includes more than one biologically distinct species, alpha diversity is underestimated, and conservation priorities are improperly assigned because endemic species are overlooked (Lohman *et al.* 2010).

Nowadays, the detection of cryptic diversity based on molecular markers allows promising outcomes for species that require differing conservation efforts. Rueness *et al.* (2011) refer to the grey wolf (*Canis lupus*) inhabiting Africa as a cryptic species, with an urgent need to assess the possibility of several cryptic species included in that taxonomic complex that require special policies of conservation. Coincidently, there is a perception that species with wide distribution ranges are frequently subdivided into genetically distinct forms, often not discriminated by morphology and that are therefore ‘cryptic’, as the field vole, *Microtus agrestis,* which was divided in three species (Paupério *et al.* 2012). Several species in tropical countries have a limited number of studies to describe the genetic diversity of small mammals, and thus the putative hidden diversity

was not yet been assessed, which might hamper the conservation of these mammals in key regions with high levels of biodiversity (Bradley 2004, Corley *et al.* 2011, Bagley and Johnson 2014, Pérez and Vázquez-Dominguez 2015, Bradley *et al.* 2016).

In terms of cryptic species among terrestrial small mammals in Costa Rica, in practice there is no information for the vast majority of species. Villalobos-Chaves *et al.* (2016) identified this gap of knowledge for rodents since there are only a few publications that allow identification of these species with high levels of certainty, and therefore cryptic variation is probably quite high. This pattern is similar for the rest of species of terrestrial small mammals based on similar information available for the country.

For Costa Rica, the number of cryptic species is probably high due to the variety of environmental gradients as highlighted in previous sections. Consequently, high local ecological adaptation may have a deep effect on species evolution since adaptation depends on the current environmental demands, forcing distinctive features by selection pressure (Kardong 2012). McCain and King (2014) found that mammals are good indicators of climate variation and its effects over different altitudinal ranges. They showed that species restricted to high elevations decrease their distribution, while species associated to low elevations expanded their distributions ranges toward high elevations.

1.7 Elevation gradients and species distribution

Studies of terrestrial small mammals have referred to the need to increase knowledge about the specificity of ecological ranges that determine species richness. For instance, McCain and Grytnes (2010) mentioned that evaluation of biotic and abiotic conditions across altitudinal gradients has great potential to reveal patterns of species` distribution and richness. In this sense, Stevens *et al.* (2019) proposed four patterns of gradients that influence mammalian biodiversity distributions: the species-area relationship., latitudinal gradients., elevational gradients., and geohistorical gradients. Herein, following the advice of those authors, elevation is considered a complement for geophysical, environmental, and physiographic factors that can influence fundamental processes and ultimately the location and overlap of species ranges, resulting in the geographic variation typically associated with mammals.

Regarding information available for terrestrial small mammals, distribution in an elevation gradient for this guild group display a mid-elevation peak in diversity. The mid-domain effect (MDE) is defined by several authors (Brehm 2007, Cowell *et al.* 2016, Xu

et al. 2021) as an overlap in the distribution range of species along altitudinal gradients., with the highest intensity of overlap at intermediate elevations. However, this idea has been widely debated. Hence, several studies are consistent with the proposal that terrestrial small mammals follow a mid-elevation peak (McCain 2005, McCain and Grytnes 2010, Wu *et al.* 2013). On the other hand, additional data for small mammals showed a partial mid-elevation peak, only for some studied species, with the recommendation for broader comparative studies to examine whether the results presented are common in other taxa, regions and scales (Chen *et al.* 2017). Contrarily, Bateman *et al.* (2010) reported that small-mammal richness peaked towards the summit of the gradient and not at one-half the maximum altitude predicted by MDE theory.

The distribution of species in altitudinal gradients respond to natural combinations of environmental factors, but clearly direct and indirect human impact also define the distribution of terrestrial small mammals. Wildlife populations are affected by human activities in different ways including habitat loss, fragmentation, fire and overhunting. However, climate change is considered one of the greatest stressors, because animals, plants and other organisms can be impacted by dramatic changes in physical conditions, namely temperature and humidity (Mills 2013). Organisms respond directly to this variation through physiological changes, uncommon behaviours, abundance, and modifications to distribution (Franks and Hoffmann 2012, Liu *et al.* 2015).

The vulnerability of species is related to climate change through changes in climatic variables and extreme conditions, which affect physiological limits, habitat or trophic specializations, natural history characteristics, or interactions with other species (Moritz and Angulo 2013). Those traits directly impacted by climatic modifications will have more effect at the individual level and consequently the particular reaction of each species is related to the limitations imposed by the environment (McCain and King 2014).

1.8 Climate change and future research

Response to climate change is an interaction between different elements where some species have high sensitivity. In contrast, other species have mechanisms to adapt easily and successfully face new scenarios. For instance, Buckley *et al.* (2015) showed four scenarios to assign a body temperature within the range of potential operative temperatures: (i) lizards use microclimates randomly (thermoconformer); (ii) lizards use microclimates randomly but avoid extremes outside their critical thermal limits (avoid extremes); (iii) lizards thermoregulate as close to their preferred temperature as possible

when the benefit outweighs the cost (thermoregulation with costs); and (iv) lizards thermoregulate as close to their preferred temperature as possible without a cost (thermoregulation without costs).

In the case of mammals, responses to this phenomenon are less evident than in reptiles. However, Moore *et al.* (2015) demonstrated how mammals can have direct and indirect consequences. They demonstrated an indirect effect of climate change in some herbivorous mammals, with plants that constitute part of the diet of these animals, concentrating their toxicity due to the increases in temperature and this alteration making them inaccessible items in their normal diet. In the same study, they showed how these animals reduce their liver size as acclimation to the increase in the temperatures and consequently their ability to metabolize these toxic substances is reduced.

Mammals with small populations and reduced birth rates have a high risk to produce deleterious mutations and unfit progeny (Bromham 2016). The ability to produce offspring is one of the most important factors to reduce the risk of effects related to molecular variation. An understanding of successful adaptation to a quickly changing climate requires knowledge of the following factors: 1) generation time, 2) population size and 3) population structure (Hoffmann *et al.* 2015).

Due to their population parameters, small mammals have been considered appropriate to evaluate the effects of climate change mainly for two reasons: limited dispersal ability and short life-cycles (Rowe *et al.* 2014). Accordingly, small mammals have been used in models of metapopulations, to evaluate the effect of the climate change (Haby *et al.* 2013). Additionally, small mammals provide interesting perspectives in relation to the effect of climate change on populations associated with tropical ecosystems (Prost *et al.* 2013).

Phylogenetic diversity and phylogeography enable researchers to develop inferences about complex evolutionary processes and facilitate the understanding of evolutionary history using multiple traits of species to face climate change (Pio *et al.* 2014). For this matter, fresh samples and historic samples (from museums) help to understand the effect on climate change in the genetic diversity (Bi *et al*. 2013) using NGS (“Next-generation sequences”) tools (Rowe *et al.* 2011, Bi *et al.* 2013, Nachman 2013, McLean *et al.* 2015).

During the last decade, DNA sequencing welcomed a revolution with NGS analysis (Fig. 1.8), specially to evaluate biodiversity in complex biological and ecological matrices

(Galimberti *et al.* 2015). NGS analysis facilitates robust studies about demographic history of populations, adaptive variation related with the fitness, and local adaptations (Steiner *et al.* 2013). For instance, in the case of American pika (*Ochotona princeps*), Lemay *et al.* (2013) identified variation in the genomic sequences along an altitudinal gradient using NGS analysis. They founded differential gene activity (associated with haemoglobin alpha chain) only expressed in particular conditions through altitudinal gradients related mainly with temperature.

Based on the above evidence, lack of information for terrestrial small mammals of Costa Rica can be preliminarily solved using global open databases. These databases, provide valuable information to assess temporal knowledge gaps, namely in species distribution and relative abundance. The GBIF (Global Biodiversity Information Facility) offers significant historical and recent presence records, mostly provided by museums, which can enable studies to evaluate species distributional trends over time. In terms of biomes, Holdridge's Life Zones System (1967) consider multiple environmental factors and constitute a complementary tool to validate the species distribution patterns of small mammals.

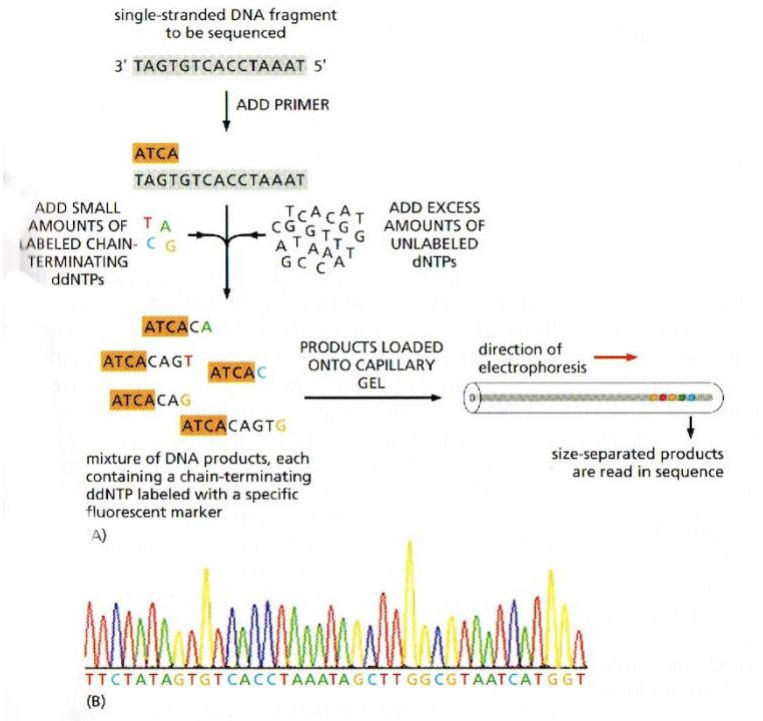


Figure 1.8. Automated dideoxy sequencing relies on set of four ddNTPs, each bearing a uniquely colored fluorescent tag. (A) To determine the complete sequence of a single-stranded fragment of DNA (gray), the DNA is first hybridized with a short DNA primer (orange). The DNA is then mixed with DNA polymerase (not shown), an excess amount of normal dNTPs, and a mixture containing small amounts of all four chain-terminating ddNTPs, each of which is labeled with a

fluorescent tag of a different color. Because the chain-terminating ddNTPs will be incorporated only occasionally, each reaction produces a diverse set of DNA copies that terminate at different points in the sequence. The reaction products are loaded onto a long, thin capillary gel and separated by electrophoresis. A camera reads the color of each band on the gel and feeds the data to a computed that assembles the sequence (not shown). The sequence reads from the gel will be complementary to the sequence of the original DNA molecule. (B) A tiny part of the data from such an automated sequencing run. Each colored peak represents a nucleotide in the DNA sequence. (Adapted from: Alberts *et al.* 2019).

1.9 Objectives and Hypothesis

1.9.1 Rationale

Terrestrial small mammals are considered essential organisms to keep the natural balance in the ecosystems, mainly by the quantity of biomass that this group represents, their role as prey and as seeds dispersers. These organisms are found everywhere in the world and have adapted to a wide variety of ecosystems. These adaptations arise from the relatively rapid ability to modify their morphological, behavioural, and physiological traits in response to environmental instability at specific moments in their evolution. Some characteristic factors of these species facilitate this rapid adaptation, such as their limited dispersal capacity and short life cycles. All these features suggest that species of small mammals are good indicators of the characteristics of the ecosystems that they inhabit. In this process of adaptation, not only emerging species with a great dispersal capacity, but also other species, are limited in their ability to colonize new environments since they are highly dependent on local conditions. In this sense, a topic with great potential for research in terrestrial small mammals is the effect on their distribution along elevation gradients. Moreover, a sensitive issue of conservation is the endemic species distribution, which can be associated with different gradients in an altitudinal range of distributions. This is the main reason for the need to delimit adequately the species distribution to address specific policies in conservation and management. Several studies indicate that small mammals follows a distribution based on the principles of the Mid-domain Effect (MDE). However, several studies have contradicted this effect, and various of them found a partial effect only for some species and contrarily, there are other findings with inconsistent results about postulates for this theory. In addition, Costa Rica might host a considerable number of endemic species due to its unique geomorphological characteristics, making this small territory a refuge for many species throughout the evolutionary history of several species. The influence on species distribution caused by the effect of altitudinal gradients has been the subject of research in numerous studies worldwide. In particular, Costa Rica is a country considered a laboratory on this subject for different taxonomic groups due to its marked

characteristics in terms of relief. The specific case of Cordillera de Talamanca in southern Costa Rica where a variety of factors are mixed such as: a marked altitudinal gradient, the bridge effect between two ancient continental masses and the direct influence of the effect of two oceans in a narrow territory. This area offers a complex scenario for evolution of species not properly explored yet, since few research efforts had been made. A lack of resources for detailed research probably explains the existing under-evaluated number of cryptic species, not only for this area in Costa Rica but also for the rest of the Neotropical region. Human disturbance has directly (loss of habitat by deforestation) or indirectly (climate change) affected the distributions of terrestrial small mammals. Climate change alters the natural dynamics of species distributions, mainly influenced by two factors in the case of terrestrial small mammals: temperature and precipitation. Normally, species distributed in places where there are temperature increases move to high elevations. Implications for organisms and their habitat of these alterations have been not properly evaluated in Costa Rica. Conservation genetics offers a wide range of research to understand complex ecosystems under a context of tropical conditions, but also provides tools to solve inconsistencies at the taxonomic level, and thus to better support adequate conservation policies towards the protection of species and their ecosystems.

Our definition of small mammals is derived from Lim and Pacheco (2016) for non-volant small mammals. They categorize this group based on similar ecological requirements, with the majority being predominantly nocturnal and terrestrial. We refined this definition using the criteria outlined by Rowe *et al.* (2015), specifically focusing on animals weighing less than 500g. By applying this criterion, we excluded two other groups traditionally considered small mammals: squirrels (family Sciuridae) and bats (order Chiroptera). Therefore, our target taxa were non-volant small mammals (< 500 g), particularly including shrews, small marsupials, rodents, and one carnivore.

1.9.2 Objectives

General objective

This study aimed to assess the diversity and patterns of distribution of terrestrial small mammals in Costa Rica, while examining the influence of environmental conditions and human disturbance, based on elevations and biome characterisations and databases for Costa Rica.

Specific Objectives

To address the general goal, several specific objectives were defined, namely:

i. to identify the distribution patterns of small mammals along altitudinal gradients according to variation in environmental conditions.,
ii. to implement baseline information for small mammal studies and field work in Costa Rica.,
iii. to determine genetic diversity and phylogenetic relationships of small mammals across Costa Rica and along altitudinal gradients.,
iv. to determine putative genetic cryptic diversity within small mammals.,
v. to propose conservation measures based on the analysis of genetic composition and distribution of small mammals according to possible effects of climate change.

1.9.3 Hypothesis

Terrestrial small mammals have low dispersal, a short life span and a high breeding rate, therefore they are good indicators to address the effect of climate change on ecosystem biodiversty. Costa Rica is located in a biodiversity hotspot region, and due to its physiography with a steep mountain system together with a wide river system along the country, it has the highest density of species per square kilometre in the world. Thus, we hypothesise that terrestrial small mammals species show different distribution patterns along altitudinal gradients, high genetic diversity across the country and high levels of cryptic diversity

1.9.4 Structure of the thesis

This thesis is composed by four chapters, and four articles (submitted or under preparation for publication). Chapter 1 provides a general introduction to this work, conducting a comprehensive review of various historical and current factors that have influenced the distribution of small mammals in Costa Rica. These factors include extensive deforestation processes from the 1960s to the 1990s, as well as the development of the current system of protected areas that reflects Costa Rica’s legislation on biodiversity and the preservation of natural ecosystems. This chapter also examines the evolution of the country's territory in geomorphological terms and its impact on endemism in small mammals. Furthermore, it emphasizes the significance of combining taxonomic studies with contemporary genetic tools to comprehend the

landscape of cryptic species and propose appropriate management strategies for them. Finally, this chapter presents the research's objectives, hypotheses and thesis strcture.

Chapter 2 focuses on defining and evaluating the diversity and distribution patterns of small terrestrial mammals in Costa Rica, while examining the influence of environmental conditions and human disturbances. Data on small mammals were obtained from the GBIF database, local museums, and field work, and analyzed primarily using Holdridge's Life Zones system. The study also considers the impact of human disturbances during two periods: deforestation (1960-1984) and the establishment of protected areas (1994-2018). The dataset comprises 2324 records of 46 species which were used for all analyses (in total, the current literature includes 52 species). This work comprises Article 1. Additionally, Chapter 2, includes Article 2, where a capture protocol specifically designed for small mammals, highlighting the species distribution within the Valle del Silencio sector of La Amistad International Park as one of Mesoamerica's most pristine areas, is presented. This article aims to provide basic information for studies on terrestrial small mammals from Costa Rica.

In Chapter 3, Article 3 provides details regarding the genetic analysis of samples collected during captures performed during this study, across various locations in Costa Rica, in addition to samples obtained from the mammal collections of the National Museum of Costa Rica and the Museum of Zoology of the National University from Costa Rica. Supplementary sequences from the GenBank were also utilized, offering an overall phylogenetic analysis of genera and species of terrestrial small mammals occuring in Costa Rica, using mtDNA. In addition, Article 4 investigates the genetic variation on *Scotinomys* genus and the extent of divergence between its two constituent species: *Scotinomys teguina* and *Scotinomys xerampelinus*.

Chapter 4 encompasses a General Discussion, by compiling and synthetizing the main findings obtained throughout this work, its discussion, and the main conclusions. Finally, the principal annexes associated with the various sections of the presented articles, are presented after Chapter 4.

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Chapter 2: Distribution of species and protocol for captures.

Chapter 2: Article I. Distribution patterns of terrestrial small mammals along altitudinal gradients and biomes in Costa Rica

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2.1.1 Abstract

Increasing knowledge of species diversity and distribution patterns is fundamental for biodiversity conservation under current climate change scenarios. Costa Rica holds one the highest levels of biodiversity in the world, but the information available on terrestrial small mammals is still minimal. Therefore, this study aimed to assess the diversity and

distribution of terrestrial small mammals, whilst examining the influence of environmental conditions and human disturbance. To achieve this aim, presence records of small mammals were compiled from the GBIF database and data from local museums in Costa Rica. Location was classified according to the Holdridge Life Zone system and by elevation. Human disturbance was analyzed using the seven species with the highest number of available records and partitioned into two time periods: deforestation (1960-1984) and establishment of protected areas (1994-2018). The final dataset included 2324 records from 46 species. For numbers of records and species richness we observed a “mid-domain effect” with high values at intermediate elevations (1218-2436 m.a.s.l) but abundance and richness values were also high in the lowlands (0-1218 m.a.s.l). Clearly lower abundance and richness was found in the highlands. Comparison of the two time periods for the selected seven species showed two main trends: an increase in average altitudes within distribution ranges by moving up to intermediate altitudes from the lowlands, and a decrease in average altitudes by moving down from the highlands. This expansion and contraction of altitudinal ranges between the periods may be explained by responses of small mammals to current environmental change. The terrestrial small mammals in Costa Rica appear to be good indicators of habitat and climate change.

Keywords

Mid-domain effect, species richness, species distribution, GBIF, climate change, conservation biology

2.1.2 Introduction

Costa Rica has approximately 5% of the world’s biodiversity, with more than 95,000 species described to date (Bermudez and Obando 2021), being considered one of 36 hotspots around the world (MINAE, 2016). This diversity is the result of the geographic position of Costa Rica in the Neotropics (influenced by two oceans and two continental landmasses) and its geomorphology, including a mountain system that provides a range of different microclimates (Obando 2007). Costa Rica was also influenced by the massive faunal interchange after the formation of the Panamanian land bridge, 2-7 million years ago (Wainwright 2007), with Central America acting as a species filter (Bemmels *et al*. 2018, Coates 1992, Kirby and MacFadden 2005).

These factors contribute to the high number of mammal species (249) currently recognized in Costa Rica (Rodríguez-Herrera *et al*. 2014) and underlie different aspects

of their ecology, genetics, physiology and behavior (DeMattia *et al.* 2004, Fleming 1974, McCain 2004, McCain 2005, Ribble and Rathbun 2018).

Furthermore, Costa Rica has long been considered an optimal location in the tropics to test different hypotheses to explain gradients of species richness (Blake and Loiselle 2000, Boyero *et al.* 2011, Colwell and Hurtt 1994, Janzen and Wilson 1991, McCain and Bracy 2013, Stevens, 1989). For example, Rapoport's rule focuses on two factors, latitude and elevation, to explain species range sizes (Stevens 1989), although, based on studies in Costa Rica the rule has been questioned for birds, frogs and salamanders in relation to altitude (McCain and Bracy 2013). Instead Szewczyk and McCain (2016) suggested a complex web of explanatory factors beyond simply altitude.

In the case of small mammals, reported peaks in species richness at intermediate elevations may result from a productivity-diversity relationship, where temperature decreases and precipitation increases with elevation (McCain *et al.* 2018). Microclimate conditions represent a fundamental factor for small mammals reflecting conditions near or under the ground moderated by changes in temperature and humidity at different elevations (McCain and King 2014). Thus, over recent years terrestrial small mammals have been considered a key group to test the "mid-domain effect" explaining species richness distributions (Bateman *et al.* 2010, Chen *et al.* 2017, Jones *et al.* 2019, Rowe *et al.* 2015, McCain 2004, McCain 2005). Studies on small mammals suggest that changes in community composition and species richness not only reflect environmental conditions and biological limitations along the altitudinal gradient, but also the combinations of neighboring habitats and resources offered at each elevation (Kamenišťák *et al.* 2020). Thus, the north-south mountain system along the center of Costa Rica creates biogeographic variation that promotes a high diversification of biomes that have not been fully explored (Enquist, 2002). In Costa Rica, terrestrial small mammals represent around 20% of the full mammalian diversity, but information available for this group is very scarce (Rodríguez-Herrera *et al.* 2014). It is also known that the great majority of small mammal species have a crucial role as primary prey for many vertebrate predators, as well as being key pollinators and seed dispersers (Lim and Pacheco 2016). For this reason, conservation of small mammals is critical to maintain these ecological processes. Additionally, distribution patterns of these species and their range shifts over time have been reported as predictors to assess the effect of current climate change (Boutin and Lane 2013, Santos *et al*. 2017).

Despite this ecological relevance, there has been little effort to understand the diversity, distribution and evolutionary history of the small mammals of Costa Rica (González-Maya *et al.* 2015, Janzen and Wilson 1991, Reid 2009, Rodríguez-Herrera *et al.* 2014, Villalobos-Chaves *et al.* 2016, Wainwright 2007). Here we begin to address this knowledge gap by accessing global databases. The GBIF (Global Biodiversity Information Facility) provides a wealth of historical and recent records, mostly provided by museums, which can enable analyses of species distributional trends over time. In terms of biomes, the Holdridge Life Zone System (1967) considers multiple environmental factors and constitutes a complementary tool to evaluate species distribution patterns.

Thus, in this study we aimed to assess the diversity and patterns of distribution of terrestrial small mammals in Costa Rica, in the context of environmental conditions and human disturbance, based on altitudinal and biome records and databases for Costa Rica.

2.1.3 Materials and methods.

Study area

Costa Rica is a small, narrow landmass with a continental area of 51,100 km^2 located in Central America (8°0'-11°14'N and 82°32'-85°56'W) (González-Maya *et al.* 2015) in between two seas (Caribbean and Pacific) and with extraordinary altitudinal complexity. Costa Rica's origins date back 35 million years when the tallest mountain range in southern Central America, Talamanca, was the first island to appear between North and South America, due the activity of the Cocos and Caribbean tectonic plates (Wainwright 2007). Gradually, other islands arose to constitute the current irregular and narrow territory of Costa Rica, with a variety of geomorphological landscapes between zero to 3820 meters above sea level (Bergoeing 2017). This complex physical geography confers a complexity of biomes well described by Holdridge (1967) in his wide-ranging Life Zone System. The Holdridge Life Zone system is simple and objective, requiring only data on precipitation, temperature and elevation (Lugo *et al.* 1999). It divides Costa Rica into 12 life zones and 11 transitions zone (Kohlmann *et al.* 2010).

Forests in Costa Rica and their biodiversity were seriously threatened during the 1960s and 1970s due two main factors: accelerating population growth and expansion of crops (mainly banana and coffee). Consequently, during this period there was around 80% deforestation over the country (one of the highest in the world during this period)

creating a subsequent environmental crisis (Rosero-Bixby and Palloni 1998). To reverse this disastrous situation, the Biodiversity Law of Costa Rica was constituted during the 1990s, aligning with a variety of international treaties (Law N° 7788, 1998). This law defines a clear and strict policy to protect natural resources through a National System of Conservation Areas, which divides the country in 11 conservation administrative areas, and approximately 25% of the total territory into different management categories (national parks: 11.1%; biological reserves: 0.42%; forest reserves: 5.5%; protected zones: 3.1%; wildlife refuges: 3.4%; wetlands: 1.8%) (Valverde 2018).

Target species

The target species for the present survey were terrestrial small mammals with an average mass of no more than 500 g, following Rowe *et al.* (2015). In the case of Costa Rica, that definition applies for small mammals included in four orders: Didelphimorphia, Eulipotyphla, Rodentia and Carnivora. An exhaustive list of small mammals from Costa Rica was generated based on six previous works: Goodwin (1946), International Union for Conservation of Nature [IUCN] (2021), Reid (2009), Rodríguez-Herrera *et al.* (2014), Rodríguez and Chinchilla (1996), Villalobos-Chaves *et al.* (2016). Our new list includes a compilation of regional endemism (Costa Rica, Nicaragua and Panama) from Lower Central America (Bagley and Johnson 2014) and data from IUCN categories. The list was complemented with information available in the Genbank (https://www.ncbi.nlm.nih.gov/genbank/) on Cytochrome *b* (Cyt*b*), since this gene has proved to be sensitive and powerful for species identification (Parson *et al.* 2000). Three categories were consigned according to data from GenBank: complete Cyt*b* sequences (T), partial Cyt*b* sequences (P), information only available for other genes (OG), non-existent genetic information (NI). Seven species—*Marmosa nicaraguae*, *Peromyscus nicaraguae*, *Reithrodontomys cherrii*, *Reithrodontomys garichensis*, *Ichthyomys tweedii*, *Diplomys labilis*, and *Cryptotis monteverdensis*—were partially included in the present analysis because they are either new or reclassified species, and therefore, the recorded information was limited. From those last species, only *Ichthyomys tweedii* and *Diplomys labilis* have information in the UICN Red List.

Database sources and data selection.

Considering the new list of small mammals of Costa Rica defined in the previous section, four databases were used to compile the complete set of records for the species recognized. The main database accessed was the Global Biodiversity Information

Facility (GBIF., https://doi.org/10.15468/dl.kk3jpe), filtered by records of mammal species located in Costa Rica. Also, two additional databases were used, based on collections of local museums: the National Museum of Costa Rica and the Museum of Zoology of the National University of Costa Rica. Finally, we included data from a field survey conducted between 2016 and 2018 as part of our research project on terrestrial small mammals of Costa Rica. In this project, small mammals were captured using Sherman traps following all ethical and permit regulations (permit: R-052-2017-OT-CONAGEBIO) of Costa Rica current law.

Records were organized into three datasets according to a standardized data curation procedure. First, a Raw dataset including all records of small mammals where taxonomic identification was available at the species level. Second, a Corroborated dataset excluded those records from the Raw dataset lacking adequate locality information or geographical coordinates. Lastly, a Final dataset discarded records in the Corroborated dataset where there was redundant information (i.e. records duplicated with the same date and coordinate information). If a locality was clearly described but lacked geographical coordinates, these details were added in the Final dataset. Geographical coordinates and localities were verified using QGIS, free Geographic Information System software, version 2.18.20 (QGIS Development Team 1991). The map used to corroborate the data was the Costa Rica Canton's 2007 map in the Atlas Digital Project of Costa Rica (ADPCRC) (Orozi, 2015). Elevations were defined for each record using QGIS, based on the ALOS Global Digital Surface Model version 2.1 (Tadono *et al.* 2014). Holdridge's Life Zones map included in ADPCRC allowed life zones to be assigned to each record in the Ultimate dataset through Point Sampling Tools in QGIS, based on elevation.

Elevation distribution analyses

All elevation records from the Ultimate dataset were analyzed using Box-Plot in the *gglot2* package (R Development Core Team 2020), by species to determine the abundance patterns for all altitudinal range.

The Life Zone System of Holdridge (1967) defines six altitudinal belts for tropical regions based on variation of biotemperatures along an altitudinal range between sea level to 4750 m.a.s.l. Geographic position and climatic information (Regional Forecast in Meteorological National Institute of Costa Rica: https://www.imn.ac.cr) of Costa Rica allowed the Holdridge system to be used. Thus, altitudinal records from the Ultimate

dataset permitted terrestrial small mammals to be assigned to four altitudinal belts: Basal, Premontane, Lower Montane and Montane. Further analyses were conducted in R (R Development Core Team, 2020). In order to determine the similarity between belts, all altitudinal records were transformed to log(x+1) using the function *log ( )*. The function *matrix ( )* was used to create a matrix from the transformed dataset. Also, the altitudinal belts were compared with covariance matrix executed through the function *cov2cor ( )*.

Analysis of Life Zones

As already indicated, the life zone system proposed by Holdridge (1967) was used to characterize the pattern of distribution of records for terrestrial small mammals in Costa Rica according to the Figure 2.1. This classification is based on meteorological records: evapotranspiration (which is a projection of the potential evapotranspiration ratio), precipitation (estimation of annual average of precipitation in millimeters), and biotemperature (considering only the temperatures effective in plant growth in a range between 0 °C and 30 °C). This system delineated twelve distinct World Life Zones for terrestrial small mammals in Costa Rica. For each life zone, the number of species and the number of records were included as numerator and denominator respectively, inside of each hexagon. Numbers of exclusive species by life zone were indicated within parentheses. The altitudinal belts were defined by elevational zonation based on an average rate of change of 6°C per 1000 meters. The classification of latitudinal regions was based on the International Institute for Applied Systems Analysis. The distribution of all records allowed 12 life zones to be defined (Fig. 2.1) according to the map description in section 2.3: **rp-SA**: Rain Forest (Paramo) Subalpine, **wf-M**: Wet Forest Montane, **rf-M**: Rain Forest Montane, **mf-LM**: Moist Forest Low Montane, **wf-LM**: Wet Forest Lower Montane, **rf-LM**: Rain Forest Lower Montane, **mf-P**: Moist Forest Premontane, **wf-P**: Wet Forest Premontane, **rf-P**: Rain Forest Premontane, **mf-T**: Moist Forest Tropical, **wf-T**: Wet Forest Tropical, **df-T**: Dry Forest Tropical.

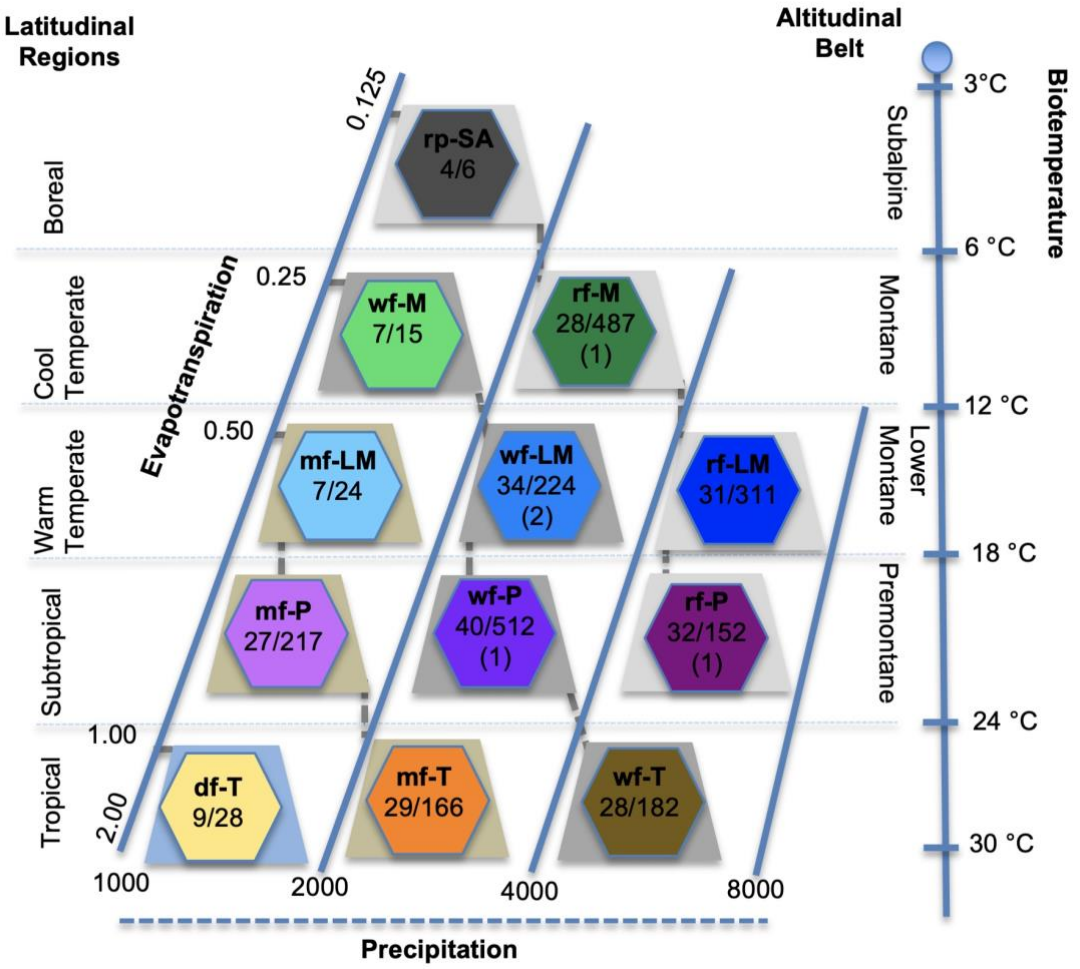

Figure 2.1. Twelve World Life Zones (Holdridge, 1967) identified based on records for terrestrial small mammals of Costa Rica. Hexagons reflect each life zone defined in the World Life Zones classification: **rp-SA**: Rain Forest (Paramo) Subalpine, **wf-M**: Wet Forest Montane, **rf-M**: Rain Forest Montane, **mf-LM**: Moist Forest Low Montane, **wf-LM**: Wet Forest Lower Montane, **rf-LM**: Rain Forest Lower Montane, **mf-P**: Moist Forest Premontane, **wf-P**: Wet Forest Premontane, **rf-P**: Rain Forest Premontane, **mf-T**: Moist Forest Tropical, **wf-T**: Wet Forest Tropical, **df-T**: Dry Forest Tropical. This classification is based on meteorological records: **evapotranspiration** – inclined axis (which is a projection of the potential evapotranspiration ratio), **precipitation** – x axis (estimation of annual average precipitation in millimeters), and **biotemperature** – y right axis (measurement of the heat which is effective in plant growth in a range between 0 °C and 30 °C). Altitudinal belts were defined by an elevational zonation based on average rate of change of 6°C per 1000 meters (y right axis divisions). Classification of latitudinal regions (y left axis divisions) was based on the International Institute for Applied Systems Analysis. Inside each hexagon is the number of species and the quantity of records, thus the numbers of exclusive species by life zone are indicated within parentheses.

The data on life zones was further analyzed using nonmetric multidimensional scaling (NMDS) in R (R Development Core Team 2020). Dexter *et al.* (2018) indicate that this analysis is typically used to evaluate the fit of an NMDS ordination via ordination "stress" (i.e. data distortion) against a commonly accepted set of heuristic guidelines with a stress <0.05 providing adequate results. Accordingly, the NMDS analysis was run with the *vegan* package in R and from this package the functions *vegdist* and *metaMDS* were

used together with method *bray*, in order to evaluate the similarity in the composition of species for each life zone. Also, the NMDS dataset was plotted with the function *plot ()* in R to visualize the arrangement of data by life zone.

Evaluation of human impact

To describe changes in species distribution over time, altitudinal records from the Ultimate dataset by species were divided in two sub datasets: the deforestation period (1960-1984) and the protected areas period (1994-2018). These reflect the intensive deforestation period during the 1960s and 1970s, and the establishment of the current protected areas system in Costa Rica from 1980s and 1990s to the present (Wainwright, 2007). A selection of records was made considering only species where there were more than 30 occurrences for each period.

The gap between deforestation and forest protection in Costa Rica has been estimated as 1977 to 2000 with a tendency for forest recovery after 1997 (State of the Nation Program 2002). Hence, we instituted a data gap period of ten years (1984-1994) assuming that basic habitat conditions for most terrestrial small mammals takes between 3 to 5 years to develop (depending on the type of forest), which offers a minimum continuous woody canopy based on estimation of forest regeneration for Costa Rica (Centro Agronómico Tropical de Investigación y Enseñanza [CATIE] 2016).

In order to detect fluctuations in distribution of small mammal species over time, randomization test in R (R Development Core Team 2020) were conducted to detect difference in altitudinal means by period for each species using the *mosaic* package (functions *means* and *diff*). A null distribution with 1000 permutations was obtained using the function *shuffle* to create a permutation dataset. Significance between means by period was obtained with the function *prop* based on simulated and observed datasets. Difference in means was plotted with the package *ggplot2* through histograms. Box-plot diagrams were used to illustrate the patterns in the distribution of both periods.

2.1.4 Results

Species information

Our exhaustive listing of the small mammals of Costa Rica generated an initial total of 92 species reported historically (Appendix 1). The final list of terrestrial small mammals currently occurring in Costa Rica includes 52 species (Table 2.1). However, data for the present investigation were only available for 46 of these species. The species

Reithrodontomys mexicanus has been removed from the list of mammals of Costa Rica and has been reclassified into two separate species: *Reithrodontomys cherrii* and *Reithrodontomys garichensis* (Reid and Gómez 2022, Ramírez-Fernández *et al.* 2023). Therefore, data collected for *R. mexicanus* were treated as *Reithrodontomys* spp for the present analysis. The list result contains seven species of Didelphids (marsupials), five species of shrews, 39 rodents and one carnivore. Of these, 19 species (38%) are regional endemics, of which six species only occur in Costa Rica (*Heteromys oresterus, Heteromys nubicolens, Reithrodontomys rodriguezi*, *Reithrodontomys musseri*, *Reithrodontomys cherrii*, *Cryptotis monteverdensis*), four species occur in Costa Rica and Nicaragua (*Marmosa nicaraguae*, *Peromyscus nicaraguae*, *Reithrodontomys brevirostris, Reithrodontomys paradoxus*), and eight species are found in Costa Rica and Panama (*Scotinomys xerampelinus, Reithrodontomys creper, Cryptotis gracilis, Cryptotis nigrescens, Nephelomys devius, Oligoryzomys vegetus, Rheomys raptor, Rheomys underwoodi)* (Table 2.1). Regarding risk of extinction, only one species is considered 'vulnerable' (*Cryptotis gracilis*) and there are 16 species in the data deficient or not evaluated category (Table 2.1). All other species are listed as 'least concern' by the IUCN. Regarding genetic data, only 37% of species (n = 19) have complete Cyt*b* sequences. Additionally, 22 species (42%) have partial Cyt*b* sequences, and four species (8%) only have information for other genes. Finally, there are seven species (13%) without any genetic data.

Data sources

The records of terrestrial small mammals for Costa Rica were organized into three datasets according to the data curation process described earlier (Table 2.2). The Ultimate dataset only includes single records for occurrences with unique latitude, longitude and date and was the main database for the rest of the analysis with 46 species and 2324 records. Also, the Ultimate dataset includes the elevation and life zone for each record.

Mid-elevation peak in numbers of species

According to the median of records for each species, the highest number of species (22) occur in the mid elevation band between 1218 – 2436 m.a.s.l., somewhat fewer in the lowlands (0 – 1218 m.a.s.l., 19 species) and very few at high elevations (2436 - 3654 m.a.s.l., 5 species) (Fig. 2.2). Four species occurred only once each (*Cryptotis merriami*, *Tanyuromys aphrastus, Metachirus nudicaudatus* and

Reithrodontomys musseri). Several species showed a high number of outlier occurrences, probably associated with identification errors: *Cryptotis nigrescens*, *Reithrodontomys creper*, *Reithrodontomys rodriguezi*, *Scotinomys xerampelinus*. Despite this situation, we include these records in the analysis.

Table 2.1. Terrestrial small mammals of Costa Rica. Compilation of regional endemism, IUCN category and GenBank information available.

Small mammal classification	**Regional Endemism**			**IUCN Category**	**GenBank Information**
	CR	PA	NI		
Class Mammalia					
Order Didelphimorphia					
Family Didelphidae					
Subfamily Caluromyinae					
Caluromys derbianus (Waterhouse, 1841)	-	-	-	LC	T
Subfamily Didelphinae					
Tribe Mormosini					
Marmosa alstoni (J. A. Allen, 1900)	-	-	-	LC	OG
Marmosa mexicana Merriam, 1897	-	-	-	LC	T
Marmosa nicaraguae O. Thomas, 1905	✓	-	✓	NE	NI
Marmosa zeledoni Goldman, 1911	-	-	-	NE	P
Tribe Metachirini					
Metachirus nudicaudatus (Desmarest, 1871)	-	-	-	LC	P
Tribe Didelphini					
Philander melanurus (O. Thomas, 1899)	-	-	-	NE	P
Order Rodentia					
Suborder Castorimorpha					
Family Heteromyidae					

Subfamily Heteromyinae					
Heteromys desmarestianus Gray, 1868	-	-	-	LC	T
Heteromys nubicolens Anderson & Timm, 2006	✓	-	-	NE	P
Heteromys oresterus Harris, 1932	✓	-	-	LC	P
Heteromys salvini O. Thomas, 1893	-	-	-	LC	T
Suborder Myomorpha					
Family Cricetidae					
Subfamily Neotominae					
Tribe Baiomyini					
Scotinomys teguina (Alston, 1877)	-	-	-	LC	T
Scotinomys xerampelinus (Bangs, 1902)	✓	✓	-	LC	T
Tribe Reithrodontomyini					
Peromyscus nicaraguae (J. A. Allen, 1908)	✓	-	✓	NE	T
Peromyscus nudipes (Allen 1891)	-	-	-	NE	T
Reithrodontomys brevirostris Goodwin, 1943	✓	-	✓	LC	P
Reithrodontomys cherrii (J. A. Allen, 1891)	✓	-	-	NE	P
Reithrodontomys creper Bangs, 1902	✓	✓	_	LC	T
Reithrodontomys garichensis Enders & O. P. Pearson, 1940	✓	✓	-	NE	P
Reithrodontomys gracilis J. A. Allen & Chapman, 1897	-	-	-	LC	T
Reithrodontomys musseri Gardner & Carleton, 2009	✓	-	-	NE	NI
Reithrodontomys paradoxus Jones & Genoways, 1970	✓	-	✓	DD	NI
Reithrodontomys rodriguezi Goodwin, 1943	✓	-	-	LC	NI
Reithrodontomys sumichrasti (Saussure, 1861)	-	-	-	LC	T
Subfamily Sigmodontinae					
Tribe Ichthyomyini					
Ichthyomys tweedii Anthony, 1921	-	-	-	DD	P
Rheomys raptor Goldman, 1912	✓	✓	-	LC	T
Rheomys underwoodi Thomas, 1906	✓	✓	-	LC	NI
Tribe Oryzomyini					
Handleyomys alfaroi (J. A. Allen, 1891)	-	-	-	LC	P
Melanomys chrysomelas (Allen 1897)	-	-	-	NE	P

Nephelomys devius (Bangs, 1902)	✓	✓	-	LC	OG
Oecomys trinitatis (J. A. Allen & Chapman, 1893)	-	-	-	LC	P
Oligoryzomys costaricensis (J. A. Allen, 1893)	-	-	-	NE	P
Oligoryzomys vegetus (Bangs, 1902)	✓	✓	-	LC	P
Oryzomys couesi (Alston, 1877)	-	-	-	LC	T
Sigmodontomys alfari J. A. Allen, 1897	-	-	-	LC	T
Tanyuromys aphrastus (Harris, 1932)	-	-	-	DD	P
Transandinomys bolivaris (J. A. Allen, 1901)	-	-	-	LC	T
Transandinomys talamancae (J. A. Allen, 1891)	-	-	-	LC	T
Zygodontomys brevicauda (J. A. Allen & Chapman, 1893)	-	-	-	LC	T
Tribe Sigmodontini					
Sigmodon hirsutus (Burmeister, 1854)	-	-	-	LC	T
Subfamily Tylomyinae					
Tribe Nyctomyini					
Nyctomys sumichrasti Saussure, 1860	-	-	-	LC	P
Tribe Tylomyini					
Ototylomys phyllotis Merriam, 1901	-	-	-	LC	P
Tylomys watsoni Thomas, 1899	-	-	-	LC	T
Suborder Hystricomorpha					
Family Echimyidae					
Subfamily Echimyinae					
Tribe Echimyini					
Diplomys labilis (Bangs, 1901)	-	-	-	LC	P
Tribe Myocastorini					
Hoplomys gymnurus (Thomas, 1897)	-	-	-	LC	OG
Proechimys semispinosus (Tomes, 1860)	-	-	-	LC	OG
Order Eulipotyphla					
Family Soricidae					
Subfamily Soricinae					
Cryptotis gracilis Miller, 1911	✓	✓	-	VU	P
Cryptotis merriami (Choate, 1970)	-	-	-	LC	P

Cryptotis monteverdensis Woodman & Timm, 2017	✓	-	-	NE	NI
Cryptotis nigrescens (J. A. Allen, 1895)	✓	✓	-	LC	P
Cryptotis orophilus (J. A. Allen, 1895)	-	-	-	NE	NI
Order Carnivora					
Suborder Carniformia					
Family Mustelidae					
Subfamily Mustelinae					
Neogale frenata (Lichtenstein, 1831)	-	-	-	LC	P

Regional endemism according to geographical distribution of the IUCN Red List: Costa Rica (CR), Panama (PA) and Nicaragua (NI) (https://www.iucnredlist.org/species/19487/22354257). The three IUCN categories of level of threat for each species are: Vulnerable (VU), Least Concern (LC) and Data Deficient (DD). Species without information in the IUCN database are categorized as Not Evaluated (NE). Data from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) is provided as follows: complete Cyt*b* sequence (T), partial Cyt*b* sequence (P), data for other genes only (OG), no genetic information (NI).

Data sources

The records of terrestrial small mammals for Costa Rica were organized into three datasets according to the data curation process described earlier (Table 2.2). The Ultimate dataset only includes single records for occurrences with unique latitude, longitude and date and was the main database for the rest of the analysis with 46 species and 2324 records. Also, the Ultimate dataset includes the elevation and life zone for each record.

Table 2.2. Number of records and species for terrestrial small mammals in Costa Rica based on a compilation of different datasets.

Dataset	Number of records	Number of species
Raw dataset	8485	67
Corroborated dataset	7892	45
Ultimate dataset	2324	46

Patterns of species occurrences by elevation category

All elevation records for each species were placed into four categories according to Holdridge's (1967) system of altitudinal belts for the tropics: Basal, Premontane, Lower Montane and Montane (Table 2.3).

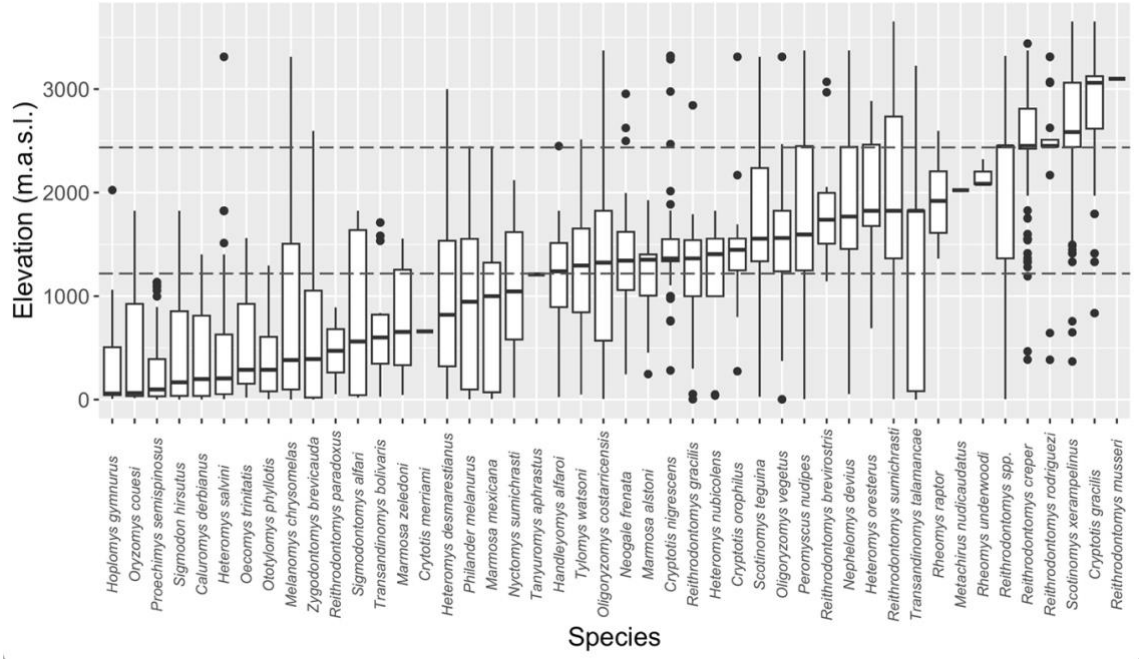

Figure 2.2. Distribution of records along the elevation gradient for each terrestrial small mammal species in Costa Rica. The elevation range was defined by the total of altitudinal records between zero meters above sea level (m.a.s.l) to 3654 meters. Box and whisker plots represent the quartiles from 25th to 75th and from 9th to 91th percentiles, respectively. Data outside of this range were considered atypical records (●). The horizontal line inside each box indicates the median. The dashed lines represent the borders of the intermediate area between 1218-2436 m.a.s.l for all altitudinal range of records.

Table 2.3. Terrestrial small mammal species occurrence in Costa Rica according to the altitudinal belt categories of Holdridge's World Life Zones System. Additional information (species representation, number of records, number of exclusive species) by category is also presented.

	Species occurrence by elevation belt			
Small mammal classification	**Basal**	**Premontane**	**Lower Montane**	**Montane**
CLASS MAMMALIA				
Order Didelphimorphia				
Family Didelphidae				
Subfamily Caluromyinae				

Caluromys derbianus	✓	✓	–	–
Subfamily Didelphinae				
Tribe Marmosini				
Marmosa alstoni	✓	✓	–	–
Marmosa mexicana	✓	✓	✓	–
Marmosa zeledoni	✓	✓	–	–
Tribe Metachirini				
Metachirus nudicaudatus	–	–	✓*	–
Tribe Didelphini				
Philander melanurus	✓	✓	✓	–
Order Eulipotyphla				
Family Soricidae				
Cryptotis gracilis	✓	✓	✓	✓
Cryptotis merriami	*	–	–	–
Cryptotis nigrescens	✓	✓	✓	✓
Cryptotis orophila	✓	✓	✓	✓
Order Rodentia				
Suborder Castorimorpha				
Family Heteromyidae				
Subfamily Heteromyinae				
Heteromys desmarestianus	✓	✓	✓	–
Heteromys nubicolens	✓	✓	–	–
Heteromys oresterus	✓	✓	✓	–
Liomys salvini	✓	✓	–	✓
Suborder Myomorpha				

Family Cricetidae				
Subfamily Sigmodontinae				
Tribe Ichthyomyini				
Rheomys raptor	–	✓	✓	–
Rheomys underwoodi	–	–	✓*	–
Tribe Sigmodontini				
Sigmodon hirsutus	✓	✓	–	–
Tribe Oryzomyini				
Handleyomys alfaroi	✓	✓	✓	–
Melanomys chrysomelas	✓	✓	–	✓
Nephelomys devius	✓	✓	✓	✓
Oecomys trinitatis	✓	✓	–	–
Oligoryzomys fulvescens	✓	✓	✓	✓
Oligoryzomys vegetus	✓	✓	✓	✓
Oryzomys couesi	✓	✓	–	–
Sigmodontomys alfari	✓	✓	–	–
Tanyuromys aphrastus	–	✓*	–	–
Transandinomys bolivaris	✓	✓	–	–
Transandinomys talamancae	✓	✓	–	✓
Zygodontomys brevicauda	✓	✓	✓	
Subfamily Tylomyinae				
Nyctomys sumichrasti	✓	✓	✓	–
Ototylomys phyllotis	✓	✓	–	–
Tylomys watsoni	✓	✓	✓	–
Subfamily Neotominae				
Tribe Baiomyini				

Scotinomys teguina	✓	✓	✓	✓
Scotinomys xerampelinus	✓	✓	✓	✓
Tribe Reithrodontomyini				
Peromyscus nudipes	✓	✓	✓	✓
Reithrodontomysbrevirostris	–	✓	✓	✓
Reithrodontomyscreper	✓	✓	✓	✓
Reithrodontomysgracilis	✓	✓	✓	–
Reithrodontomysmexicanus	✓	✓	✓	✓
Reithrodontomysmusseri	–	–	–	✓*
Reithrodontomysparadoxus	✓*	–	–	–
Reithrodontomysrodriguezi	✓	–	✓	✓
Reithrodontomyssumichrasti	✓	✓	✓	✓
Suborder Hystricomorpha				
Family Echimyidae				
Hoplomys gymnurus	✓	✓	✓	–
Proechimys semispinosus	✓	✓	−	−
Order Carnivora				
Suborder Caniformia				
Family Mustelidae				
Neogale frenata	✓	✓	✓	–
Total number of species	**40**	**40**	**28**	**18**
Species representation (%)	**87**	**87**	**61**	**39**
Number of records	**844**	**853**	**462**	**165**
Exclusive species	**1**	**1**	**2**	**1**

Four categories of elevation were considered: Basal, Premontane, Lower Montane and Montane. Check (✓) and minus (−) signs indicate the presence or absence for each species by category, respectively. Exclusive species by category were highlighted with an asterisk (*).

There is a clear difference in species occurrence according to altitudinal belt, with few species at higher altitude (Table 2.3). Although the Basal and Lower Montane categories had the same number of species (Table 2.3), there was considerable divergence between them (35%) (Table 2.4). The Lower Montane and Montane categories showed the greatest similarity (83%). Intermediate values of similarity (53%) were observed between Premontane category and Lower Montane, and between Premontane and Montane. The lowest similarity values were found for the extreme altitude comparisons: Basal and Lower Montane (10%) and Basal and Montane (15%).

Table 2.4. Covariance matrix of similarity for terrestrial small mammals of Costa Rica according to the altitudinal belt categories of Holdridge's World Life Zones System.

Category	Basal	Premontane	Lower Montane	Montane
Basal	-			
Premontane	0.65	-		
Lower Montane	0.10	0.53	-	
Montane	0.15	0.53	0.83	-

Distribution of species by life zones

Based on the small mammal species occurrences, according to non-metric multidimension scaling (NMDS; stress = 0.055), most life zones (8) grouped together (Fig. 2.3). This suggests that a wide range of life zones substantially share the same species of terrestrial small mammals. Four life zones were totally separated from the rest and from each other, due to the small quantity of records in each. The eight life zones that group together show intermediate values for the main factors (precipitation, biotemperature and evapotranspiration) considered by the Holdridge Life Zone System (Fig. 2.3). Thus, precipitation appears to be a key factor in the distribution of terrestrial small mammals. Most of the life zones that grouped together (four) showed the same precipitation range (2000-4000): rf-M, wf-LM, wf-P, mf-T (Fig. 2.3). Conversely, the four life zones that separated out by NMDS analysis (rp-SA, wf-M, mf-LM, df-T) plus mf-P showed the lowest range of precipitation (1000-2000). Regarding biotemperature, most of species and records were associated with life zones at an intermediate range of

biotemperature between 12 °C – 24 °C. Thus, life zones with a low number of species and occurrences were associated with extreme temperatures both high and low. Life zone wf-P had the highest number of species (87%., 40 species) and records (22%., 512 records). This life zone particularly showed intermediate values for precipitation, temperature, evapotranspiration and altitudinal belt (Fig. 2.1). In contrast, the rp-SA life zone only had four species (10%) and six records (0.25%). This life zone is associated with extreme values of the environmental conditions mentioned above.

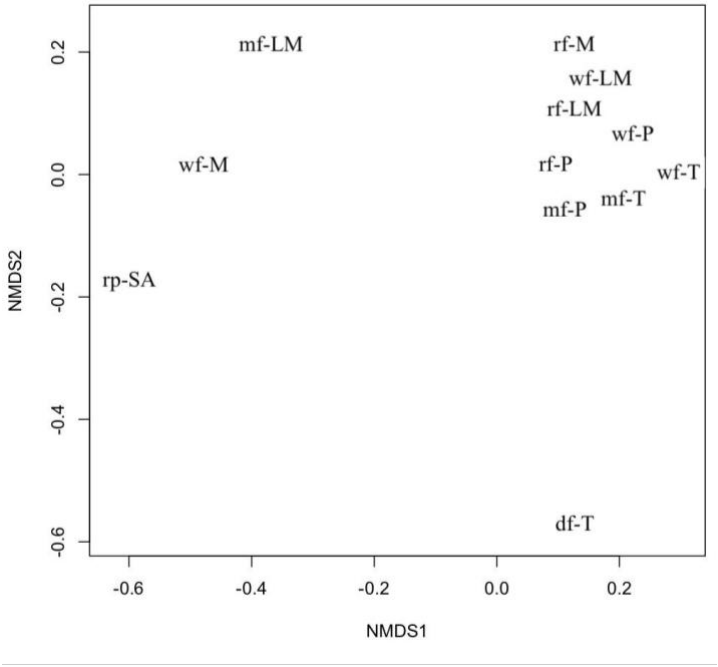

Figure 2.3. Non-Metric Multidimensional Scaling Analysis (NMDS) based on distributions of terrestrial small mammal species in Costa Rica, according to Holdridge's Life Zones. Life zone codes: **rp-SA**: Rain Forest (Paramo) Subalpine, **wf-M**: Wet Forest Montane, **rf-M**: Rain Forest Montane, **mf-LM**: Moist Forest Low Montane, **wf-LM**: Wet Forest Lower Montane, **rf-LM**: Rain Forest Lower Montane, **mf-P**: Moist Forest Premontane, **wf-P**: Wet Forest Premontane, **rf-P**: Rain Forest Premontane, **mf-T**: Moist Forest Tropical, **wf-T**: Wet Forest Tropical, **df-T**: Dry forest Tropical.

Comparison of the presence of small mammals between the period of deforestation and the period of conservation in Costa Rica

Only seven species with more than 30 records by period were available to conduct a comparative analysis of species distributions along the elevation gradient between the periods of deforestation (1960-1984) and the implementation of protected areas (1994-2018) in Costa Rica: *Heteromys desmarestianus*, *Nephelomys devius*, *Peromyscus nudipes*, *Reithrodontomys sumichrasti, Reithrodontomys creper*, *Scotinomys teguina* and *Scotinomys xerampelinus* (Fig 2.4). Two species showed a non-significant tendency to move to higher elevations between the two periods: *N. devius*

(p=0.083), *P. nudipes* (p=0.123), whilst *H. desmarestianus* was the only species showing a significant elevation increase (p=0.001). Two species showed a significant decline in elevation between the two periods: *R. creper* (p=0.004) and *S. teguina* (p=0.003), while *S. xerampelinus* showed a non-significant tendency in the same direction (p=0.864). *R. sumichrasti* (p=0.455) did not showed any tendency to change elevation between periods.

Summarizing what is known on the distribution ranges of the seven species described above over the whole period of data collection (Fig. 2.4) and their changes in ranges between periods, it is clear that four species are found at mid elevations: *N. devius*, *P. nudipes*, *R. sumichrasti, S. teguina*. Three of these species did not change significantly between periods (*N. devius*, *P. nudipes*, *R. sumichrasti*), which could mean that those species are strongly affiliated to intermediate elevations, whilst *S. teguina* exhibited a significant decline in elevation, while still remaining a mid elevation species (Fig. 2.4). Higher in the elevational range, *R. creper*, which occurs at the border of mid and high elevations, showed a significant decline in elevation between periods. The distribution of *S. xerampelinus* did not show a significant change between periods, which could mean that this species is strongly linked to high elevations. *H. desmarestianus* was the only species represented from the lowlands, and its distribution showed significantly increased elevation between the deforestation and protected areas period.

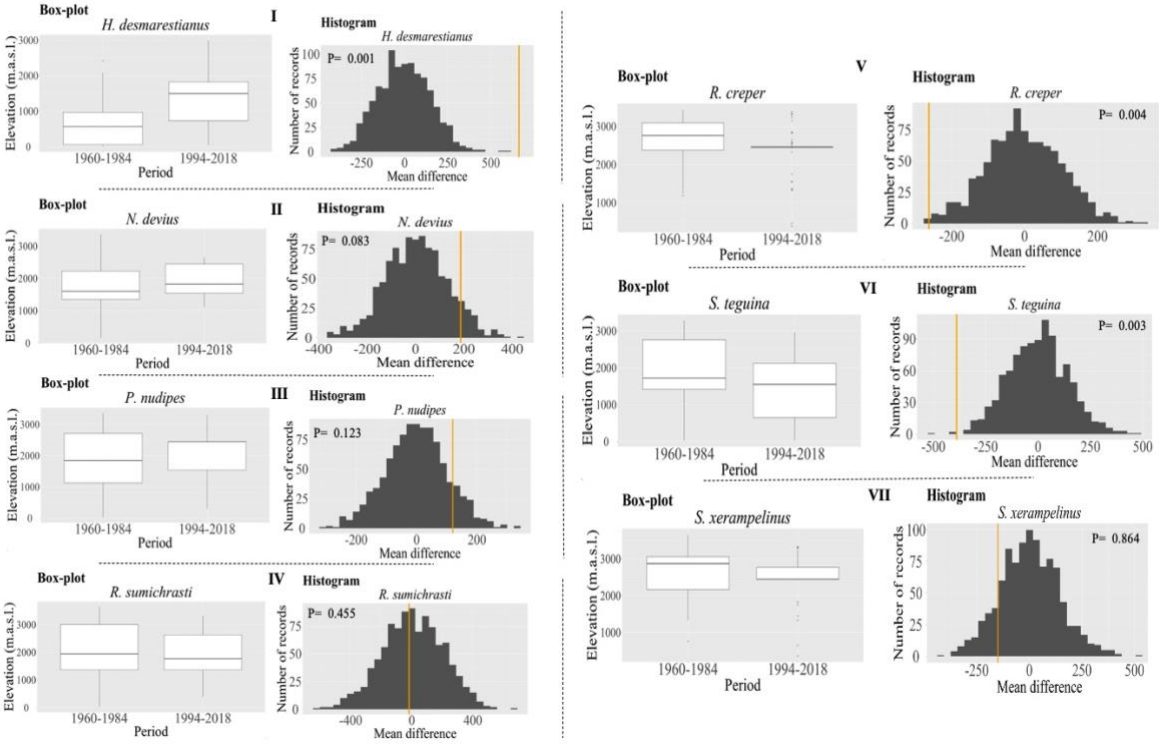

Figure 2.4. Occurrences by elevation for seven species of terrestrial small mammals between two periods with different use of forest in Costa Rica (deforestation: 1960-1984 and protected areas: 1994-2018). The box-plot of elevation for each species shows the trends of records by period considering the median and percentiles (25-75% for each box). Histograms represent the randomized data distribution calculated for each species between both periods. The orange line refers to observed mean values. Differences between randomized means and observed means was indicated as p-values. Roman numerals indicated the paired graphics (boxplot and histograms) for each species.

2.1.5 Discussion

The terrestrial small mammal species of Costa Rica and degree of endemism

There is a large diversity of terrestrial small mammals in Costa Rica but the available data on these species is scarce, and there are taxonomic uncertainties for several species (Villalobos-Chaves *et al.* 2016). In the present study, occurrences and distribution information were gathered from 52 terrestrial small mammal species of Costa Rica. Among these, high regional endemism (Costa Rica, Panama and Nicaragua) was detected (19 species, 37%). Although only seven species are endemic to Costa Rica (*Heteromys nubicolens*, *Heteromys oresterus*, *Reithrodontomys cherrii*, *Reithrodontomys musseri*, *Reithrodontomys paradoxus*, *Reithrodontomys rodriguezi*, *Cryptotis monteverdensis*), this finding may be misleading due to the lack of genetic data. Taking the presence of the complete sequence of the well-studied marker, *Cytb*, in GenBank as the standard, only 19 species (36%) have complete *Cytb* while 42% of species (22) just have partial genetic information and seven species (13%) do not have any available genetic information.

The data retrieved for this work corresponds to 92 terrestrial small mammals historically registered in the main existing general lists of mammals from Costa Rica or regional compilations (Appendix 1). Inconsistences between the current number of recognized species and those historically recorded only confirm the overall lack of available information. This pattern has already been reported by Reid (2009), who also referred to the necessity of data beyond just species presence and abundance, including regional research in subjects like ecology, behavior, or natural history. This author also highlighted the importance of using modern systematic methods to expand the number of species recognized in Central America. In this context, despite the large number of existing species in this region, the overall lack of available genetic data hinders the resolution of existing taxonomic controversies.

In addition, most of the existing genetic data come from other regions, and there is a considerable local unawareness, which could be hiding putative cryptic species. *R.*

mexicanus represents a good example of this. This species has historically been considered a single species among the *Reithrodontomys* complex of species in Costa Rica. However, this genus has been subdivided into several cryptic species based on morphological traits by Gardner and Carleton (2009), suggesting two endemic species for Costa Rica.

In addition to the need for clarity in taxonomy, studies in ecology, conservation and management are also required for Costa Rican small mammals. The lack of information is clear when considering the records available for the IUCN red list (Table 2.1). In this list, 18% of species (8 species) appear with 'deficient data' or without any type of reference information ('not evaluated'). Some taxonomically complex groups, like *Oligoryzomys*, need particular attention. Rogers *et al.* (2009) indicated notable regional endemism for this genus and their results support a high likelihood of cryptic species.

The description above implies an undoubted lack of specific information for terrestrial small mammals in Costa Rica, as already pointed out by others (Reid 2009, Rodríguez-Herrera 2014, Wainwright 2007). This mirrors a scarcity of data for bats of Costa Rica recognized over two decades ago by Timm and Laval (1998). Research based on current molecular tools is a good way to identify species without needing detailed morphological identification, allowing a focused effective conservation and management process (Paupério *et al.* 2012) within the context of the extraordinary habitat and climatic diversity in Costa Rica (Wainwright 2007).

Mid-domain Effect

The dissection of Costa Rica by mountain ranges has previously been mentioned as a key factor for diversification of terrestrial small mammals there. Colwell and Lee (2000) define the mid-domain effect as 'the increasing overlap of species ranges towards the centre of a shared geographic domain due to geometric boundary constraints in relation to the distribution of species' range sizes and midpoints'. According to early naturalists, the types of habitats and the number of terrestrial small mammal species changes predictably with increases in latitude and elevation, typically attributed to a symmetrical mid-elevation peak known as the Mid-Domain Effect (McCain and Grythnes 2010). The variation in altitude and environmental conditions permitted small mammal distribution patterns to be determined by McCain (2004) and McCain (2005) for several specific mountains in Costa Rica. Subsequently, this pattern was identified for terrestrial small mammals in other Neotropical forests as well (Ferro and Barquez 2014).

The results of the present research also suggest a peak in diversity at middle-elevations, probably in association with changes in climatic variables at different elevations (McCain, 2004). However, high small mammal diversity and records are also found in the lowlands (Fig. 2.1), which could be an effect of oversampling as a result of easier access to those regions of the country. Likewise, the high diversity of terrestrial small mammals both at middle-elevation and the lowlands could reflect a strong dependence of these animals to specific precipitation patterns for tropical mountains described by McCain and Grytnes (2010). Precipitation may play an essential role defining distribution patterns for these species according to the data obtained in our study, as detailed below. Compared to the lowland records, data for this study suggest a decline in abundance of records and species richness towards high elevations. This pattern has been broadly recognized in other studies based on terrestrial small mammals (Lomolino 2001, McCain 2004, McCain 2005, McCain *et al.* 2018).

The same pattern of small mammal occurrence at low and mid elevations was identified by considering the distribution of all records according to the altitudinal categories defined by Holdridge (1967). This system defines four categories applicable to all records based on latitude and altitude parameters: Basal, Premontane, Low Montane and Montane. The Basal and Premontane categories recorded the same quantity of species and a similar quantity of records (Table 2.3), nonetheless the similarity of the populations of small mammals between them was measured as only 65%, whilst the highest similarity reached 83% between Lower Montane and Montane (Table 2.4). Based on these similarity values, species with large geographical ranges could be most prevalent in the Premontane category influencing species composition at top of the elevational distribution and in the lowlands, which means that the Premontane category could offer conditions to support higher richness and abundance than other elevational categories. If so, this pattern is totally consistent with the peak of distribution described for terrestrial small mammals at intermediate elevation proposed by McCain *et al.* (2018). Furthermore, the similarities of Premontane with Lower Montane and Premontane with Montane at around 50% (Table 2.4) could reinforce the idea that species with large to medium sized geographical ranges overlap at intermediate elevations more than at the base or top of the mountains, and consequently this would induce a spatial constraint (Colwell *et al.* 2004, White *et al.* 2018).

The pattern in elevation ranges of small mammals and the intermediate distribution of records are totally coincident with previous evaluations of the mid-domain

effect in Costa Rica (Kluge *et al.* 2006, McCain 2004). In the case of the analysis of life zones (Fig. 2.1), it was found that the Premontane and Lower Montane are the elevation categories that have the most suitable climatic conditions (biotemperature, evapotranspiration and precipitation) with the highest values of richness and abundance observed within these life zones at intermediate elevation ranges (Fig. 2.1). However, several live zones have shown a tendency to support high productivity habitat for terrestrial small mammals as well, because most species occur at specific intermediate range of climatic factors (Fig. 2.4). Likewise, the lowest values of richness and abundance were associated to life zones with extreme conditions of precipitation and biotemperature. Thus, precipitation looks like a restrictive factor for the distribution of terrestrial small mammals. Specifically, intermediate values of precipitation (2000 – 4000 mm) resulted in the highest values of richness for grouped categories in the NMDS analysis (Fig. 2.3). According to this, when the values of precipitation decrease, the richness and abundance decreases substantially more than any other factor considered.

Terrestrial small mammals as indicators of habitat modification

Based on previous reviews, species richness of terrestrial small mammals could provide a good maker of habitat modification due to the association of this measure with site productivity and climatic zones (McCain and Grytnes 2010). Small terrestrial mammals have a special interest in their role in predator-prey systems (Lim and Pacheco 2016) as well as other functions such as seed dispersal. The occurrence of particular species in defined elevational ranges revealed in this research (Fig. 2.2) likely reflects adaptive responses of those species to local conditions. The data show that all species in the highlands are exclusive to this zone (5 species), and this exclusivity also applies to most of the lowland species (11 species), confirming earlier findings by Chen *et al.* (2017) Another feature relating to small mammals, relates to geographical range size. For instance, small-ranged species are sensitive in terms of conservation because these species are living in areas of restricted climatic suitability and, consequently, their ability to disperse probably is extremely restricted (White *et al.* 2018). This should be considered an important feature for local surveys in future research in Costa Rica. The above features of terrestrial small mammals reflect the potential of these animals to act as a tool to evaluate habitat quality based on their high sensitivity in relation to the habitat-species equilibrium that they display.

Species distribution and effectiveness of conservation

Comparison of the distribution of species between the two periods (deforestation and protected areas) suggests a positive effect of the protected areas period, which presumably guarantees habitat suitability for terrestrial small mammals at the mid elevation range. An elevation decreases or increases or an unchanging species distribution between the periods all converge towards that intermediate elevation. *S. xerampelinus* was the unique species confined exclusively to high elevations during both periods. The protected areas period has involved a stringent and particular forest use system over the last 30 years (Stan and Sanchez 2019) and it might be guaranteeing good conditions at intermediate elevations for multiple species of terrestrial small mammals. This idea supports the current definition for the mid-domain effect (Prillwitz and Blasius 2020). Responses of species to climate change may be an additional explanation for the trend of elevation ranges contracting or expanding between periods. McCain and King (2014) suggest that mammals differentially respond to current climate change., negatively (e.g. local extirpation, range contraction), positively (e.g. increasing range and population size) or no response at all, which reflects closely the patterns identified for all the terrestrial small mammals studied (Fig. 2.3).

Conclusions

GBIF open access databases were used to complement other data sources (local museum collections and field work) to provide information on the distribution and habitat of 46 species of terrestrial small mammals along the elevation gradient in Costa Rica from 1960 to the present day.

Overall, the data available for terrestrial small mammals is highly deficient, both regional and locally, which creates gaps to implement adequate policies of management and conservation for habitats and species.

Endemism maybe higher than would be recorded if systematic research advances using genetic tools were used to complement morphological analysis and ecological and behavioral studies., such an approach would uncover cryptic species.
Microclimatic and topographic variation in Costa Rica may generate very particular local conditions suitable for certain terrestrial small mammal species, promoting evolution of local morphotypes. These morphotypes could explain the inconsistencies at the taxonomic level evident in the species datasets generated in this study.

Results support the Mid-domain effect, with a peak of richness and abundance at intermediate elevations. However, there was also high richness and abundance in the lowlands that could be caused by oversampling, taxonomical uncertainties or

precipitation conditions similar to those tropical mountains, although this has not been evaluated properly yet.

Several of the life zones under consideration appear to offer suitable conditions for most of terrestrial small mammals, but premontane and lower montane generated the highest numbers of species and records. Premontane and Lower Montane are life zones defined by intermediate values of climate conditions: biotemperature, evapotranspiration and precipitation. Definitively, precipitation looks like a key climatic factor for the distribution of terrestrial small mammals and helps explain the observed distribution of small mammals in lowlands and mid altitudes.

Terrestrial small mammals in Costa Rica are good indicators of habitat alterations and responses to current climate change. However, they have not been used systematically to evaluate the recovery of ecosystems by the protected areas policy. Hence, small mammals could have a considerably positive influence on forest recovery in a way that has not yet been appreciated. Again, there is a tendency for species considered between the periods from deforestation to protected areas to converge on the mid-range elevation, consistent with the Mid-domain effect.

Conservation recommendations

Use open databases to complete and recreate information on biodiversity for tropical countries that currently have limited information on species and their ecosystems.

Increase initiatives to identify cryptic species using genetics to reduce the current inconsistences in taxonomy.

Promote a research focus to guarantee adequate identification of endemic species, to establish conservation and management policies for species and their habitat from local and regional level.

Systematically focus on the effect of topographical and climatic variability on species and their ecosystems to clarify the basis of the high diversity in the country, since it has not yet been properly evaluated.

Validate the Mid-domain effect by considering multiple environmental factors involved in explaining the current distribution of terrestrial small mammal species.

Verify through specific studies the relevance of precipitation as a limiting factor in the distribution of small mammal species.

Evaluate, through long-term research, the relevance of terrestrial small mammals as an indicator group for the alteration of ecosystems and the effect of climate change.

Promote collaboration between national and international institutions to reduce information gaps by supporting research on the diversity of small mammals present in Costa Rica.

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Chapter 2: Article 2. Protocol for capture of terrestrial small mammals in a pristine Neotropical montane oak forest in an International Park in Costa Rica.

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2.2.1 Abstract

Costa Rica, with its diverse landscape, boasts exceptional biodiversity, due in part to its mountainous terrain. The high-altitude regions have limited surface areas, low temperatures, and ample precipitation, promoting unique adaptations among resident species, resulting in endemism and distinctive community structures. In the Valle del Silencio within the La Amistad International Park, located in the Talamanca Mountain range, we conducted a capture-recapture study on the small mammal community. We employed a standardized live-trapping method and the Cormark-Jolly-Seber model for open populations to assess species abundance. The most prevalent species identified

were *Peromyscus nudipes* and *Reithrodontomys creper*, while *Nephelomys devius*, other *Reithrodontomys* spp., *Scotinomys teguina*, and *Scotinomys xerampelinus* were less abundant. Similar species have been documented in previous high-altitude studies in Costa Rica, but only *P. nudipes* and *R. creper* consistently appeared across all studies. The Valle del Silencio findings may reflect a typical high-altitude community in the country due to minimal human intervention in the area. By characterizing this ecosystem, our paper aims to provide impactful guidance for optimizing the capture, handling, and release of small mammals, prioritizing the well-being of the animals and the safety of researchers. We present this as a case study to inform future research on small mammals. While our focus has centered on Costa Rica's high-altitude community, we believe that our protocols hold broader applicability for small mammal studies.

Keywords

Small mammal sampling, Neotropical oak forests, capture-recapture methodologies, Cormark-Jolly-Seber, field techniques, Valle del Silencio, Talamanca, La Amistad International Park.

2.2.2 Introduction

Costa Rica is a country with one of the highest levels of biodiversity globally (Obando 2007., Bermudez and Obando, 2021., Kholmann *et al.*, 2010). Several factors explain this abundance based on the country's geographical position and characteristics of its narrow territory, including the steep altitudinal gradients of the Neotropical Mountain system and the influence of two large oceans on the climatic conditions (Kappelle 2016, Suzart de Albuquerque *et al.* 2015). Combinations of all those factors create environmental gradients that influence species distribution patterns (Scatena 2002).

Mountainous areas host many species that live at a delicate ecophysiological transition or on the edge of their distribution and are therefore very susceptible to environmental change and local extinction (McCain and Colwell 2011). This is particularly relevant in the tropics due to the high specificity of animals and plants in any given area (Janzen 1967, Christmann and Olivieras 2021). In an altitudinal gradient, high elevations are especially vulnerable ecosystems due to the extreme montane conditions providing suitable habitat for only a few species (Schmeller *et al.* 2022).

Ecosystems in highland areas tend to be poorly studied due to several factors, but mainly because the topographic and climatic conditions make such places inaccessible. In Mesoamerica, the lack of study further reflects availability of inadequate

resources, despite the high biodiversity (Myers *et al.* 2000). This is a critical problem in conservation because insufficient research means that many species could become extinct without being properly identified or characterized.

These issues apply to species of terrestrial small mammals (i.e. mammals with an adult weight less than 500 g) (Rowe *et al.* 2015) present in Costa Rica. This group currently comprises 52 species belonging to four large taxonomic groups: shrews, marsupials, rodents, and one species of carnivore (*Neogale frenata*) (Wainwright 2007., Reid, 2009, Rodríguez *et al.* 2014, Villalobos *et al.* 2016, Reid and Gómez 2022, Ramírez-Fernández *et al.* 2023b). The identification of these species in Costa Rica has largely been based on traditional taxonomy, and there is likely a degree of crypticity, which currently creates some inconsistencies in species identification (Gómez-Lépiz et al, submitted).

Cryptic species are defined as two or more distinct species that are erroneously classified (and hidden) under a single species name because they cannot be distinguished easily on the basis of external morphology (Bickford *et al.* 2007, Zúñiga-Reinoso and Benitez 2015). Identification of cryptic species allows specific conservation policies to be developed which reduce the extinction risks for potentially sensitive species, due to their intrinsic features or the fragility of the ecosystems in which they occur (Pauperio *et al.* 2012, Moutinho *et al.* 2020). Currently, tools used in conservation genetics allow the accurate detection of cryptic diversity (Allendorf *et al.* 2013)., thus these methods can increase the numbers of identified endemic species.

Costa Rica, like many countries in Mesoamerica, may possess substantial endemism that has not yet been detected fully, particularly in groups like terrestrial small mammals (Hansen *et al.* 2013). Despite the research efforts there have been in the past, there remains insufficient studies that utilize genetic tools to support traditional taxonomy. Consequently, inconsistencies persist in the available classification of small mammal species within the country. Additionally, there are major gaps in knowledge of their natural history, behavior, and ecological interactions (Janzen and Wilson 1991, Wainwright 2007, Reid 2009).

When information on the full range of species for any particular group of organisms is available, it provides the foundation for the field of applied population biology to estimate accurately the vital rates of any given population. Regarding vital rates, Mills (2013) refers to the abundance or population size as the most sought-after

piece of information. This author suggests using robust capture-mark-recapture methods for estimating abundance, applying models for closed populations (if populations do not have additions or losses) or open populations (when it is impossible to assume that there are no additions or losses).

Valle del Silencio, located within the La Amistad International Park, provides an ideal setting for studying the population parameters of small mammals. With minimal direct human intervention, this location allows natural population trends of species to be analyzed in pristine highland habitat (Brenes *et al.* 2004, Chaverri *et al.* 2016).

Terrestrial small mammal research typically involves live-trapping and further manipulation of the individuals caught (Gordon 1998, Brouard *et al.* 2015). The procedure requires adherence to a series of steps that maximize the welfare of each animal. To date, there are no detailed protocols designed by Costa Rican research to guide the proper procedure for such studies in the country. However, general well described considerations are included in literature for small mammals, for example: Lim and Pacheco (2016) or Umetsu *et al.* (2006).

Through our studies of small mammals in a Costa Rican high altitude setting, we have developed a standardized methodology for capture of small mammals and for estimating critical population parameters. This involves a series of steps for collecting, manipulating and identifying small mammals during fieldwork in tropical pristine highland ecosystems and analysis of the field data obtained.

2.2.3 Methods and Materials

Study area

Valle del Silencio is an isolated and pristine area located within the La Amistad International Park in Costa Rica. This park is a World Heritage Site protected for the high diversity of flora and fauna. It is in a region separated from North America and South America by the Talamanca Mountain system – consequently, there is a high level of endemism (PILA – SINAC 2019). In addition, surrounding areas of the park have substantial cultural value due to presence of four isolated groups of indigenous people (UNESCO 2023).

Valle del Silencio is located on the Caribbean side of the mountain ridge (9°06'42" N, 82°57'42" W) (Figure 2.5) yet at high altitude, at 2500 m.a.s.l. (Chaverri *et al.* 2016), and in the montane humid forest life zone following Holdridge (1967). The protected area

includes several poorly-studied ecosystems, mainly due to their inaccessibility. However, investigations conducted at the site indicate the presence of many endemics (Chaves *et al.* 2009, Gonzalez-Maya *et al.* 2012, Chaverri *et al.* 2016, Carrion-Bonilla 2020).

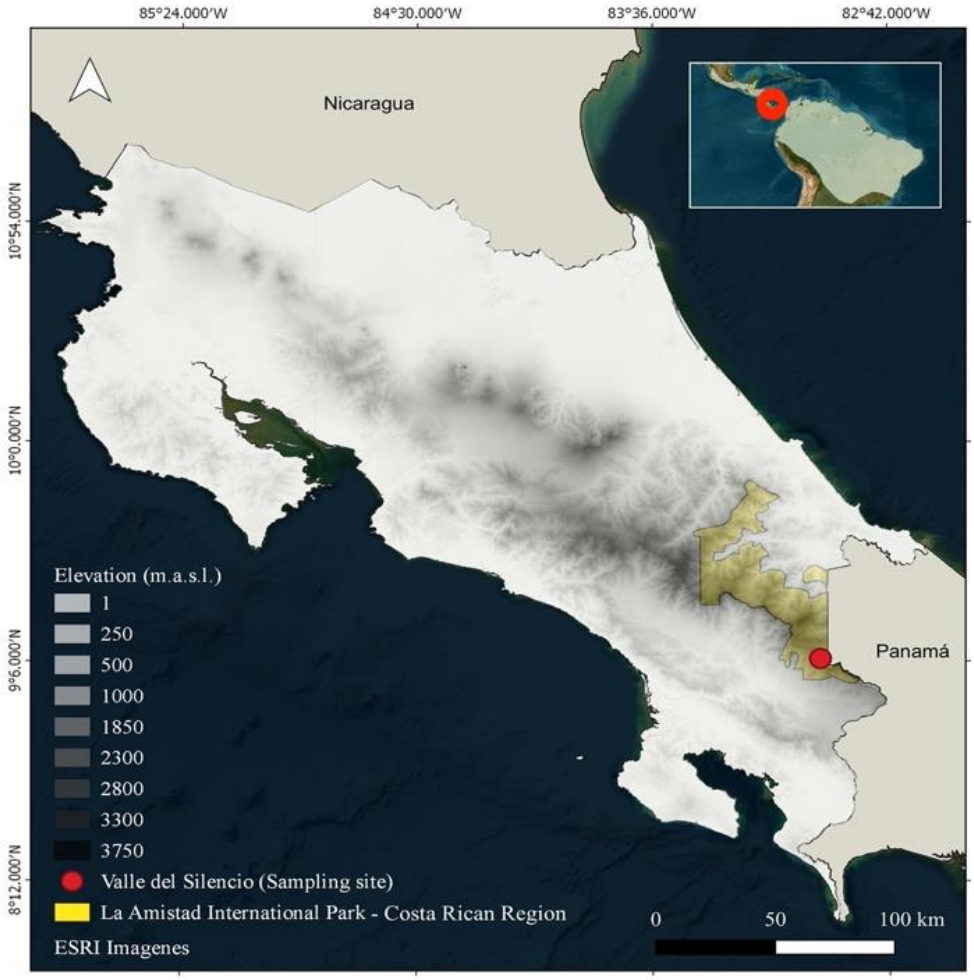

Figure 2.5. Map showing the location of Valle del Silencio inside La Amistad International Park, Cosa Rica, Limón Province. The shaded area in yellow represent the Costa Rican region of La Amistad International Park and the red circle highlights the study area (Valle del Silencio area). Map designed by Christian G. Herrera (christian.herrera@agbcr.org) using QGis 3.4.15. Elevation was defined as meters above sea level: m.a.s.l.

In terms of landscape, Valle del Silencio shows impressive relief: the steepest slopes have inclines of 45% - 60% along several sections of the 15-kilometers trail from Altamira ranger station according to Brenes *et al.* (2004). The area is forested with domination by oaks (*Quercus* spp.) that form root-bases hosting a dense layer of bryophytes, along with fungi and lichens, which help absorb nutrients directly from the environment. These root-bases are a crucial and fragile habitat for insects, small reptiles,

and amphibians, some birds as well as terrestrial small mammals. Similar structures with similar functions are also created in the branches of the trees. Gonzalez-Maya *et al.* (2012) showed that Valle del Silencio is characterized by other high-elevation plants including bromeliads, *Chusquea* spp., *Drymis* spp., and *Prunus* spp. Another fragile ecosystem in this valley is peat bog, which is dominated by *Sphagnum* spp. (Clymo 1984) and large ferns - this is located in a sector known as “El Jardin”.

Equipment

A total of 120 Sherman traps of two sizes were used for live trapping: 23 cm (l) x 10 cm (w) x 11 cm (h) and 3 cm (l) x 11 cm (w) x 10 cm (h) (length: l, width: w and height: h). Each individual captured was implanted with a high-quality Bio-glass microchip (Microchip 1.25 · 7 mm, ISO11784, FDX-B standard, AgnTho`s AB, Sweden) recommended for all sizes of terrestrial small mammal. A Compact Max Pocket Sized Scanner (ISO 9001 and ISO 11784/11785 certified) was used to scan recaptured animals. An ear punch (AgnTho`s 2,75 mm OD, Stainless Steel) was used to collect samples for genetic analysis, with storage of tissue in 1.5mL Eppendorf tubes with 96% ethanol. Pesola® scales (20 g, 50 g, 100 g, 500 g, 1000 g) were used to weigh the animals. Body measurements were taken with an Electronic Digital Vernier.

Species

Of the terrestrial small mammals in Costa Rica, only individuals belonging to the order Rodentia were captured in Valle del Silencio. The protocol described below applies for marsupials captured in the other sampling sites. Shrews require a different approach that was not considered in this protocol since no individuals were captured. In the case of the small carnivore *Neogale frenata* (which was also not captured during the field work for this research), according to previous capture experiences, this same protocol can be considered for this species as well. Table 2.5 in the Results section below show species captured in Valle del Silencio.

Ethical Statement

All trapping and handling procedures with terrestrial small mammals in the present research were approved by CONAGEBIO (National Commission for Biodiversity Management), the official entity from the Ministry of Environmental of Costa Rica by permit number: R-052-2017-OT-CONAGEBIO. The procedures were supervised by a

veterinary official (National Id: 1074). All equipment used during the manipulation of small mammals follows internationally accepted standards.

Protocol for capture of small mammals (rodents and marsupials)

- **General considerations**

Optimal handling of captured small mammals is best carried out under adequate indoor conditions, whenever possible. The room should be sealed to prevent animal escape during handling and any obstacles that would allow hiding space should be avoided. The room should be cool and protected from rain, sun, or other stress-inducing effects on the animal. Ideally, the room should be located in a house close to the trapping area in order to return the animal as soon as possible to the original capture site (Figure 2.6). If a room is not available, a table in a protected area without direct sunlight should be used to provide good conditions for animal handling.

During the current investigation, the traps were always placed within 300 m of the ranger station, which included a room for animal manipulation as described above (Fig. 2.6). Before starting any procedure, it is essential to ensure that all necessary equipment is available in sufficient quantities. It is advisable to carry out pilot sampling to establish the approximate capture success rate, which will help in estimating the materials required based on the procedures to be performed.

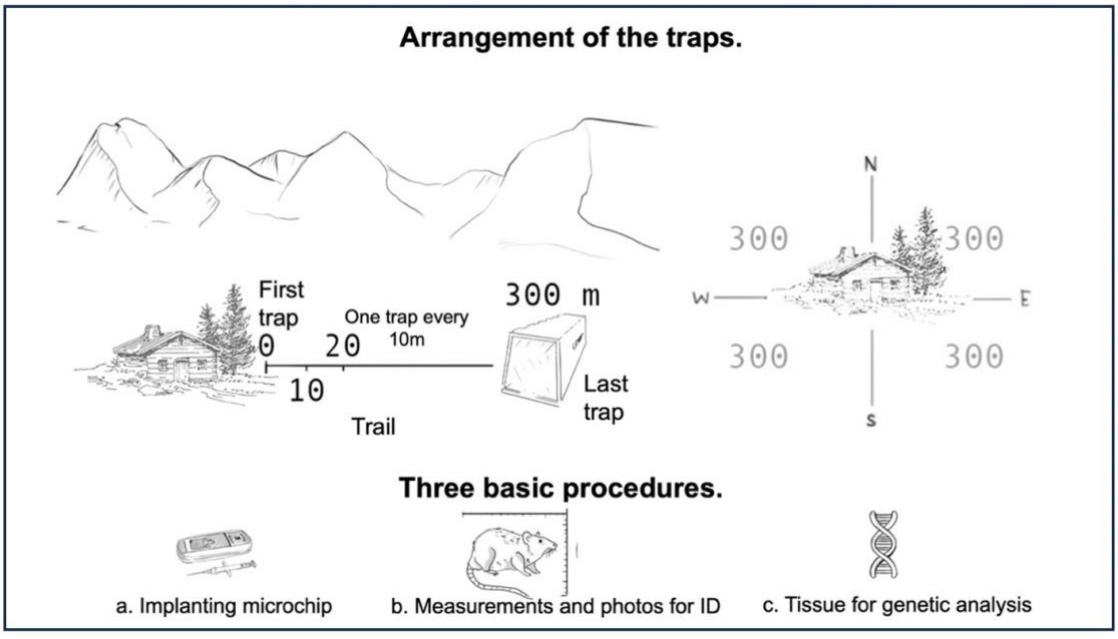

Figure 2.6. Arrangement of traps for sampling during fieldwork for the present research in Valle del Silencio. Four transects were oriented in the compass directions from the ranger station. Each trap was placed every 10 m for a total of

30 traps by transect. Three procedures were adopted for each animal captured: a. a microchip was implanted for subsequent identification in capture-mark-recapture analysis, b. measurements and photographs were taken for species identification and c. tissue was collected for genetic analysis.

The materials needed for the procedures implemented here are displayed in Fig. 2.7.

Figure 2.7. Materials required for the three main procedures. 1: Sherman trap, 2: pens and masking tape, 3: paper towel, 4: guides for identification and notebooks, 5: disinfectant gel, 6: electricians' gloves (to avoid bites) and latex gloves, 7: anesthetic isoflurane (100%), 8: chip reader, 9: sugar water (to hydrate the animal), 10: scissors, 11: iodine, 12: Vernier calipers, 13: face mask, 14: test tube with cotton wool (to apply isoflurane), 15: ear punch, 16: Eppendorf tubes (for tissue sample), 17: 96% ethanol (to fill the Eppendorf tube with the sample), 18: cotton wool, 19: Pesola scales, 20: transparent bags (to manipulate the captured animals), 21: injection needle with chip, 22: lighter (to sterilize equipment)., 23: clamp.

For the current investigation, items were needed for three main procedures (Fig. 2.6):

a) Implanting a microchip for capture-mark-recapture analysis.

b) Measuring body dimensions and collecting photographic records to identify and document the species.

c) Collecting tissue for genetic analysis.

Traps were placed in transects oriented towards the four compass directions from the ranger station. For each transect, 30 traps were placed every 10 m (Fig. 2.6). It is crucial to check the functioning of the traps, as incorrect closure of the trap doors can lead to serious injury or death of captured animals. Another important issue to address in tropical countries is the high likelihood of ants invading the traps, which can deplete the bait or cause serious injury or stress to the captured animal due to ant bites. Also, it is advisable to place cotton wool in the traps to provide greater comfort to the animal and reduce the effects of nighttime temperatures.

- **Bait**

Bait for traps for the present research was prepared by mixing peanut butter (200 g approx.), vanilla extract (50 ml approx.), vegetable shortening (500 g approx.) and oat flour (500 g approx) to achieve the desired consistency (Figure 2.8). The final consistency results in dough that should not stick to fingers. The mixture should be used to generate small balls of approximately 10 g, which is enough to sustain individuals captured overnight. There are other possible baits. Small pieces of apples are commonly used, while tuna and sardines work for some species with more carnivorous or piscivorous habits.

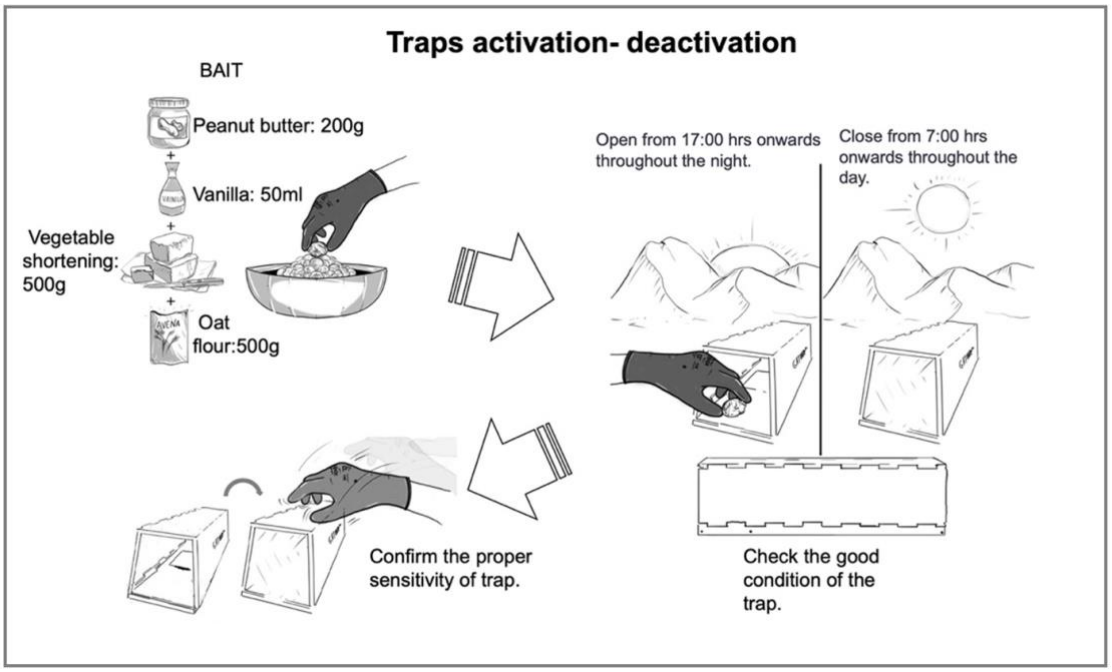

Figure 2.8. Basic procedure for trap activation and deactivation. A bait mixture was prepared to generate 10 g balls to add to the traps. Traps were open from 17:00 hrs onwards throughout the night and closed again from 07:00 hrs onwards throughout the day. Before use, each trap must be checked for its general condition and sensitivity.

- **Trap activation**

The traps were opened one hour before sunset. Each trap was filled with cotton wool and a ball of bait was placed before activation. It is crucial to check the sensitivity of the traps. For Sherman traps, the trigger should neither hold the door too tightly nor be so loose that movement of the trap triggers it. It is recommended to check again that the trap is open and ensure that the trap is in perfect condition before leaving the trapping point. Once the trap is set in place, a small test can be performed by gently tapping the top of the trap with your hand to confirm that it activates quickly (Figure 2.8).

- **Checking traps**

The traps were checked one hour after sunrise and were closed for the remainder of the day to prevent any other animals entering them (Figure 2.8). Traps with captured animals were quickly checked to ensure that the animal was in good condition but also to scan all captured individuals with the microchip reader to confirm if they were recaptures allowing immediate release (Figure 2.9). Afterwards, the trap with a new capture was taken to the next step in the handling procedure. If the animal was not in good condition, it was monitored until it recovered, or released directly at the capture site and monitored until recovery. In only a few extreme cases where the individual did not recover, it was euthanized using isoflurane. It is important to check the traps as early as possible in the day in lowlands and intermediate elevations due to the high temperatures. If possible, traps should be covered with vegetation to avoid direct sun exposure.

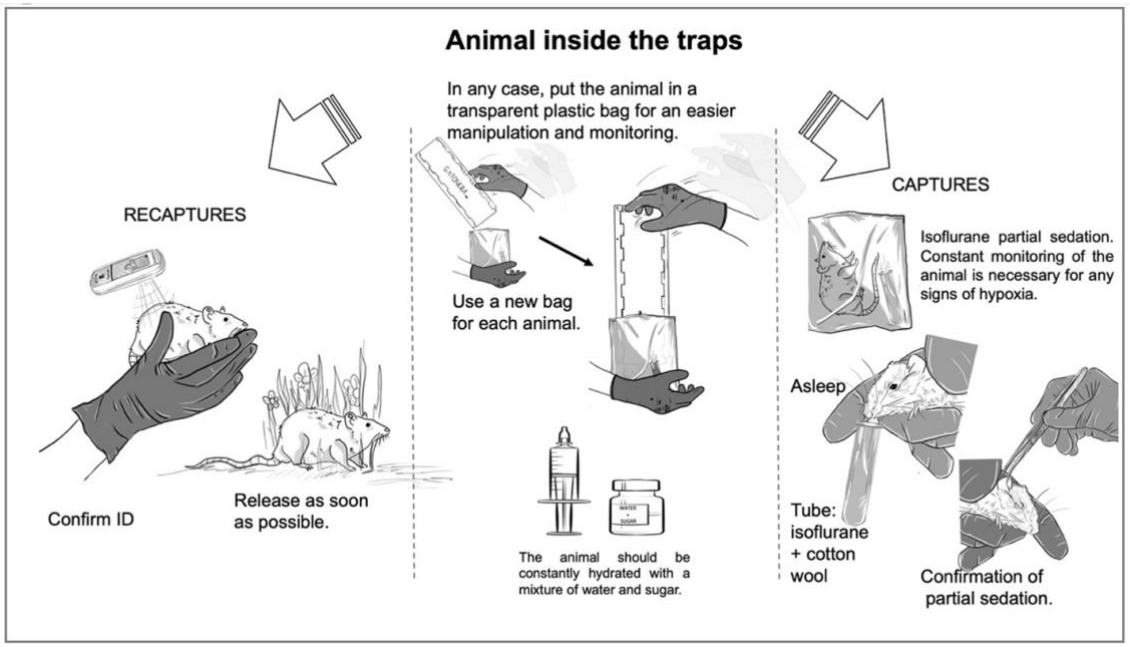

Figure 2.9. Any animal captured should be checked with microchip scanner to determine if it is a recapture or a new capture. Recaptures were released immediately, and new captures were prepared for the procedure. New animals were manipulated in a transparent plastic bag. Partial sedation was implemented with a 20 ml test tube with cotton wool and some isoflurane. The animal should be constantly hydrated with a mixture of water and sugar.

- **Starting animal manipulation**

To minimize any effect of manipulation, immediate handling should be prioritized. For adequate handling and constant observation, each individual should be placed in a transparent plastic bag, with one bag per individual (Fig. 2.9) and each bag discarded after the procedure to prevent any transmission of diseases or parasites. Partial sedation

using isoflurane should be initiated as soon as the animal is placed in the bag. This anesthetic is highly effective in partially numbing small mammals and reduces stress during the procedure. For this study, isoflurane was applied in a test tube with a lid and cotton wool, which was then brought close to the nose until the animal became unconscious (Fig. 2.9). It is important to constantly observe the animal and continue the inhalation for a few more seconds once its eyes start to be completely closed. Several gentle ear pinch tests can confirm sedation before proceeding. During the procedure, the animal should be restrained in a way that minimizes movement, for example, by supporting the head and one forelimb. To prevent sudden unexpected escape reactions due to the animal being only partially sedated, it is important to cover the animal's eyes whenever possible. It is good practice to have some transparent plastic boxes to observe frequently any animal that require special monitoring to maintain their comfort.

- **Microchip implantation**

Each animal captured was implanted with a microchip for individual identification. Before microchip implantation, the captured animal was carefully scanned to ensure that it was not a recapture (Figure 2.9). Each microchip should be scanned before implanting to guarantee that it works properly and registers the ID code (Fig. 2.10). The lock and cap should be removed from the syringe. The microchip was injected under the skin on the middle of the back. The skin was gently lifted to secure and fix the injection site where the microchip should be inserted. Immediately after microchip injection, the wound site was cleaned with iodine to disinfect it and pressure was applied to stop any bleeding (Fig. 2.10). The animal was scanned to confirm that the implantation procedure and chip identification were done properly. In terms of the general care for each animal, a mixture of water and raw sugar was provided occasionally to prevent dehydration.

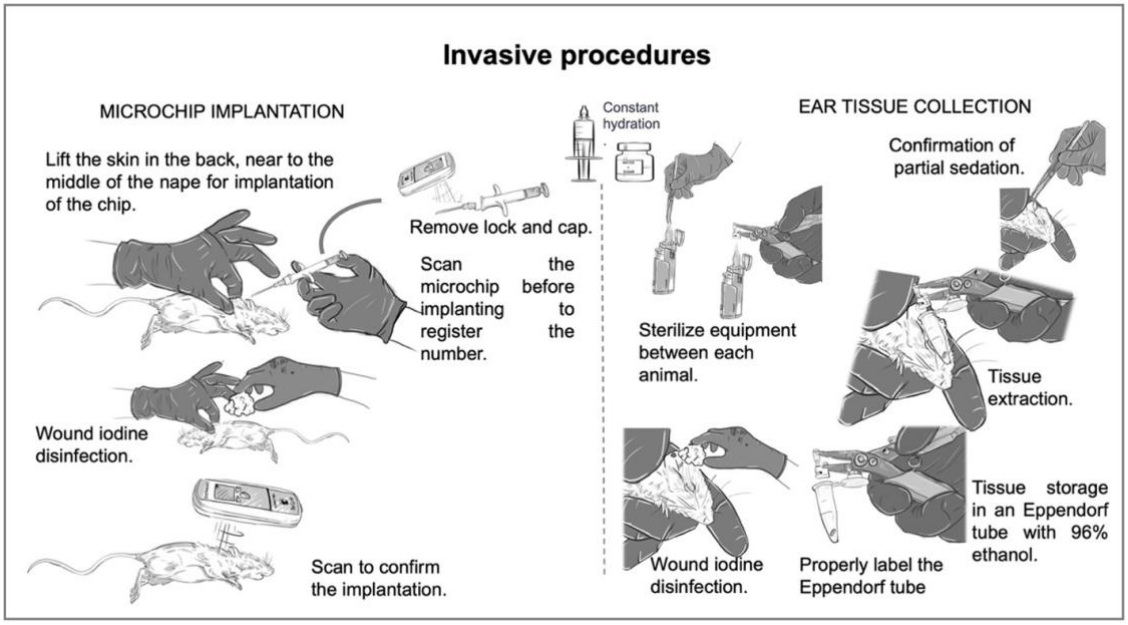

Figure 2.10. Two invasive procedures were performed: microchip implantation and ear tissue collection. The partial sedation can be confirmed by applying repeated pressure to the ears with forceps. For microchip implantation the skin on the back should be lifted, close to the middle of the nape. The animal should be scanned to confirm the implantation. Ear tissue collection requires sterilization of all equipment with a lighter, the ear should be punched to collect the tissue in the Eppendorf tube with 96% ethanol. Both wounds should be disinfected with iodine. The animal should be constantly hydrated with a mixture of water and sugar.

- **Ear tissue collection**

Tissue for genetic analyses was collected using a punch especially designed to extract samples from the ear. The punch has been designed to minimize animal discomfort. The extracted sample was stored in an Eppendorf tube filled with 96% ethanol (Fig. 2.10). It is critical to sterilize all equipment that will be re-used by cleaning with ethanol and flaming with a lighter (e.g., tweezers and punch) and to discard materials such as plastic bags or cotton wool between individuals. After the ear punch, the wound was also disinfected with iodine.

- **Body measurements and photographic records**

Body measurements and photographic records were used to confirm the identification of the captured species. Measurements of body length (without the tail), and tail, ear, forefoot and hind foot length (Fig. 2.11) were taken, as was the body weight of each individual and if it was possible, the animal was also sexed. Regarding photographic records – images of the head, ventral side, dorsal side, forefoot, hind foot and tail were recorded for each animal. Although this information was not used in the present analysis, it is of value for the proper identification and documentation of the species.

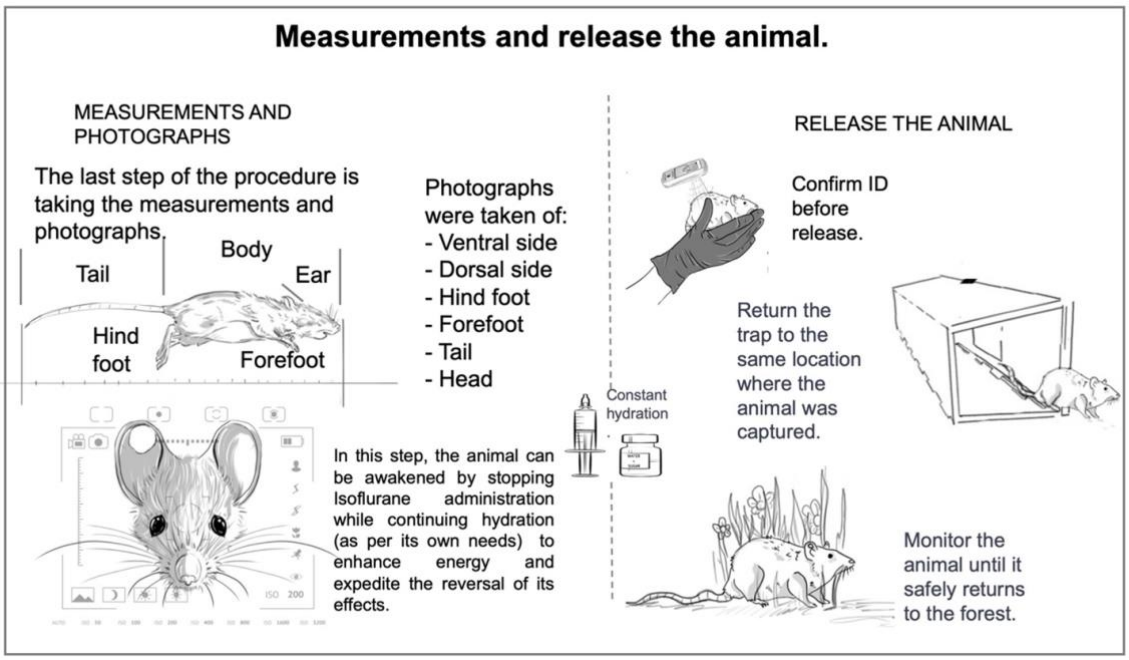

Figure 2.11. Final steps of the procedures were body measurements (tail, body, ear, hind foot) and photographs (head, dorsal side, ventral side, lateral side, hind foot) before releasing the animal. The animal should be scanned to confirm the functionality of the microchip. The animal was monitored until it safely returns to the forest.

- **Release of animals**

Once the procedure was completed, the animal was released as soon as possible in the same place as it was collected. Animals released were monitored until they had made their way back into the forest (Fig. 2.11). The trap that recaptured the animal was placed in the same position and it is highly recommended to wash the trap before that.

- **Safety of participants**

All terrestrial small mammals are potential reservoirs of dangerous wildlife diseases. Direct contact can lead to disease transmission through wounds caused by bites or scratches, contact with the blood of animals, urine, or feces, or the inhalation of contaminated airborne particles. Therefore, researchers and their assistants should always wear protective clothing, including a mask, during the procedure. It is suggested to use dedicated clothing during animal contact. The risk of transmission can be reduced by carrying out intensive cleaning before, during, and after handling animals, with adequate hand washing and/or disinfection using an alcohol gel. The use of gloves is mandatory during the procedure but also choosing the proper gloves is a key step. Both latex and thicker gloves, such as electricians' gloves, can be used to prevent injuries while handling the animals. However, when selecting gloves, the size of the animal and the difficulties in handling it must be considered. Furthermore, it is recommended to wear

long-sleeved shirts and pants as a basic protective measure when participating in the process. At least two trained persons are recommended for all procedures.

Statistical analysis

According to the model of analysis by Mills (2013) for estimation of population vital rates, six primary sampling periods (months) were conducted with three continuous nights each (secondary sampling periods): I-January 2017, II-April 2017, III-August 2017, IV-October 2017, V-December 2017, VI-January 2018. The method described by Coach and White (2021) was followed to record capture histories of each individual by species. In this method, animals were represented in rows, while the capture condition (capture or recapture) and the respective frequency for each of the six capture sampling sessions were represented in columns.

The analysis model was defined as Cormark Jolly-Seber for an open population, under the assumptions that each individual has the same probability of capture and survival between periods whether it is tagged or not, there are no losses of tags, emigrations and immigrations can occur, and the spatial area in which the study is carried out is invariant over time (Pollock *et al.*1990, Kanive *et al.* 2021).

Rmark in the R package (R Development Core Team, 2020) was used to estimate the population size of each species caught. The Comark-Jolly-Seber (CJS) analysis was performed for each capture history in parallel with the analysis procedure described by Giménez (2016) and Laake *et al.* (2022). A modification of the initial data reading command was done to integrate frequency using the code described by Laake (2015).

For each species, four models of heterogeneity were generated as described by Pollock *et al.* (1990): constant survival and recapture during the study (M1), constant survival with time-dependent recapture (M2), time-dependent survival with constant recapture (M3), and time-dependent survival and recapture (M4). The selection method was based on the Akaike's information criteria (AICc) system since it represents the best goodness of fit in relation to the model and the number of parameters (Amstrup *et al.* 2010). Ninety-five percent confidence intervals were generated, through 500 iterations.

2.2.4 Results

During the field phase of this study, a large number of captures and recaptures were made in Valle del Silencio, with a sampling effort of 25,920 trap hours over 18 nights. The average number of captures per night, based on the 120 traps placed, was

29.4 individuals (24.5% of the traps), with 12.33 individuals (10.27% of the traps) being new captures and 17.05 individuals (14.21% of the traps) being recaptured. In total, 529 captures were made, representing 222 individual captures.

Six species of rodents were captured corresponding to: *Nephelomys devius, Peromyscus nudipes, Reithrodontomys creper, Reithrodontomys* spp., *Scotinomys teguina,* and *Scotinomys xerampelinus.* Table 2.5 shows the captures and recaptures by species.

Table 2.5. Numbers of captures and recaptures by species.

Species	Captures	Recaptures
Nephelomys devius	14	17
Peromyscus nudipes	61	124
Reithrodontomys creper	74	109
Reithrodontomys spp.	44	21
Scotinomys teguina	13	13
Scotinomys xerampelinus	18	24

Records in Valle del Silencio indicate that two species are particularly well-represented: *Peromyscus nudipes* and *Reithrodontomys creper,* which dominated significantly (Z= 21.76, d.f= 5, P< 0.001***), while the other species are not clearly different from each other (Fig. 2.12).

The arrangement of transects during the field phase facilitated estimation of species density by calculating the total area covered by four transects. Four approximately 300-meter transects were positioned in a cross pattern, overlapping at the center, enabling estimation of the total area using the formula: total area (A) = width (w) x total length (L). Thus, the width (w) was calculated as the product of the length of the transect and the cosine of 45 degrees, using the formula w = 30 m x cos (45 degrees), which gave an approximate value of 21.2 m. Using that width value, the formula gave A = 21.2 x 120 m (4 transects of 30 m), which equals an approximate total area of 2544 m^2.

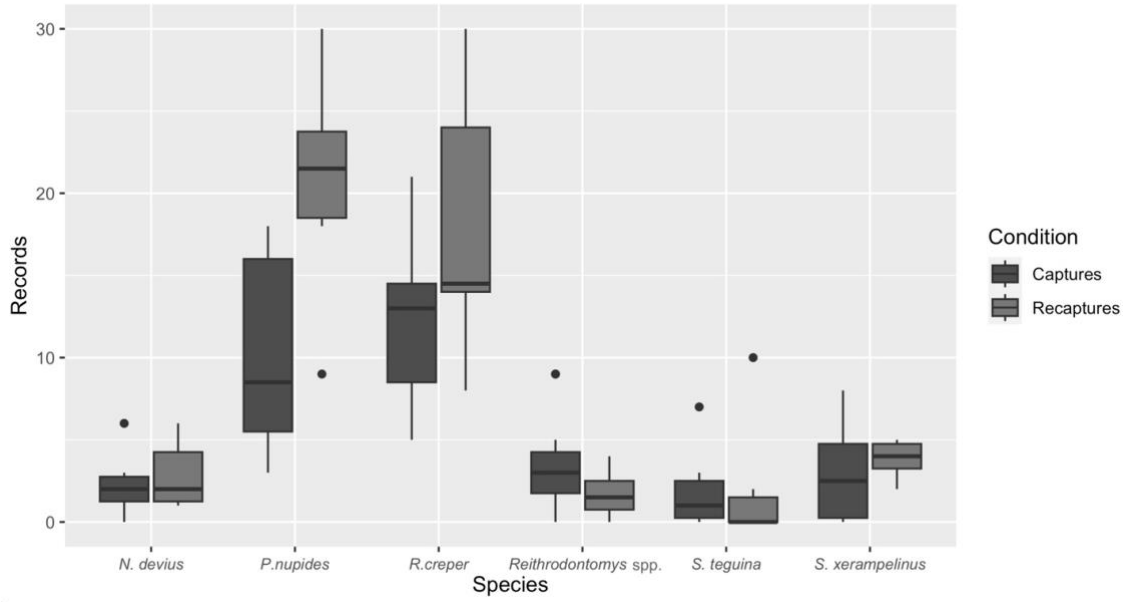

Figure 2.12. Boxplot of records for capture and recapture by species.

The Jolly-Seber model indicates that most species had adequate estimations based on Bonferroni intervals (Table 2.6). In the case of *N. devius*, estimations of the Jolly-Seber model were not possible to apply for the second sampling period due to a lack of capture or recaptures, however, for the remaining periods the estimations were sufficiently accurate. For *S. teguina*, the resulting interval estimations were inaccurate for all periods.

Table 2.6. Population size and density estimates by species, based on the Jolly-Seber model and Bonferroni intervals.

Species	Model	Period sampling — Estimated Value | Bonferroni Intervals				AIC (No. individuals)	Density (Individuals/m²)
		II	III	IV	V		
N. devius	M2	NA	09.00 (03.00-12.00)	10.45 (02.61-18.29)	04.45 (04.45-44.47)	96	0.04
P. nudipes	M4	23.00 (16.00-30.00)	33.08 (25.06-40.32)	16.00 (10.00-24.00)	17.04 (10.60-24.94)	583	0.23
R. creper	M4	25.38 (14.50-38.06)	42.12 (26.01-47.27)	34.05 (28.63-49.32)	32.39 (23.75-41.84)	630	0.25
Reithrodontomys spp.	M3	12.53 (07.97-31.89)	25.06 (13.68-34.19)	25.06 (10.55-26.37)	12.53 (08.43-32.39)	197	0.08
S. teguina	M1	NA	NA	NA	NA	22	0.01
S. xerampelinus	M2	11.64 (03.88-21.34)	07.00 (03.00-11.00)	04.24 (01.41-09.88)	03.00 (01.00-07.00)	165	0.06

Estimates of population size according to the Akaike Information Criterion (AICc) revealed two highly dominant species in terms of population size: *P. nudipes* (583 individuals) and *R. creper* (630 individuals) (Table 2.6). Two other species exhibited a stable number of individuals in terms of population size, but well below the estimates for the two dominant species: *N. devius* (96 individuals) and *S. xerampelinus* (165

individuals). *Reithrodontomys* spp. (197 individuals) may represent several cryptic species. Therefore, in this case, despite the high estimated population number, conclusions may be imprecise until the species classification is adequately resolved. Estimations for the population size of *S. teguina* were low compared to the other species and were inaccurate for all periods. As expected, based on the estimations of population size, the estimations of density by species indicated high densities for *R. creper* and *P. nudipes*, but low densities for the rest of the species (Table 2.6).

2.2.5 Discussion

Protocol considerations

Several studies of small mammals have been conducted in Costa Rica based on live capture procedures (e.g. Kettle *et al.* 2016, Bradley *et al.* 2016, Ribble and Rathbun 2018, Ramírez-Fernández *et al.* 2023a). However, most studies only describe procedures in a general way or do not provide detailed information on measures taken for animal welfare and researcher protection. The present work provides important guidelines for capturing and handling small mammals in tropical areas, representing the first effort to describe in detail the steps needed for the proper handling of small terrestrial mammals captured in Costa Rica. It provides a comprehensive guide for researchers and field-technicians on the correct procedures to capture and handle these animals, ensuring their welfare while minimizing potential harm during experimentation. While designed for a particular study in a particular region, the methods have wider applicability.

Manipulating animals for capture, marking and release represents a particular responsibility for researchers. Clear protocols must exist to ensure that each individual is released in the best possible condition (Gordon 1998). The high rates of recapture (>50%) in our study provides evidence that the procedure we present minimizes negative impacts on the animals caught. This is despite the use of invasive procedures including implantation of a microchip and extraction of tissue through ear-punching.

The high recapture rates may reflect the sedation and other careful procedures that we adopted, thereby avoiding future trap shyness (Pollock *et al.* 1990, Pacheco *et al.* 2013), which can occur as an expression of detrimental procedures during animal capture and release. Although the presence of veterinarians during the procedure is not strictly necessary in situ, they are essential for supervision and advice at every stage of the process.

In accordance with the previous point, despite the low mortality rates recorded in the present investigation (<0.03%), the involvement of a veterinarian is crucial for developing the protocol and adhering to best practices for the well-being of each captured animal, including dealing with emergency situations.

Furthermore, it is necessary for medical experts to oversee the training to avoid potential health risks for researchers participating in these procedures (Gordon 1998). Within Mesoamerica there are no clear estimates of the degree to which people come into contact with small mammals (e.g. through field practices, museum collections, park ranger activities, farmyard situations and others). This is despite the fact that terrestrial small mammals can serve as reservoirs or vectors for various diseases that can be transmitted to humans through contact with their saliva, feces, secretions, bites, inhalation of particles during respiration, and other means. These hazards to researchers warrant special attention, especially given that limited information on such matters is available in Mesoamerica and the potential for diseases associated has been poorly studied for the highly diverse mammalian fauna. Additionally, the region often lacks adequate public healthcare services provided by national authorities and appropriate measures for people involved in these procedures to protect themselves from high-risk diseases such as rabies, tetanus, leptospirosis, and toxoplasmosis.

Species dominance

Species dominance is an ecological criterion commonly referred to simply as the abundance of species without considering elements such as the role that these species play in the ecosystem or their effect on surrounding ecological communities or environmental conditions (Avolio *et al.* 2019). The distribution pattern of the species observed for the small mammal community present in Valle del Silencio indicates a dominance of two species: *P. nudipes* and *R. creper*. Knowledge about these species including their function and effect on the ecosystem is practically absent. To date, in the case of *P. nudipes*, only aspects related to its taxonomic classification have been studied, and there is hardly anything known of its natural history (Reid 2009). For *R. creper*, the taxonomy is well defined, but the narrow range of its distribution in highland areas makes it difficult to investigate, and therefore little is known about its field biology despite its importance as a locally common endemic (Reid and Gómez 2022). The presence of both species together could be important at the community level, and is interesting in relation to adaptation to the hostile conditions prevailing in high-altitude mountain ecosystems, occurring in limited geographic areas (Körner 2007).

The species composition observed in Valle del Silencio suggests a typical ecological relationship between the dominant species and other taxa, which may be due to the minimal impact of human activity (Caceres *et al.* 2011). Other studies conducted in high-altitude zones above 2200 m.a.s.l. in the country also provide insight into the relationship between community structure and the degree of human intervention (which has impacts on vegetation structure etc.). Interestingly, *P. nudipes* and *R. creper* were the only species in common among all the studies, as shown in Table 2.7.

Table 2.7. Reported species of terrestrial small mammals in five studies in the highlands of Costa Rica (>2200 m.a.s.l.).

Species	Study				
	* J & V, 1993	* VdB & K, 1998	* R & B, 2007	*PS, 2017	*R-F *et al*, 2023
Heteromys desmarestianus	√	√	-	-	-
Heteromys oresterus	-	√	-	-	-
Nephelomys devius	-	√	√	√	-
Peromyscus nudipes	√	√	√	√	√
Reithrodontomys creper	√	√	√	√	√
Reithrodontomys rodriguezi	-	-	√	-	-
Reithrodontomys spp.	-	-	-	√	-
Reithrodontomys sumichrasti	√	√	√	-	√
Scotinomys teguina	-	-	√	√	-
Scotinomys xerampelinus	√	√	-	√	√

Regarding the other species found in the present study (*N. devius*, *Reithrodontomys* spp., *S. teguina*, and *S. xerampelinus*), conventional ecology theory underscores the importance of determining the role that each species plays in an ecosystem in order to quantify their contribution to the composition of the community and to assess whether they facilitate or inhibit the establishment of new species (Gilbert *et al.* 2009). In the case of the distribution of species in Valle del Silencio, the delicate balance between the two dominant species, *P. nudipes* and *R. creper*, and other taxa likely depends on the availability of resources. Alterations in the conditions for species highly dependent on forests and exclusive to high altitude ecosystems, such as *R. creper* (Johnson and Vaughan 1993), could potentially affect the dynamics of the less dominant species and consequently impact the community structure.

Ground level microhabitat

A key factor that must be taken into account to preserve the ecosystem are the microhabitats at ground level, especially in sites such as Valle del Silencio. These microhabitats in montane oak forests play a vital role in providing highly specific refuges for terrestrial small mammals (Fig. 2.13). The ground level refuges offer abundant food

sources, including macrofungi and insects for small mammals (Kappelle and van Uffelen 2001), as well as the protection and cover of the root-bases of the oak trees and the associated bryophyte growth (which offers the possibility of building tunnels). This combination of features ensures that organisms such as small mammals survive and thrive (Mueller *et al.* 2001). Thus, safeguarding these ground level microhabitats becomes essential for the long-term preservation of these delicate ecosystems. It is likely that the endemism of *R. creper* and *S. xerampelinus* in higher altitude areas relates to the presence of the ground level microhabitat.

Another element to consider is temperature as a fundamental factor in the use of microhabitats, for example in *Peromyscus* species. As species that do not hibernate, they select microhabitats that ensure suitable microclimates buffered from the hostile temperature fluctuations in highland areas (Hayward *et al.* 2022). Behavioral responses to environmental temperature stimuli, such as the use of cover (as provided by the bryophyte layer), nest building, and huddling, are highly efficient, reducing the importance of insulation (fur, peripheral circulation changes) and physiological adaptations (metabolic rate) to climatic temperature (Hayward, 1964).

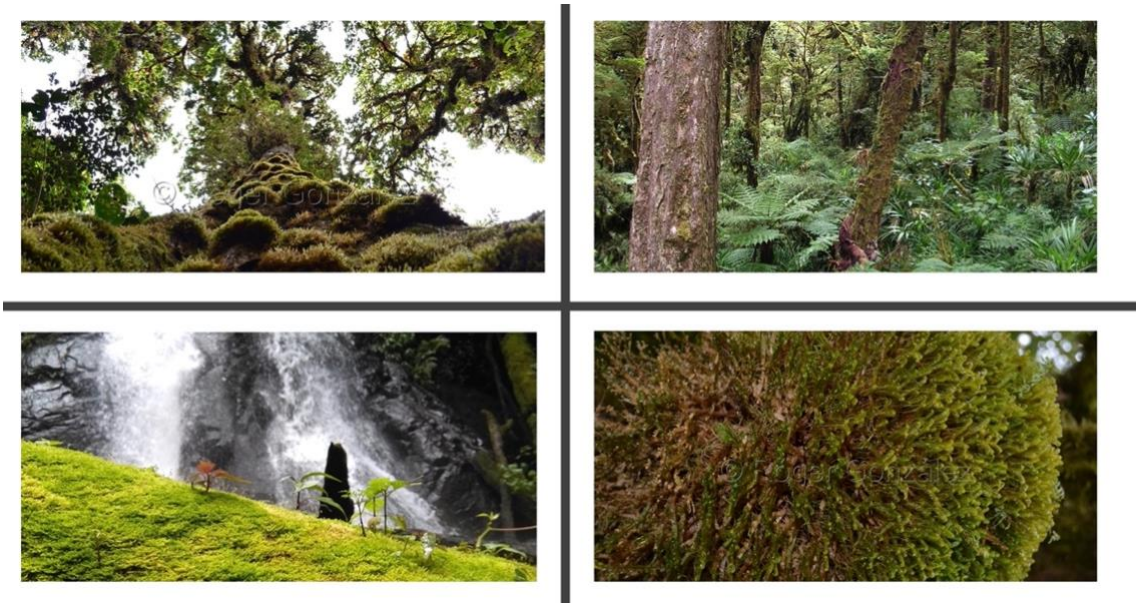

Figure 2.13. Oak forest habitat in Valle del Silencio emphasizing the characteristic presence of a bryophyte layer. Pictures: M.Sc. Roger González Tenorio (roger.gonzalez@sinac.go.cr).

It is important to investigate interactions among the genera *Peromyscus*, *Reithrodontomys*, and *Scotinomys* in high-altitude areas as part of our understanding of

the natural history of these species (Janzen and Wilson 1991). However, accessing these high-altitude areas and conducting thorough research is hindered by limited resources, posing challenges for understanding the dynamics of these taxa. The low density observed for *S. teguina* may be attributed to the sampling method, which focused exclusively on nocturnal sampling. It is worth noting that this species, unlike the others observed, is potentially primarily diurnal (Reid and Gómez 2022), which contrasts with the observed behavior of *S. xerampelinus*, which exhibits greater behavioral flexibility (both diurnal and nocturnal).

Furthermore, the specimens categorized as *Reithrodontomys* spp. in this study represent a group of several species that were not identifiable using the available reference information at the time of capture. These species could represent a group of cryptic species existing high in the mountains and that could be resolved using molecular techniques (Allendorf *et al.* 2022). Proper taxonomic classification is essential for differentiating rare species and understanding their specific requirements within highly fragile ecosystems. This knowledge is crucial for implementing appropriate conservation measures (Smith and Knapp 2003, Dos Anjos *et al.* 2023). Thus, collecting tissue samples and conducting genetic analyses is a critical component of the protocol developed (Gómez-Lépiz *et al.* submitted).

2.2.6 Conclusions and recommendations

- There is a lack of research on terrestrial small mammals in Costa Rica and in Mesoamerica in general. This means that there is a lack of information across fields such as physiology, behavior, and genetics, among others. Insufficient resources, both financial and in specialized facilities, have lessened research opportunities on small mammals, but the capture-recapture procedures described here provides an example of a relatively inexpensive way of getting new information. It is imperative to implement systematic studies for this group, given their intimate relationship with the surrounding environment and their potential as reliable indicators of ecosystem health.
- Research in high altitude forests, such as the Valle del Silencio, is extremely limited, despite the vulnerability of these ecosystems to the impacts of climate change. Consequently, there is a critical need for research on wildlife in montane forests, to provide data to justify the protection of these ecosystems and the preservation of their biodiversity. The institutions responsible for safeguarding

these resources must facilitate a coordinated response to investigate and conserve these highly fragile ecosystems.

- The present study highlights existing information gaps on small mammals, not only with regards to species and their ecosystems, but also the lack of capabilities to forge new research directions. The absence of protocols for field study is one basis of our lack of understanding of small mammals in Costa Rica, which we hope to have helped rectify here. More resources are also needed. It is crucial to promote funding for research within the region to create favorable conditions for research by fostering collaboration among diverse private and public institutions. Such initiatives would facilitate the acquisition of resources necessary for comprehensive studies aimed at bridging the knowledge gaps in this field.
- The Valle del Silencio provides an ecosystem for stable populations of small mammals, with the species concerned existing in a delicate ecological balance with their surroundings. Alterations to this ecosystem would perturb this equilibrium. The prevalence of oak forests in this area provides diverse microhabitats for terrestrial small mammals. These microhabitats hold great potential as natural laboratories for studying the impacts of climate change on temperature-sensitive species, including those belonging to the genus *Peromyscus.* Adequate research in this context is crucial for understanding the effects of climate change on small mammals in montane systems.
- In conventional ecological theory, the concept of species dominance serves as a reference to understand community structure. In the case of *R. creper* and *S. xerampelinus*, their regional endemism and exclusive distribution in highland areas make them particularly intriguing. Investigating the physiological aspects of these two species could potentially establish them as indicators of climate-induced alterations, given their specialization and adaptation to life in high-altitude environments. Consequently, conducting thorough studies on these species, as well as other organisms associated with high altitude forests, becomes crucial, particularly at the microclimatic level. Such investigations would allow for a comprehensive understanding of how physiological and behavioral aspects of the biology of small mammals respond to changes in environmental conditions. Additionally, examining the distributions of these species in relation to the different available climatic zones could provide valuable insights into their adaptive strategies.

- The potential relationship between the dominant species and other small mammal taxa in Valle del Silencio could serve as an exemplar for understanding species assemblages commonly found in similar ecosystems. This line of research could focus on evaluating the habitat preferences and relationships between the species present in a particular site and the corresponding vegetation cover. Such assessments would provide valuable indicators of ecosystem health. However, it is important to emphasize again the lack of experience in data analysis techniques in Costa Rica and neighboring countries, specifically regarding the capture and recapture of species. As a result, standardized comparisons of results across sites with similar conditions are challenging, limiting the ability to make informed conservation decisions for both the species and their ecosystems. Addressing these deficiencies by establishing robust research protocols and data collection methodologies (such as those we have described) is essential to improve our understanding of the species present and their conservation needs.
- It is crucial to prioritize training programs provided by authorities in the country and region to ensure proper fieldwork practices that prioritize the well-being of researchers, participants, and the small mammals that are captured. Given the high recapture rates for the protocols that we developed, it can be inferred that they are adequate for most purposes. However, adjustments should be made as appropriate to improve the experience and minimize risks for organisms during capture, handling, and subsequent release. Strict vaccination protocols should be enforced to prevent the spread of known diseases. Additionally, stringent cleanliness measures must be implemented to minimize the risk of transmission of both known and unknown diseases.
- The application of current molecular techniques to study cryptic species in high altitude areas of Costa Rica is necessary. Complementing existing taxonomy with molecular approaches can help clarify species identification and prevent inconsistencies (Gómez-Lépiz *et al.*, submitted). This approach should be prioritized to enhance our understanding of the species present in these and other ecosystems in the country.

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2.2.6 References

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Chapter 3: Genetic diversity

Chapter 3: Article III. A first species-wide phylogenetic analysis of small mammals from Costa Rica using mitochondrial cytochrome *b*

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3.1.1 Abstract

Within the Mesoamerican biodiversity hotspot, Costa Rica shows high species richness, due to its biogeographical, tectonic and climatic history. Small mammals (species of less than 500g) are a very diverse group in Costa Rica with about 52 native species. This high diversity, recent records of new species and molecular studies detecting cryptic genetic diversity highlight this group and this region as an important target for the DNA barcoding approach. Here we used 131 tissue samples across multiple localities in Costa Rica and sequenced the complete mitochondrial cytochrome *b* (1140 bp). These samples represented 17 recognized species (including *Didelphis marsupialis*, which is technically not a small mammal) and two taxa of uncertain status. The new sequence data were supplemented with previously published data from International Nucleotide

Sequence Database Collaboration (INSDC). Our phylogenetic analyses are consistent with and extend upon recent revisions in *Heteromys*, *Peromyscus* and *Reithrodontomys* and suggest possible new cryptic forms within what are currently named *Melanomys chrysomelas*, *Nyctomys sumichrasti* and *Proechimys semispinosus.* The previously named "*Heteromys* sp" is indeed likely a new species requiring a full taxonomic description. To confirm the presence of cryptic species and major genetic forms in *Heteromys*, *Peromyscus*, *Reithrodontomys*, *Melanomys*, *Nyctomys* and *Proechimys* there needs to be greater sampling, additional genetic markers, morphometrics and other studies. *Scotinomys* also shows interesting phylogenetic subdivision, requiring further investigation.

Keywords: DNA barcoding · Phylogenetics · Taxonomy · Mitochodrial DNA · Conservation genetics

3.1.2 Introduction

Even for a well-studied group like mammals, small mammals are poorly studied in some countries especially in the tropics. Thus, there are regions of the world where we are unlikely to know the full range of species and major genetic forms within species. The DNA barcoding approach developed by Hebert *et al.* (2003) exploits phylogenetics of simple mitochondrial markers to uncover a previously unknown diversity of life forms. For mammals, the mitochondrial gene of choice for such studies is cytochrome *b* (cyt*b*., Tobe *et al.* 2009). Here we describe an investigation using a barcoding approach with cyt*b* to learn more about the diversity of small mammals in one particular country, Costa Rica.

The need to uncover previously undescribed diversity is especially important in areas already known to be rich in species – which typically are in understudied tropical regions at high risk of habitat destruction and species loss (Bradshaw *et al.* 2009). The tropical biodiversity hotspots, inferred by Myers *et al.* (2000) highlighting such high species diversity and high risk, are therefore places where the DNA barcoding approach is particularly important to adopt. The Mesoamerican biodiversity hotspot that includes Costa Rica and Panamá, plus nearby islands, represent one of these high diversity regions. It is one of the most physically and biologically complex areas on the planet, with among the highest levels of biodiversity per km^2 worldwide (Reid and Miller 1989., Bagley and Johnson 2014) and yet only covering ~0.09% (127050 km^2) of the earth's land area (Bagley and Johnson 2014). A considerable proportion of this region (36%) is a tropical forest biome, incorporating vegetation zones ranging from lowland wet and dry

forests to mangrove estuaries, rolling savannahs and grasslands, and once-pristine montane habitats (Marshall 2007). The mainland forms a long (~1170 km), narrow isthmus that is ~240 km wide across Costa Rica but only 65 km wide at the Panama Canal basin. The Talamanca Cordillera mountain range reaches its highest peak at 3820 m in Chirripó Hill (Costa Rica), creating sky-islands of isolated montane habitat (Bagley and Johnson 2014). This mountainous area has the largest forest cover in the country, with the greatest number of life zones and the largest national park (~404500 ha., La Amistad International Park) (Rodríguez-Herrera *et al.* 2014). Costa Rica has a diversity of mammals comparable to much larger countries (such as Mexico and USA) likely due to its geographical position within the tropics and at a location that has acted as a bridge and barrier to several groups of mammals coming from North and South America over ~3 Mya (Cody *et al.* 2010., Wilson *et al.* 2014).

When considering mammalian diversity with an eye to conservation, small mammals (species less than 500g according to Lim and Pacheco (2016) and Rowe *et al.* (2015)) are particularly understudied and undervalued (Fisher 2011). This is unfortunate given their merit as study systems in relation to environmental and conservation issues (e.g. Rodríguez-Estival and Smits 2016., Pardini *et al.* 2005., Jumeau *et al.* 2017., Sullivan *et al.* 2012). Considering the ecological significance of small mammals, it is important to understand their diversity and their interactions in ecosystems. Numerous studies have focussed on the phylogeny (e.g. Jaarola *et al.* 2004., Jansa and Weksler 2004., Piaggio *et al.* 2013) and phylogeography of this group (e.g. Jaarola and Searle 2002., Tougard *et al.* 2013., Barbosa *et al.* 2017), highlighting the importance of molecular tools for understanding their evolutionary history and for their conservation. Multiple studies have also found cryptic diversity in some named small mammals (e.g. Lecompte *et al.* 2005., Paupério *et al.* 2012., Demos *et al.* 2014., Rivera *et al.* 2018).

According to Reid (2009), Reid and Gómez (2022) and Ramírez-Fernández *et al.* (2023), there are about 52 native species of small mammals occurring in Costa Rica, from three orders. The marsupial order Didelphimorphia has only one family, the Didelphidae, which includes all Central American opossums. There are five genera of didelphids occurring in Costa Rica: *Caluromys* (one species), *Marmosa* (four species), *Metachirus* and *Philander* (both with one species), and two species of *Didelphis*, which are technically not small mammals (being larger than 500g). The placental order Eulipotyphla is only represented by one family, the shrews (family Soricidae) and one genus, *Cryptotis*, in Costa Rica, comprising five species. The placental order Rodentia

shows much more diversity at all levels than the other mammalian orders found in Costa Rica, with the 39 species distributed among three families and 23 genera (Table 3.1). The derivation of Costa Rican mammals from both North and South America (Cody *et al.* 2010) can be seen in the small mammal groupings listed above, with the didelphids and the echimyids (members of a rodent family: Table 3.1) with ancestry from South America and all other Costa Rica small mammals with ancestry from North America.

Furthermore, there are six species of endemic small mammals within the political boundaries of Costa Rica, five rodents and one soricid: *Heteromys oresterus*, *Heteromys nubicolens*, *Reithrodontomys cherrii*, *Reithrodontomys musseri*, *Reithrodontomys rodriguezi* and *Cryptotis monteverdensis*. This number increases to 15 species when considering the geographical region consisting of the highlands of the Talamanca Mountain range in Costa Rica and the Chiriquí zone in the west of Panamá. In the extreme north of the country, four other species are only shared with Nicaragua: *Marmosa nicaraguae, Peromyscus nicaraguae*, *Reithrodontomys brevirostris* and *Reithrodontomys paradoxus*, increasing the total number of endemic species to 19. Of these, 14 are distributed in high altitudinal areas (above 1500 m) and mainly in the Talamanca Mountain range (Rodríguez-Herrera *et al.* 2014).

Our understanding of the diversity of small mammals of Central America is based on a mixture of traditional taxonomy and molecular phylogenetic studies, particularly using cyt*b*. For this molecular approach, while there have been many excellent broad-scale studies over the whole region, there have been relatively few that have involved intensive study of Costa Rica (although there are exceptions, e.g. the study of *Heteromys* by Rogers and Vance 2005 and Rogers and González 2010). Given the high diversity of small mammals in Costa Rica, and the associated high likelihood of cryptic diversity, we have conducted a major effort to collect tissue samples of small mammals from localities throughout Costa Rica (Fig. 3.1) for complete cyt*b* sequencing. The sequence data obtained was subjected to a species-wide phylogenetic analysis, following the DNA barcoding approach (Hebert *et al.* 2003). Our rationale was to establish whether the genetic subdivisions that we find in this phylogenetic analysis mirror the current taxonomy, or whether there are undescribed genetic lineages (new major genetic forms within species or new species). One rodent genus stands out as particularly species-rich: *Reithrodontomys* (Table 3.1). We subjected that genus to an additional, separate analysis, to establish whether cyt*b* could confirm already described species boundaries and to investigate if there is even greater species level diversity in this genus.

It needs to be emphasised that the DNA barcoding approach that we adopted should only be seen as the first step in the taxonomic investigation of the small mammals of Costa Rica. More detailed investigations involving further genetic markers and integration with morphological and other analyses are needed to generate convincing descriptions of new species (DeSalle *et al.* 2005).

Table 3.1. The genera and number of native rodent species present in Costa Rica according to Rodríguez-Herrera *et al.* (2014) and Ramírez-Fernández *et al.* (2023)

Order	Family	Genera	Number of species
Rodentia	Heteromyidae	*Heteromys*	4
	Cricetidae	*Handleyomys*	1
		Icthyomys	1
		Melanomys	1
		Nephelomys	1
		Nyctomys	1
		Oecomys	1
		Oligoryzomys	2
		Oryzomys	1
		Ototylomys	1
		Peromyscus	2
		Reithrodontomys	9
		Rheomys	2
		Scotinomys	2
		Sigmodon	1
		Sigmodontomys	1
		Tanyuromys	1
		Transandinomys	2
		Tylomys	1
		Zygodontomys	1
	Echimyidae	*Diplomys*	1
		Hoplomys	1
		Proechimys	1
		Total	39

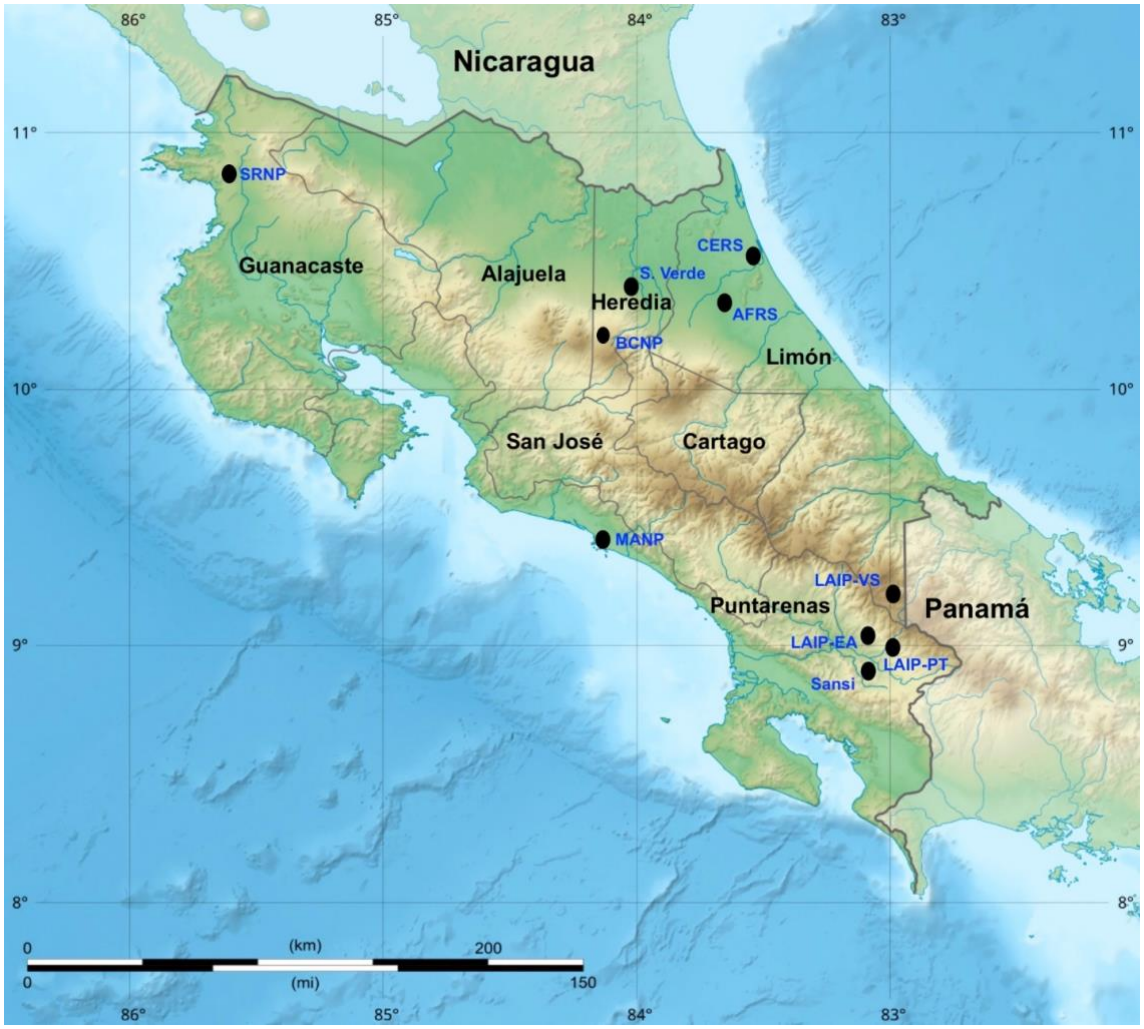


Figure 3.1. Relief map of Costa Rican provinces and within them the sampling localities for the present study (Appendix 3). LAIP: La Amistad International Park., SRNP: Santa Rosa National Park., CERS: Cuatro Esquinas Ranger Station., AFRS: Aguas Frías Ranger Station., BCNP: Braulio Carrillo National Park., MANP: Manuel Antonio National Park. Map adapted from: https://es.m.wikipedia.org/wiki/Archivo:Costa_Rica_relief_location_map.jpg

3.1.3 Materials and Methods

Sampling and DNA extraction

A total of 131 tissue samples (Appendix 3), representative of the small mammals of Costa Rica were collected from 10 sampling locations (Fig. 3.1) using live traps during 2017. In general, we followed the standard definition of small mammals (smaller than 500 g). However, because *Didelphis* entered our traps, we also included this genus in our analysis, despite them being larger than the defined size. Their inclusion also completed the representation of marsupials from Costa Rica for our study. We excluded non-native small mammals (house mice and rats). We also excluded the single

carnivoran under 500 g in Costa Rica (*Neogale frenata*) on the grounds that a single species of a major group had little value for a study such as ours because of the lack of related taxa to compare. Carnivorans are essentially not small mammals while rodents, eulipotyphlans and Neotropical marsupials largely are – so our studies were limited to these latter three taxonomic groups. In total, the samples that we obtained belong to 17 recognized species and two taxa of uncertain status (indicated as 'sp') (Appendix 3). Live individuals were identified using Reid (2009) and reference to the scientific literature available at the time of fieldwork. The tissue samples were taken with an ear punch from individuals under anaesthetic. The animals were later released. The DNA of the tissue samples were extracted using the ExtractMe Genomic DNA 96-Well kit (DNA GDAŃSK).

Amplification and sequencing

The complete cyt*b* gene (1143 bp) was amplified in all samples. Each polymerase chain reaction (PCR) was performed with a total of 10 µL using 5 µL of Qiagen© PCR Multiplex Kit Master Mix (Qiagen, Hilden, Germany), 0.4 µL of each primer (10 µM) and 1 µL of genomic DNA. Details of primers used are given in Table 3.2. The thermal cycling profile consisted of a general protocol: 15 min at 95 °C, 45 s at 95 °C, 45 s at the annealing temperature, and 1 min at 72 °C, plus 5 min at 60 °C over a total of 35 cycles. The combination of primer pair and annealing temperature differ between genera (Table 3.3).

Table 3.2 Primers used for cytochrome *b* amplification and sequencing in small mammals of Costa Rica.

Primer	Strand*	Sequence (5' to 3')	Reference
L14727-SP	L	GACAGGAAAAATCATCGTTG	Jaarola and Searle 2002
L14724	L	CGAAGCTTGATATGAAAAACCATCGTTG	Irwin *et al.* 1991
MVZ05M	L	CGAAGCTTGATATGAAAAACCATCGTTG	Smith and Patton 1993
F1 Cytb	L	TGAGGACARATATCHTTYTGRGG	Whiting *et al.* 2003
Mus 14095	L	GACATGAAAAATCATCGTTGTAATTC	González-Ittig *et al.* 2010
H15915-SP	H	TCTCCATTTCTGGTTTACAAGAC	Jaarola and Searle 2002
MVZ14M	H	TCTTCATCTTTGACTTACAAGG	Modified from Smith and Patton 1993
CBH3	H	GGCAAATAGGAARTATCATTC	Palumbi *et al.* 1991
Mus 15398	H	GAATATCAGCTTTGGGTGTTGRTG	González-Ittig *et al.* 2010

* L (light strand), H (heavy strand)

Table 3.3 Primer pairs and annealing temperatures (°C) used in each genus for cytochrome *b* optimization.

Primer (Forward)	Primer (Reverse)	Genera	Annealing temperature
L14727-SP	H15915-SP	*Peromyscus*, *Reithrodontomys*, *Scotinomys*	52, 54
		Didelphis, Handleyomys, *Heteromys, Melanomys, Nyctomys, Philander, Sigmodon*	52
MVZ05M	H15915-SP	*Reithrodontomys*, *Scotinomys*	50, 52
		Proechimys	50
		Cryptotis, *Handleyomys*, *Melanomys*	52
L14727-SP	H15915-SP	*Oecomys*, *Oryzomys*, *Oligoryzomys*, *Nephelomys*	52
		Oligoryzomys	46, 50, 52
		Oligoryzomys, *Nephelomys*	52-48
		Oligoryzomys, *Nephelomys*	56
MVZ05M	H15915-SP	*Oligoryzomys*	52
Mus 14095	Mus 15398	*Oligoryzomys*	52, 54, 62-54, 56
		Nephelomys	54, 62-54
		Melanomys	56, 58, 60, 62-54

The product obtained by PCR was purified using ExoSAP-IT® PCR clean-up Kit (GE Healthcare, Piscataway, NJ, USA) to clean the remaining nucleotides and primers that were not incorporated during the PCR, and sequences were generated with the amplification primers. Cycle sequencing reactions were carried out using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) or by Genewiz Inc. (USA). Samples were subsequently sequenced for both strands on a 3130XL automated sequencer (Applied Biosystems, USA). Forward and reverse sequences were assembled and edited in Geneious version 8.1.9.

Data analysis

The sequences obtained were analysed together with 129 cyt*b* sequences from INSDC (International Nucleotide Sequence Database Collaboration) incorporating all the taxa represented by our new samples (although there were no INSDC sequences specified as *Reithrodontomys* sp), plus an additional 24 species (Appendix 4). For the *Reithrodontomys* specific analysis, there were an additional 20 INSDC sequences included, with three extra species represented (Appendix 5). In essence, for the all-species analysis we included all species from Costa Rica where INSDC sequences are available, while for the *Reithrodontomys* specific analysis we included all species from Central America represented by INSDC sequences. Our restrictions on the numbers of sequences were as follows:

For the all-species analysis, all newly generated sequences were used except for reduced numbers of *Reithrodontomys* and *Scotinomys* (these genera were considered

fully in separate analyses elsewhere: this work and González *et al.* in prep.). For *Reithrodontomys* and *Scotinomys* a maximum of five of our own sequences per species or major genetic lineage were included, maximizing the geographic spread. For all small mammal species found in Costa Rica, we also restricted the number of INSDC sequences, with a maximum of five per species, preferably from Costa Rica or Central America. For the all species analysis, platypus and echidna were included as outgroup species.

For the *Reithrodontomys* analysis, all the sequences of this genus that we generated were incorporated. INSDC sequences were treated in a similar way as for the all-species analysis, except that all species of *Reithrodontomys* with a distribution that includes at least one of the seven Central American countries were included. Once again, for every species of *Reithrodontomys* considered, up to five INSDC sequences were included according to availability. *Peromyscus nudipes* and *Microtus agrestis* were added as outgroup sequences.

The cytochrome *b* sequences were aligned in both the all-species and *Reithrodontomys* datasets with Clustal Omega v1.2.2 (Madeira *et al.* 2022) under default parameters. Alignments were examined by eye and trimmed to 1140 bp with the subseq function in SeqKit v2.4.0 (Shen *et al.* 2016) to remove trailing gaps.

To obtain a preliminary overview of the situation regarding small mammal species in Costa Rica, a single tree analysis was conducted using maximum likelihood trees inferred using IQ-TREE 2 v2.2.0 (Minh *et al.* 2020) considering default search parameters. Our alignment was partitioned by codon position (1st+2nd and 3rd) with independent rates (Chernomor *et al.* 2016) and the best-fit substitution model for each was determined using ModelFinder (Kalyaanamoorthy *et al.* 2017). Branch support was assessed using ultrafast bootstrap approximation (Hoang *et al.* 2018) and the SH-like approximate likelihood ratio test, each with 1000 replicates.

3.1.4 Results

All-species analysis

Of the 17 recognized species that we sampled in this study, the following provided the first complete cyt*b* sequences from Costa Rica: *Didelphis marsupialis*, *Nyctomys sumichrasti*, *Oryzomys couesi*, *Peromyscus nudipes*, *Philander melanurus, Proechimys semispinosus*, *Scotinomys teguina*, *Sigmodon hirsutus*. In those instances where

complete cyt*b* sequences were already available for Costa Rica, our data often provided new locations within the country.

Combining our new data with previously published sequences in INSDC, we generated a cyt*b* phylogeny using IQ-TREE (Appendix 2). The general features of the phylogeny were as expected, with placentals and marsupials each forming monophyletic groups sister to each other, although the support for the placental grouping is weak. Within the placentals, the Eulipotyphla and the three families of rodents (Cricetidae, Echimyidae, Heteromyidae: Table 3.1) all form well-supported monophyletic groups. The relative positioning of these four groups is unresolved, so that the Rodentia do not form a monophyletic group. This may be a consequence of long-branch attraction (Bergsten 2005).

Nearly all the 41 named species analysed form monophyletic groups within the tree. Nevertheless, some are paraphyletic, or have an otherwise unclear relationship, namely: *Peromyscus nudipes*/*nicaraguae*, *Scotinomys xerampelinus* and *Transandinomys talamancae*. *Heteromys* sp shows features that sets it apart from named species, forming its own clade. Interpreting the results with *Reithrodontomys* sp is more complex. Sequences classified as *Reithrodontomys* sp mostly occupy a large independent clade. However, there is one sequence that is positioned separately with the *Reithrodontomys brevirostris* sequence as its closest relative.

The following list considers each species in turn, with reference to location data in Appendix 3 and Appendix 4 and Fig. 3.1, and referring to the phylogenetic structure in Appendix 2 (with highlighted branches in Fig. 3.2):

Caluromys derbianus (Fig. 3.2D): There are no new sequences and no phylogenetic structure with the sequences from Costa Rica, Panamá and Ecuador.

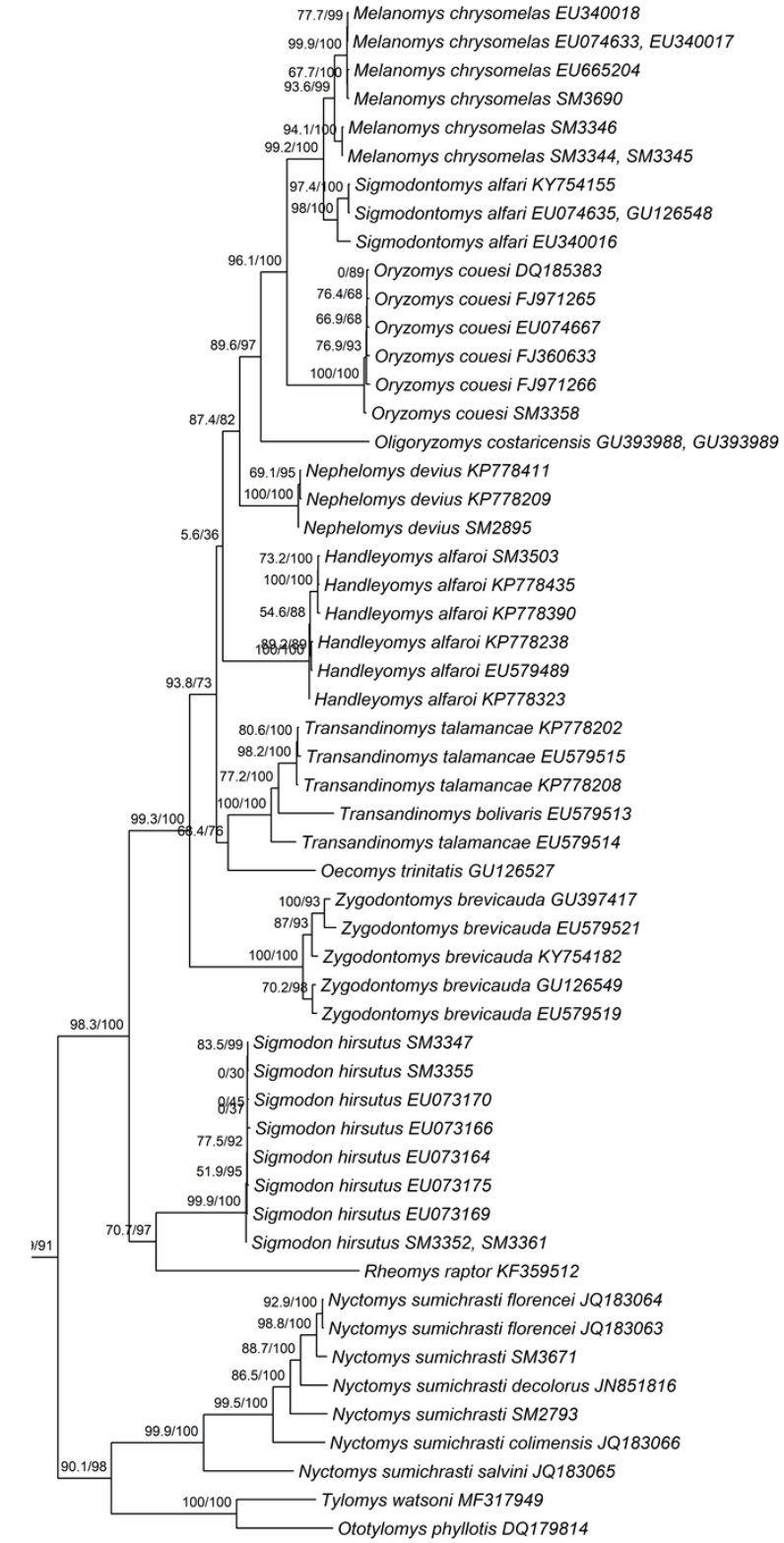
Cryptotis (3 species) (Fig. 3.2C): These three species are represented by four sequences, clustered together. We contributed a new sequence of *C. nigrescens* from BCNP in the province of Heredia, while the previous sequence was also from Costa Rica but from a different province (Puntarenas). They cluster together with long branch lengths.

Didelphis marsupialis (Fig. 3.2D): Our three sequences from Costa Rica, from close to the border with Panamá, add to previous sequences from Panamá, Brazil and Mexico. There is little structure but our sequences cluster most closely with those from Panamá and those from Mexico are most distinctive.

Didelphis virginiana (Fig. 3.2D): There are no new sequences, and minor differentiation between the sequences from Mexico and the United States.

Diplomys labilis (Fig. 3.2C): There are no new sequences, just one existing sequence from Panamá.

A



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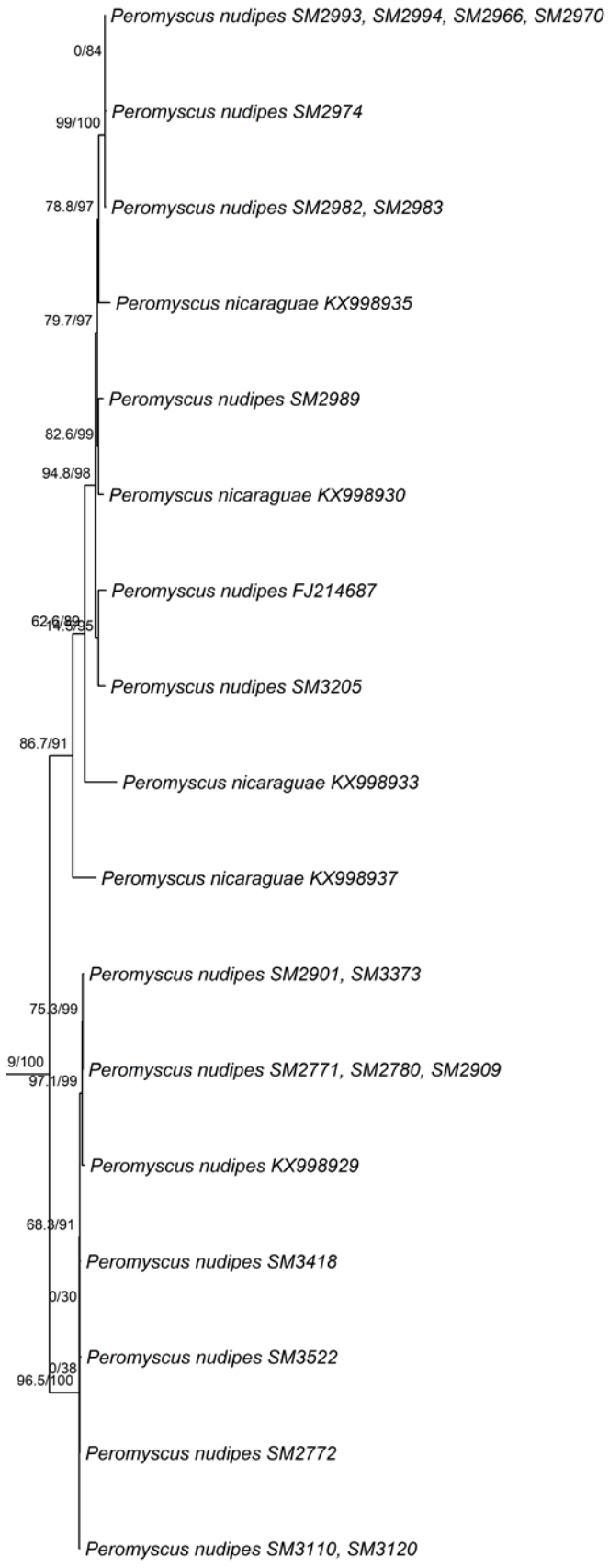
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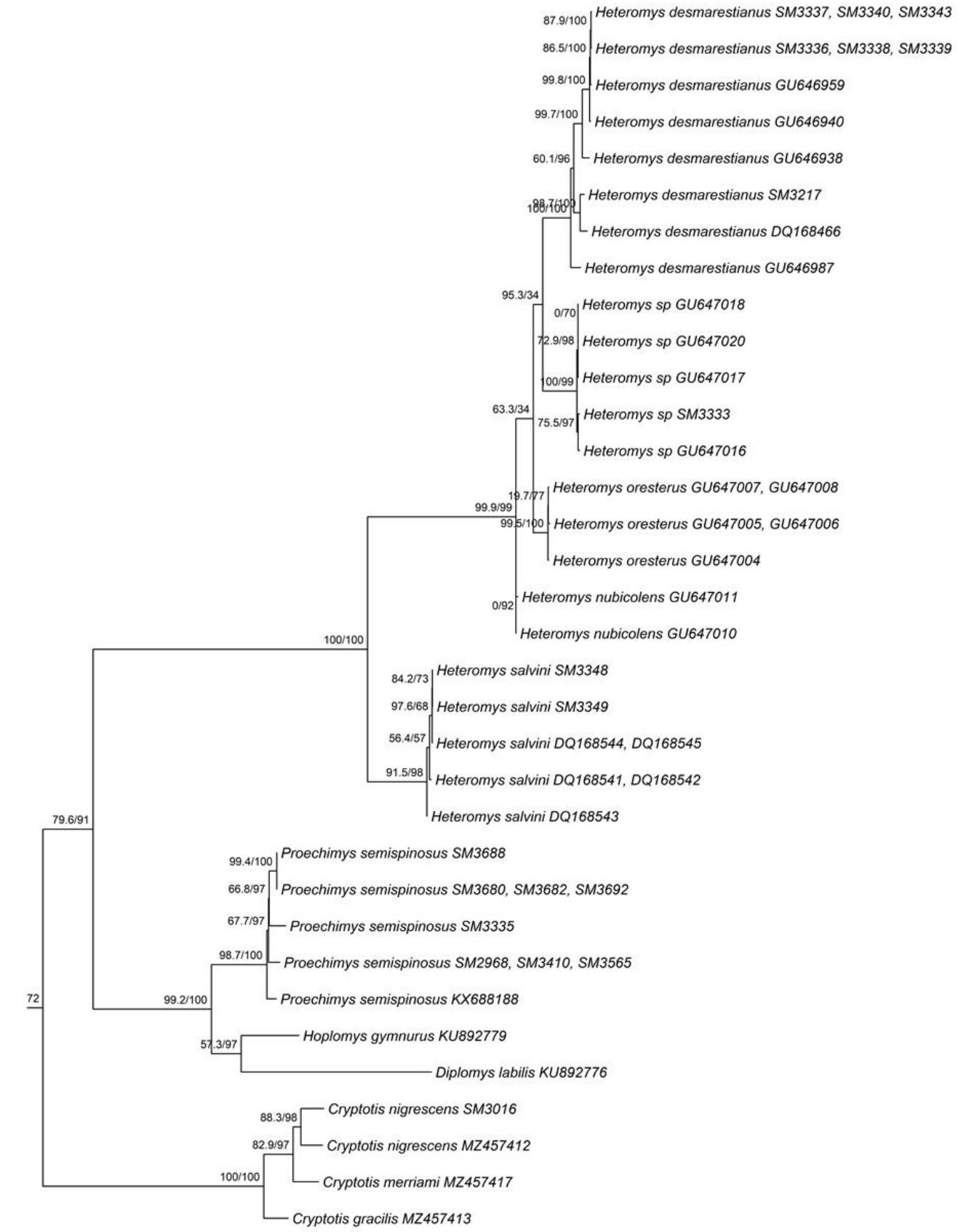
Peromyscus nudipes SM2974
99/100
Peromyscus nudipes SM2982, SM2983
78.8/97
Peromyscus nicaraguae KX998935
79.7/97
Peromyscus nudipes SM2989
82.6/99
94.8/98
Peromyscus nicaraguae KX998930
Peromyscus nudipes FJ214687
Peromyscus nudipes SM3205
86.7/91
Peromyscus nicaraguae KX998933
Peromyscus nicaraguae KX998937
Peromyscus nudipes SM2901, SM3373
75.3/99
9/100
Peromyscus nudipes SM2771, SM2780, SM2909
Peromyscus nudipes KX998929
68.3/91
Peromyscus nudipes SM3418
0/30
Peromyscus nudipes SM3522
0/38
96.5/100
Peromyscus nudipes SM2772
Peromyscus nudipes SM3110, SM3120

C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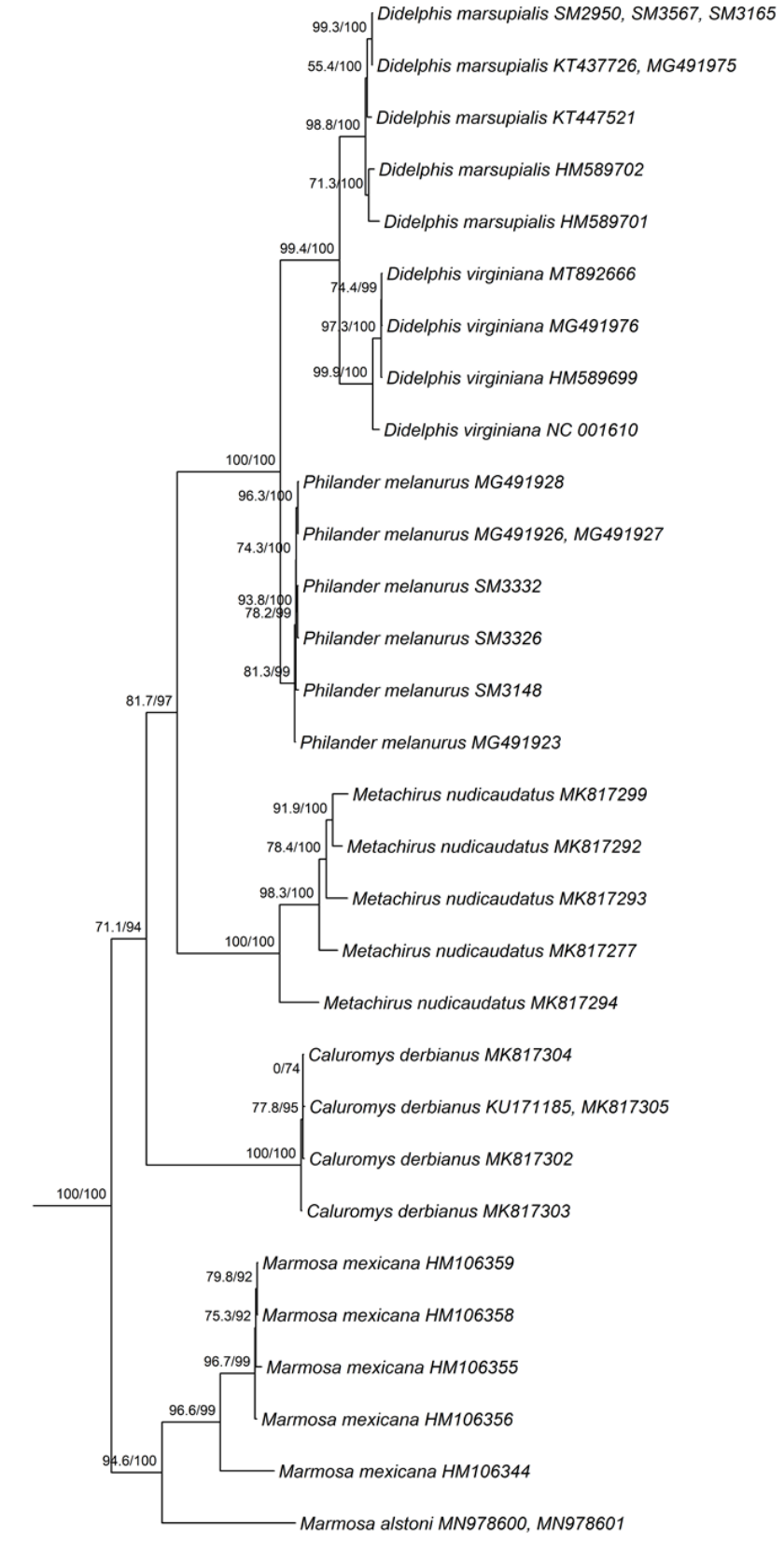
Heteromys desmarestianus GU646938
Heteromys desmarestianus SM3217
Heteromys desmarestianus DQ168466
Heteromys desmarestianus GU646987
Heteromys sp GU647018
Heteromys sp GU647020
Heteromys sp GU647017
Heteromys sp SM3333
Heteromys sp GU647016
Heteromys oresterus GU647007, GU647008
Heteromys oresterus GU647005, GU647006
Heteromys oresterus GU647004
Heteromys nubicolens GU647011
Heteromys nubicolens GU647010
Heteromys salvini SM3348
Heteromys salvini SM3349
Heteromys salvini DQ168544, DQ168545
Heteromys salvini DQ168541, DQ168542
Heteromys salvini DQ168543
Proechimys semispinosus SM3688
Proechimys semispinosus SM3680, SM3682, SM3692
Proechimys semispinosus SM3335
Proechimys semispinosus SM2968, SM3410, SM3565
Proechimys semispinosus KX688188
Hoplomys gymnurus KU892779
Diplomys labilis KU892776
Cryptotis nigrescens SM3016
Cryptotis nigrescens MZ457412
Cryptotis merriami MZ457417
Cryptotis gracilis MZ457413
87.9/100
86.5/100
99.8/100
99.7/100
60.1/96
100/100
95.3/34
0/70
72.9/98
100/99
63.3/34
75.5/97
19.7/77
99.9/99
99.5/100
0/92
100/100
84.2/73
97.6/68
56.4/57
91.5/98
79.6/91
99.4/100
66.8/97
67.7/97
98.7/100
72
99.2/100
57.3/97
88.3/98
82.9/97
100/100

D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Didelphis marsupialis KT437726, MG491975
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Didelphis marsupialis HM589702
Didelphis marsupialis HM589701
Didelphis virginiana MT892666
Didelphis virginiana MG491976
Didelphis virginiana HM589699
Didelphis virginiana NC 001610
Philander melanurus MG491928
Philander melanurus MG491926, MG491927
Philander melanurus SM3332
Philander melanurus SM3326
Philander melanurus SM3148
Philander melanurus MG491923
Metachirus nudicaudatus MK817299
Metachirus nudicaudatus MK817292
Metachirus nudicaudatus MK817293
Metachirus nudicaudatus MK817277
Metachirus nudicaudatus MK817294
Caluromys derbianus MK817304
Caluromys derbianus KU171185, MK817305
Caluromys derbianus MK817302
Caluromys derbianus MK817303
Marmosa mexicana HM106359
Marmosa mexicana HM106358
Marmosa mexicana HM106355
Marmosa mexicana HM106356
Marmosa mexicana HM106344
Marmosa alstoni MN978600, MN978601
99.3/100
55.4/100
98.8/100
71.3/100
99.4/100
74.4/99
97.3/100
99.9/100
100/100
96.3/100
74.3/100
93.8/100
78.2/99
81.3/99
81.7/97
91.9/100
78.4/100
98.3/100
71.1/94
100/100
100/100
0/74
77.8/95
100/100
100/100
79.8/92
75.3/92
96.7/99
96.6/99
94.6/100

Figure 3.2 Highlighted branches of the cytochrome *b* phylogeny for small mammals in Costa Rica (see Appendix 2 for the complete phylogeny). Maximum likelihood tree (IQ-TREE). Branch support: ultrafast bootstrap approximation/SH-like approximate likelihood ratio test results. **A:** Branch including the cricetid rodent genera *Handleyomys*, *Melanomys*, *Nephelomys*, *Nyctomys*, *Oecomys*, *Oligoryzomys*, *Oryzomys*, *Ototylomys*, *Rheomys*, *Sigmodon*, *Sigmodontomys*, *Transandinomys*, *Tylomys*, *Zygodontomys*., **B:** Branch including the cricetid rodent genus *Peromyscus*., **C:** Branch including rodent (echimyid and heteromyid) and eulipotyphlan genera., **D:** Branch including the marsupial genera.

Handleyomys alfaroi (Fig. 3.2A): Our new sequence from Costa Rica (LAIP-PT, Puntarenas) clusters with another sequence from Puntarenas and a sequence from Panamá. The sequences from Honduras, Guatemala and Nicaragua are marginally separate, but there is very little discernible subdivision.

Heteromys desmarestianus (Fig. 3.2C): Our six sequences from coastal central Costa Rica (MANP, Puntarenas) cluster closely with a previous sequence from Puntarenas and a sequence from Cartago (inland central Costa Rica). A sequence from another more northern Costa Rican province (Alajuela) is more distantly related. More distantly related still are two sequences from Honduras (one was ours), which cluster together, and a sequence from Mexico. Thus, there is some subdivision in *H. desmarestianus* over a wide geographic area.

Heteromys nubicolens (Fig. 3.2C): There are no new sequences, and the two sequences previously obtained (from Guanacaste and Puntarenas in Costa Rica) are very similar.

Heteromys oresterus (Fig. 3.2C): There are no new sequences, and the five sequences previously obtained (from San José and Cartago in central inland Costa Rica) are very similar.

Heteromys salvini (Fig. 3.2C): Our two sequences from SRNP (Guanacaste) in Costa Rica group very closely with previous sequences from Guanacaste and Puntarenas and a sequence from Honduras.

Heteromys sp (Fig. 3.2C): This provisional designation by Rogers and González (2010) was based on a distinct lineage located in Costa Rica (found in Alajuela and Limón). We added another sequence from Limón (AFRS). All sequences are very closely related. On the basis of all the cyt*b* data available for this form and in the context of our whole phylogenetic tree, *Heteromys* sp most likely represents a separate species, having a similar level of distinctiveness as other recognized species (both considering *Heteromys* and other genera in our phylogenetic tree).

Hoplomys gymnurus (Fig. 3.2C): There are no new sequences, just one existing sequence from Panamá.

Marmosa alstoni (Fig. 3.2D): There are no new sequences, just two identical existing sequences from Panamá.

Marmosa mexicana (Fig. 3.2D): There are no new sequences, and most of the five sequences previously obtained from Guatemala and Mexico are very similar to each other. One sequence from Guatemala (HM106344) stands out as distinctive.

Melanomys chrysomelas (Fig. 3.2A): The four previous sequences from Nicaragua and Costa Rica (Heredia) are closely related to each other, and one of our four new sequences is closely related to those (SM3690). However, the other three new sequences from the same locality (MANP, Puntarenas) form a somewhat distinct lineage.

Metachirus nudicaudatus (Fig. 3.2D): There are no new sequences, and the five sequences from four countries (Ecuador, Guyana, Panamá, Peru) are distinctive from each other – the sequence from Guyana particularly so. The two sequences from Panamá do form a clade though.

Nephelomys devius (Fig. 3.2A): All three sequences are from Costa Rica and our sequence (from LAIP-VS, Limón) and previous sequences from Puntarenas and Cartago are extremely similar.

Nyctomys sumichrasti (Fig. 3.2A): The five existing sequences from three different countries (El Salvador, Guatamala, Mexico) are generally distinctive from each other except the two from El Salvador that are very similar. The two sequences from Mexico are particularly divergent. Our two new sequences from Costa Rica, although both coming from the same northern inland locality (S. Verde, Heredia) are different from each other and from the other sequences.

Oecomys trinitatis (Fig. 3.2A): There are no new sequences, just one existing sequence from Peru.

Oligoryzomys costaricensis (Fig. 3.2A): There are no new sequences, just two identical existing sequences from Panamá.

Oryzomys couesi (Fig. 3.2A): Our new sequence from Costa Rica (LAIP-PT, Puntarenas) is very similar to existing sequences from Guatemala, Honduras and Nicaragua.

Ototylomys phyllotis (Fig. 3.2A): There are no new sequences, just one existing sequence from Honduras.

Peromyscus nicaraguae/nudipes (Fig. 3.2B): We contributed 18 new sequences from Costa Rica (BCNP, LAIP) and one from Honduras (SM3205). They were all labelled *P. nudipes* but in fact they fall into one of two distinct clusters of tightly related individuals.

One of these clusters, which includes our sequence from Honduras and all our sequences from BCNP in Heredia (and one from LAIP-PT, Puntarenas), also includes five previous sequences from Costa Rica (Puntarenas), Honduras and Nicaragua. Four of these previous sequences have been named *P. nicaraguae*, and it appears likely that the sequences in this cluster should all be classified as *P. nicaraguae*. The other clade with ten of our sequences from LAIP-VS (Limón) and a previous sequence from Panamá are likely appropriately named *P. nudipes*.

Philander melanurus (Fig. 3.2D): Our new sequences from Costa Rica (LAIP-EA in Puntarenas, CERS and AFRS in Limón) cluster very closely with previous sequences from Colombia and Panamá.

Proechimys semispinosus (Fig. 3.2C): Our new sequences from Costa Rica (MANP and Sansi in Puntarenas and S. Verde in Heredia) cluster with a sequence from Colombia. We have contributed eight new sequences from Costa Rica and there appears to be substantial variation among those.

Reithrodontomys: The genus-specific analysis is given below.

Rheomys raptor (Fig. 3.2A): There are no new sequences, just one existing sequence from Costa Rica.

Scotinomys teguina/xerampelinus: The combination of our new sequences from Costa Rica and existing sequences from Honduras (*S. teguina*) and Costa Rica (*S. xerampelinus*) indicate that there is more genetic subdivision than just the two named species. This is considered in more detail in González *et al.* (in prep.).

Sigmodon hirsutus (Fig. 3.2A): Our new sequences from Costa Rica (SRNP, Guanacaste) cluster very closely with previous sequences from Honduras, Mexico and Nicaragua.

Sigmodontomys alfari (Fig. 3.2A): There are no new sequences, and minor differentiation between the existing sequences from Panamá and Ecuador.

Transandinomys bolivaris/talamancae (Fig. 3.2A): There are no new sequences and these two species are represented by five existing sequences. The three *T. talamancae* sequences from Panamá cluster together. However, the *Transandinomys* from Ecuador form two further branches with the disposition making *T. talamancae* paraphyletic.

Zygodontomys brevicauda (Fig. 3.2A): There are no new sequences, and the five sequences from three countries (Bolivia, Panamá, Venezuela) show a degree of genetic subdivision, though it does not relate in a simple way to geography (e.g. the three sequences from Venezuela are not each other's closest relatives).

Reithrodontomys analysis

The sequences that we contributed to the phylogeny of the *Reithrodontomys* genus were *Reithrodontomys* sp, *R. creper* and *R. sumichrasti* (Fig. 3.3). Our sequences of *R. creper* and *R. sumichrasti* are very similar to INSDC sequences of the same species and confirm the monophyly of both those two species within the tree. For *R. creper*, the existing sequences are from the central northern interior of Costa Rica (Cartago, Heredia). Our new sequences came both from this region (BCNP, Heredia) but also at the interior border with Panamá (LAIP-VS, Limón). The previously obtained sequences of *R. sumichrasti* come from a broader geographic area (Costa Rica, Guatemala, Honduras, Nicaragua, Panamá) and our two new sequences are identical to the existing sequence from Costa Rica, being from the same general region in the central interior of the country (our sequences: BCNP, in the south of Heredia., the earlier sequence: Cartago).

The five sequences from *R. fulvescens* are all from INSDC and form a distinct monophyletic grouping at the base of the *Reithrodontomys* clade. All the sequences are from Mexico and there is considerably more structure within the lineage than in *R. creper* and *R. sumichrasti*.

The situation elsewhere within the *Reithrodontomys* lineage is more complex and our *Reithrodontomys* sp sequences are on three separate branches. One of those sequences found in three individuals (SM3143, 3149, 3150) from LAIP-EA and LAIP-PT (Puntarenas) is closely related to a published *R. brevirostris* sequence from Cartago, suggesting that those particular *Reithrodontomys* sp are *R. brevirostris.* This is supported by the inclusion of a shorter published *R. brevirostris* sequence from Alajuela in the same clade (Appendix 6). The addition of another short published sequence of *R. dariensis* suggests that the *R. brevirostris* lineage is in a larger clade consisting of *R. dariensis*, *R. gracilis* and *R. mexicanus* lineages as well (Appendix 6). The apparent misplacement of one *R. mexicanus* sequence (AF108708) clustering with *R. dariensis* is not surprising because "*R. mexicanus*" has been a standard "catch-all" designation for *Reithrodontomys* in Central America until a recent taxonomic reevaluation (Gardner and Carleton 2009).

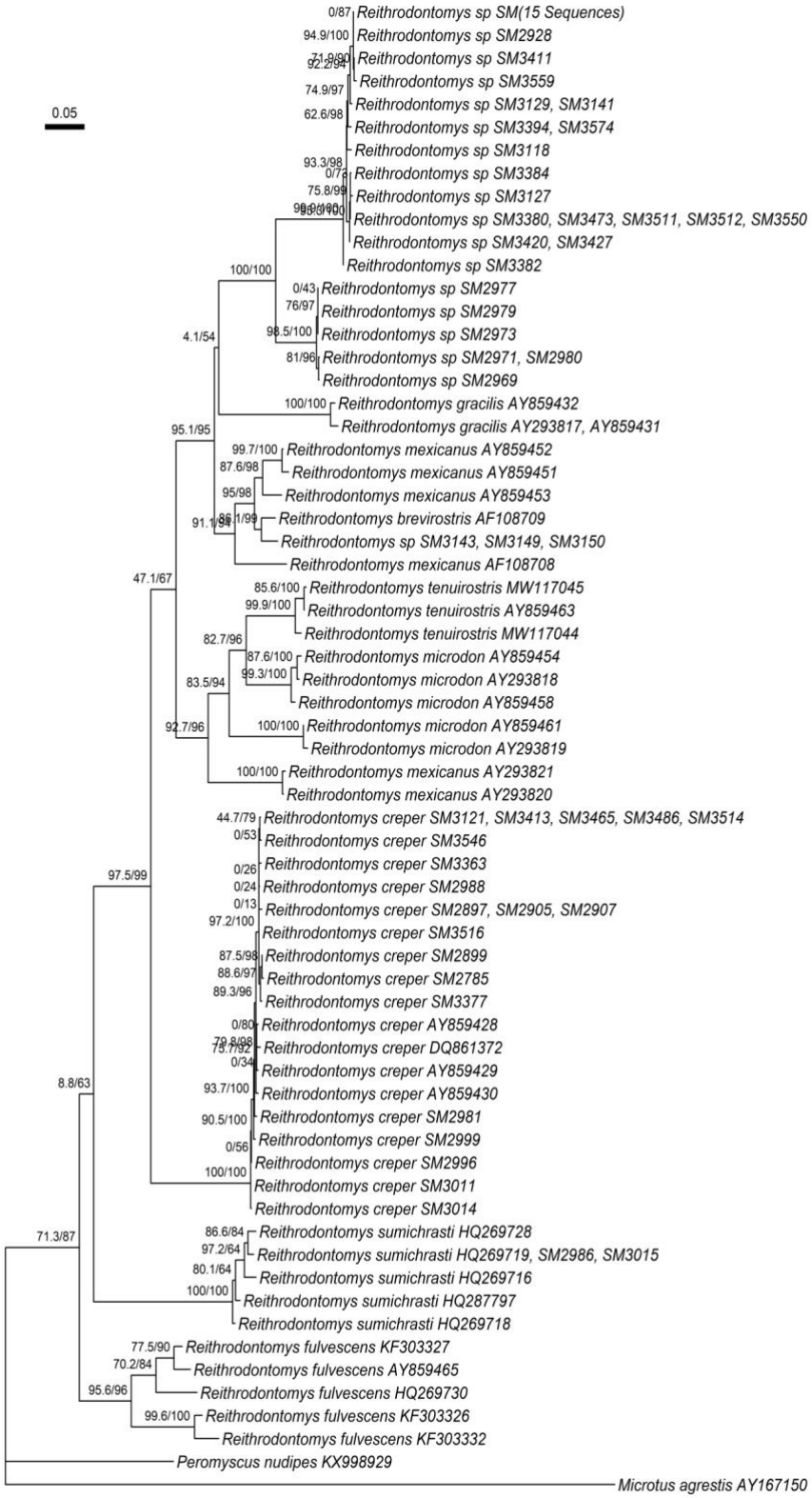

Figure 3.3. Cytochrome *b* phylogeny for *Reithrodontomys* in Costa Rica. Maximum likelihood tree (IQ-TREE). Branch support: ultrafast bootstrap approximation/SH-like approximate likelihood ratio test results. *SM(15 Sequences)*: *SM2781*, *SM2782*, *SM2903*, *SM3123*, *SM3367*, *SM3412*, *SM3417*, *SM3460*, *SM3462*, *SM3463*, *SM3470*, *SM3471*, *SM3488*, *SM3513*, *SM3535*.

A similar phenomenon may explain the clade containing two short sequences of *R. cherrii* together with *R. mexicanus* sequences all from the same province in Costa Rica (San José) (Appendix 6). That apparent *R. cherrii* lineage is grouped with *R. tenuirostris* and *R. microdon* (showing paraphyly) (Appendix 6). The sequences from these two species are from Mexico and Guatemala.

The final two lineages consisting solely of *Reithrodontomys* sp in the main analysis are notable for the lack of genetic subdivision within the branches despite large numbers of sequences (33 in one lineage, six in the other) (Fig. 3.3). Again, a short sequence suggests that the larger clade maybe *R. garichensis* (Appendix 6).

3.1.5 Discussion

The rationale for following a DNA barcoding approach for small mammals from Costa Rica was to attempt to uncover new genetic diversity that may be attributed to previously undescribed major genetic forms or even new species. This approach of taking a wide category of organisms – in our case “small mammals” – and typing reasonable numbers of specimens using a simple genetic marker has long been promoted (Hebert *et al.* 2003). It has also been found to have value in uncovering genetic and taxonomic diversity in a number of systems (e.g. Dasmahapatra and Mallet 2006., Jones *et al.* 2021, Tsoupas *et al.* 2022). For this research we used the complete cyt*b* gene as a barcoding sequence following a rich heritage of its application to phylogeographic studies of small mammals (e.g. Ditchfield *et al.* 2000, Kotlík *et al.* 2022).

Our new sequences were combined with sequences from INSDC, giving us 217 sequences to create a phylogeny of 41 named species of small mammals (plus two species of uncertain identity). Then we followed this up with a focused analysis of a particularly speciose genus of small mammals, *Reithrodontomys* (adding another 65 sequences for an analysis of eight named species plus a species of uncertain identity). The living small mammal specimens that contributed all these new sequences were identified with a field guide (Reid 2009). Clearly, this method of field identification coupled with the barcoding molecular analysis means that we have only made a “first step” at the description of small mammal diversity in Costa Rica. Cyt*b* as a sole discriminator is risky (Galtier *et al.* 2009., Alves *et al.* 2008), so further in-depth genetic and morphological studies are needed. Luckily there have been a number of excellent taxonomic studies of small mammals in Central America which have incorporated cyt*b* analysis (e.g. Almendra *et al.* 2018., Arellano *et al.* 2005., Bradley *et al.* 2016., Corley *et al.* 2011., Gutiérrez *et al.* 2010., Hanson and Bradley 2008., Hardy *et al.* 2013., Pérez Consuegra and Vázquez-

Domínguez 2015., Rogers and González 2010., Voss *et al.* 2019). In this way we have been able to build on that previous work to understand better the genetic lineages of small mammal in Costa Rica – the occurrence of previously described lineages and the identification of possible new lineages.

At the highest taxonomic levels our phylogeny fits reasonably with expectation (separation of the different rodent families, eulipotyphlans, marsupials). But more importantly for our purposes, the phylogeny showed itself to be a valuable tool for species discrimination. Almost all previously described species formed clear monophyletic groups and the clustering of sequences at the within-species level typically mirrored geography, with sequences from geographically close localities typically showing a closer relationship. Our interest was in assessing whether this geography-driven differentiation was of a sufficient magnitude to indicate presence of major genetic forms or cryptic species. Also of note are those few examples where the sequences of a particular named species do not form a clade or where relationships do not reflect geography – this may indicate previously unexposed taxonomic units. Our approach has been a species-wide barcoding analysis of small mammals and, in this spirit, we will be wide-ranging in our discussion of phylogenetic results of the different species of small mammal that we have examined.

Considering our new sequences, *Didelphis marsupialis*, *Oryzomys couesi*, *Philander melanurus* and *Sigmodon hirsutus* added complete cyt*b* sequences for the first time for Costa Rica, but with no indications of strong genetic differences between specimens in Costa Rica and in other nearby countries. In the case of *O. couesi*, this taxon was differentiated from other related forms using cyt*b* sequences, and its species status defined from this molecular analysis (Hanson *et al.* 2010). However, we found little further differentiation within the species over the range incorporating Honduras, Guatemala, Nicaragua and Costa Rica.

In the case of *Handleyomys alfaroi*, *Nephelomys devius*, *Heteromys* sp and *Heteromys salvini* our studies added to previous cyt*b* sequences from Costa Rica, but with no new indications of genetic subdivisions in the species. Previous studies on *H. alfaroi* using a range of nuclear and mitochondrial markers suggested cryptic diversity within the species (Almendra *et al.* 2018), but the differentiation that we found with just cyt*b* was not very substantial, despite using sequences from Costa Rica, Guatemala, Honduras, Nicaragua and Panamá. *Heteromys* sp is particularly interesting because Rogers and González (2010) identified it as a new cryptic species in Costa Rica on the basis of their studies with cyt*b*. Our new sequence and phylogenetic analysis supports

that contention, and we recommend a full taxonomic treatment of this form, and a new naming. *Heteromys* sp is clearly distinct from other closely related *Heteromys* found in Costa Rica on the basis of cyt*b* sequence (*H. desmarestianus*, *H. nubicolens* and *H. oresterus*: Fig. 3.2C). *Heteromys* sp was identified from the cyt*b* analysis of Rogers and González (2010) as one of several cryptic forms within what was known as *H. desmarestianus*. Certainly, considering what is currently classified as *H. desmarestianus* our new sequences reinforce the differentiation between sequences from Costa Rica and those from Honduras and those from Mexico. This differentiation is not of the same magnitude as separates *H. desmarestianus* from *Heteromys* sp, but still noteworthy.

As well as *Heteromys* sp and a Costa Rica lineage of what is currently classified as *H. desmarestianus*, there are other possible examples of cryptic forms in Costa Rica. In addition to the previously described lineage of *Melanomys chrysomelas* from Costa Rica and Nicaragua, to which we added a new sequence, we found a somewhat distinct lineage of three sequences all from the same location (MANP, Puntarenas) (Fig. 3.2A). Hanson and Bradley (2008) already described *M. chrysomelas* as a cryptic species within a wide-ranging form previously know as *M. caliginosus*. Further subdivision maybe appropriate.

In a similar way as the wide-ranging *M. caliginosus* was subdivided into species with smaller ranges on the basis of cyt*b* data, so too may it be appropriate for *Nyctomys sumichrasti*. It ranges from Mexico to Panamá (Corley *et al.* 2011) and there is much differentiation on the basis of cyt*b* sequence (Fig. 3.2A). Corley *et al.* (2011) already recognized a need for taxonomic re-evaluation of this species on the basis of data from El Salvador, Guatemala, Honduras, Mexico and Nicaragua. We have provided the first complete cyt*b* sequences from Costa Rica and our data reinforce that sentiment. One of the two sequences we provide is distinctly different from other branches in the bush of sequences for this species.

The particular utility of using complete cyt*b* sequences to uncover cryptic diversity is illustrated by our analysis of *Peromyscus* (Fig. 3.2B). At the time of collection we named our specimens as *P. nudipes*. However, Bradley *et al.* (2016) inferred the presence of *P. nicaraguae* in Costa Rica, so we included the defining cyt*b* sequence and other published Central American *P. nicaraguae* sequences in the phylogenetic tree. Our new sequences fall into two distinct clades in the tree. Judging by the presence of Bradley *et al.*'s (2016) sequences (both *P. nicaraguae* and *P. nudipes*), one of our "*P. nudipes*" clades should be classified as *P. nudipes* sensu stricto and the other as *P. nicaraguae* on their scheme.

Another species worth further taxonomic treatment on the basis of our data is *Proechimys semispinosus* for which there were no complete cyt*b* sequences for Costa Rica before our study and which shows differentiation within the country based on our work. The *Cryptotis nigrescens* sequence that we obtained was the second for Costa Rica and quite divergent from the first, so again this species is worth further treatment. Our phylogeny reveals other taxa worth further examination based purely on published sequences, particularly *Transandinomys talamancae* (which is paraphyletic in our tree) and both *Metachirus nudicaudatus* and *Marmosa mexicana* which both have single lineages clearly distinctive from the others. The importance of taxonomic revision in *Marmosa* has been recognized by Voss *et al.* (2020).

There are two other genera that require particular attention: *Scotinomys* which is obviously interesting based on the phylogenetic results in Appendix 2 (*S. xerampelinus* is paraphyletic) and *Reithrodontomys*, which is the best represented genus in our study. *Scotinomys* is considered in detail in a separate publication (González *et al.* in prep.). *Reithrodontomys* is considered below.

Reithrodontomys

Reithrodontomys were included both in the all-species analysis (Appendix 2) and in a separate analysis (Fig. 3.3). The utility of the DNA barcoding in this instance is in helping to confirm the species identity of living individuals which is challenging purely based on use of a field guide. Most of the specimens collected were classified by us as *Reithrodontomys* sp because we could not distinguish the species. That range of species is impressively large and based on equally impressive taxonomic effort over many years (Hooper 1952, Arellano *et al.* 2005, Gardner and Carleton 2009, Hardy *et al.* 2013, Martínez-Borrego *et al.* 2022, Arellano *et al.* 2023). The full analysis based on complete cyt*b* sequences allowed us to confirm the identity of *R. creper* and *R. sumichrasti* among our samples. Also, three identical sequences attributed to *Reithrodontomys* sp form a sister lineage to known *R. brevirostris* and are most reasonably attributed to that species. The use of shorter sequences (Appendix 6) suggest that the largest clade of *Reithrodontomys* sp are in fact *R. garichensis.* Also the shorter sequences help convert what we name as "*R. mexicanus*" (following the original descriptions in Smith and Patton (1999), Bradley *et al.* (2004) and Arellano *et al.* (2005) into *R. cherrii* (AY293821, AY293820), *R. dariensis* (AF108708) and *R. mexicanus* sensu stricto (AY839451, AY839452, AY839453). The inclusion of other *Reithrodontomys* species from Central America also allowed us to have a phylogeny of over ten species.

Our study on *Reithrodontomys* builds on several previous taxonomic studies making use of cyt*b* sequences. Our two *R. sumichrasti* sequences were identical to those obtained in a nearby location in Costa Rica by Hardy *et al.* (2013) and which were attributed to one of a number of lineages within the species (classified as *R. s. australis*) (Hardy *et al.* 2013, Arellano *et al.* 2023). The clade that we found that linked *R. cherrii*, *R. microdon* and *R. tenuirostris* (Appendix 6) has also been demonstrated by Martínez-Borrego *et al.* (2022) who designate it the "*R. tenuirostris* group". They also define the "*R. mexicanus* group" consisting of *R. brevirostris*, *R. dariensis*, *R. gracilis* and *R. mexicanus*, which we also find (Appendix 6) and they identify *R. creper* as an independent lineage (as we do) and another group consisting of *R. fulvescens* and *R. sumichastri* that are the earliest branching lineages in our tree. The only two lineages that have not been described by others are the two largest lineages of *Reithrodontomys* sp in our tree of complete cyt*b* sequences. From where the samples were collected, the most likely species are *R. garichensis*, *R. musseri* and *R. rodriguezi* (Gardner and Carleton 2009, Reid and Gómez 2022). Based on a single short sequence, the larger of these lineages is probably *R. garichensis*. The other is, at present, unidentified.

One interesting aspect of our phylogeny of *Reithrodontomys* is the variation in branch lengths between lineages (Fig. 3.3). *R. creper* and our two largest clades of *Reithrodontomys* sp have very short branch lengths indicating very low cyt*b* variation. This could represent biased sampling from a limited geographical area or the possibility of recent bottlenecking and expansion of this species.

3.1.6 Conclusions

Our DNA barcoding approach towards the small mammals of Costa Rica using cyt*b* has newly revealed or supported a number of possible instances of new species or major genetic forms, in *Melanomys chrysomelas*, *Nyctomys sumichrasti, Heteromys*, *Peromyscus* and *Reithrodontomys*. The work on *Scotinomys* is described elsewhere (González *et al.* in prep.) and our phylogenetic reconstructions based on INSDC sequences of *Marmosa mexicana*, *Metachirus nudicaudatus* and *Transandinomys talamancae* suggest that there may be cryptic species or major genetic forms within those taxa. Clearly this work with cyt*b* needs to be supplemented with further sampling, studies of other genetic markers, morphometrics and other studies to confirm the existence of such entities. DNA barcoding is a quick approach to find new species and major genetic forms – it has been very successful when considering the small mammals of Costa Rica. It is particularly for understudied species-rich higher taxa that DNA

barcoding might be expected to work well – and our results corroborate these expectations.

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Chapter 3: Article IV. Cryptic diversity in the singing mice, *Scotinomys* spp. in Mesoamerica.

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3.2.1 Abstract

Based on morphological studies, two species of singing mice from the genus *Scotinomys* are currently recognized in Mesoamerica: *Scotinomys teguina* and *Scotinomys xerampelinus*. *S. teguina* inhabits cloud forests and paramos at intermediate elevations (1000-2900 m) from south Mexico to west Panama and *S. xerampelinus* at high elevations (2200-3300 m) from north Costa Rica to west Panama. Previous studies detected high intraspecific diversity within *Scotinomys* denoting possible presence of cryptic diversity. We analysed one nuclear (*RAG1*) and two mitochondrial (*cytb* and *COI*) genes of 59 samples from Costa Rica together with available sequences retrieved from GenBank. A haplotype network and phylogenetic trees were constructed and intra and interspecific genetic diversity were calculated. We obtained high levels of intraspecific diversity and several well supported clades within each species, in general congruent with their geographic distribution. Five clades were distinguished within *S. teguina* and two within *S. xerampelinus*. Uncovering this genetic diversity encourages a taxonomic reevaluation within the genus *Scotinomys*. Moreover, this study contributes to the identification of conservation units and, consequently, to more effective conservation actions in highly diverse areas as Mesoamerica.

Keywords

Cryptic speciation, genetic diversity, Mesoamerica, Rodentia, *Scotinomys,* singing mice

3.2.2 Introduction

Speciation is the process by which species originate. It is usually accompanied by changes in phenotypic characteristics, reproductive isolation, as well as behavioral, ecological and molecular changes (Bickford *et al.*, 2007). However, speciation may occur without evident morphological change, leading to the categorization of different species in a single morphologically defined taxon. These species are considered as “cryptic species”, as they cannot be differentiated using only morphological data. Over recent decades, with the advance of molecular tools, it has been possible to evaluate biodiversity and speciation processes with the use of DNA sequences. According to Baker *et al.* (2006), the genetic species concept allows the recognition of cryptic species through the analyses of genetic differentiation. This concept states that speciation occurs when two lineages accumulate genetic changes leading to their genetic isolation and therefore, to different evolutionary fates. Thus, this approach focuses on genetic isolation rather than reproductive isolation - distinguishing it from the biological species concept. However, genetic data must be complemented and combined with ecological, morphological and behavioural knowledge in order to differentiate and describe cryptic species (Funk *et al.* 2011, Kucera *et al*. 2002) that would not likely be recognized based solely on morphological data (Paupério, 2012). The distinction of taxa and evolutionary units as well as the discovery of cryptic species could have major implications for conservation and natural resource protection, management, planning and prioritization, especially for endangered taxa (Bickford *et al.* 2007). Thus, describing genetic variability and population structure within species may lead to the detection and delimitation of cryptic lineages that can help us understand the mechanisms of cryptic speciation. This understanding in turn aids preservation of cryptic diversity that, according to Pfenninger *et al.* (2007), is widespread in metazoan taxa and biogeographical regions.

Currently, despite the implementation of different conservation strategies across several countries (CGIAR 2019, SINAC 2019), most organisms are facing fast climate and global changes due mostly to anthropogenic actions, such as habitat loss, land use, species introductions and increase of atmospheric CO_2. These factors cause a decrease in genetic diversity, species extinction and biodiversity loss. Species with low genetic diversity may have a lower adaptive potential and fitness and will not be able to respond

or adapt effectively to these environmental changes. Therefore, for any organism, to maintain high genetic diversity is vital for its survival and persistence (Godoy 2009). That is why it is important to maintain biodiversity and the genetic diversity, and it is essential to understand the evolutionary history of species and to assess the genetic diversity that it displays (Janecka *et al.* 2014).

Mesoamerica is among 25 biodiversity hotspots on Earth containing about 7% of the world's biological diversity in 0.5% (768,990 km^2) of the planet's land surface and 8% of the world mangrove forest (Myers *et al.*, 2000) (Fig. 3.4). Its geographic location is crucial linking North and South America and encompassing about 22 different "ecoregions" (Miller *et al.* 2001). Furthermore, Mesoamerican ecosystems provide a huge variety of primary goods and services and encompass a high level of endemism and species diversity, including a vast diversity of mammals. However, this region is experiencing great loss of habitat due to land use and resource exploitation that threaten its biodiversity (Myers *et al.* 2000). Although different conservation plans are in action (CGIAR 2019, SINAC 2019), more conservation efforts are still needed. Furthermore, the levels of genetic diversity in mammals from Mesoamerica is poorly known. Despite of the paucity of information on mammals, there are recent descriptions of new species in the region (Hernández *et al.* 2015, Hurtado *et al.* 2016) highlighting the need to collect more data. As endotherms, mammals are present in many environmental conditions and influence the diversity of other taxonomic groups (Golley *et al.* 1975). Within mammals, small mammals are a very diverse and important group, as they influence the topsoil health and ecosystem diversity (Martin 2003).

In this study we focus on the endemic rodents of the genus *Scotinomys*, singing mice, belonging to the family Cricetidae. These rodents are blackish-brown and mouse-like in appearance, are diurnal, insectivorous, all have very similar phenotypes and are best known for their vocal communication (Hooper 1972). *Scotinomys* is distributed in the Mesoamerican highlands from south of the Isthmus of Tehuantepe to western Panama in areas of low vegetation in cloud forest and paramo (Hooper 1972). This range has two main barriers that splits it in three different regions: the northern Central American mountain system, the Central American Nucleus and the Cordillera de Talamanca (Fig. 3.5).

Currently two species are recognized within *Scotinomys*: *Scotinomys xerampelinus* (Bangs 1902) and *Scotinomys teguina* (Alston 1876). *S. xerampelinus* is found from the Cordilleras Central and Talamanca regions of Costa Rica (CR) to the

Volcán Chiriquí region in West Panama, at low temperatures and wet and high elevations (2200-3300 m, Reid 2009). *S. teguina* inhabits intermediate elevations (1000-2900 m) from East Oxaca, Mexico to West Panama (Reid 2009) (Fig. 3.4). Even though they are usually found at different altitudes, in both Costa Rica and Panama their habitat overlaps to form a narrow zone of sympatry (Hooper *et al.*1976, Pasch *et al.* 2013). Although *S. xerampelinus* and *S. teguina* are morphologically similar, they can be differentiated by some specific characteristics: *S. teguina* has a shorter tail and larger eyeballs, lens and optic foramen that allows them to be more responsive to visual cues and more aggressive, and that may be important for effective avoidance of predators and detection of food (Hooper 1972, Hooper *et al.* 1976). Additionally, Campbell *et al.* (2010) detected significant differences between their songs. According to the IUCN red list, both *Scotinomys* species are classified as Least Concern. However, because they are limited to cool highlands, they are potentially at risk from climate change (Pino 2015).

Recent studies have found high intraspecific diversity in mitochondrial and nuclear genes within *Scotinomys*, pointing to possible events associated with cryptic divergence. In particular, Pino (2015) found differentiation within the genus and suggests a reevaluation of the current taxonomy. He detected high intraspecific diversity in mitochondrial Control Region (*CReg*) and cytochrome *b* (*cytb*) and nuclear beta fibrinogen intron-7 (*Bfib*) genes within *Scotinomys*, pointing to possible cryptic divergence events. He analysed samples from different Mesoamerican countries and recognized eight clades within the genus suggesting that the current taxonomy should be re-evaluated. Therefore, in our study we intend to evaluate this possibility of cryptic speciation within the genus *Scotinomys* in Mesoamerica focusing on Costa Rica. To detect intra- and interspecific genetic diversity between close related taxa, mitochondrial markers were selected for amplification because of their high evolutionary rate and variability in comparison to nuclear genes (Brown *et al.* 1979). The mitochondrial genes *cytb* and cytochrome *c* oxidase I (*COI*) have been widely employed in mammalian species identification and in determining evolutionary relationships (Martin *et al.* 2000, Castresana 2001, Ruiz-García *et al.* 2014). However, combining nuclear and mitochondrial DNA markers is more efficient and powerful to properly understand the phylogenetic and phylogeographic relationships (Toews *et al.* 2012). The recombination activating gene 1 (*RAG1*) was selected due to its phylogenetic utility in vertebrates (Steppan *et al.* 2004). Using these mitochondrial and nuclear markers, we evaluated genetic diversity, the phylogenetic relationships between *Scotinomys* species and their

phylogeography in order to clarify the cryptic diversity and contribute to the understanding of the evolutionary history of the genus *Scotinomys* in Mesoamerica.

Figure 3.4. Distribution of the genus *Scotinomys* in Mesoamerica (obtained from GBIF, 2019). The left square shows elevation (modified from Brushnell *et al.*, 2020), highlighting the main barriers and regions: 1 = Chiapas Guatemalan depression., 2 = Nicaraguan lowlands depression., NCA = Northern Central American mountain system., CAN = Central American Nucleus., CT = Talamanca Mountain Range.

3.2.3 Material and Methods

Sampling and DNA extraction

Tissue samples of the two species of *Scotinomys* were collected in the La Amistad International Park (LAIP) located at the border between Costa Rica and Panama in the Talamanca mountain range (30 samples in a high altitude site (Valle del Silencio, VS) and 22 samples in a medium altitude site (Ranger Station Pittier, PT) and in the Braulio Carrillo National Park (BCNP) - located to the north of the Central Valley in the Central Volcanic range (6 samples) (Fig. 3.5). Moreover, one sample from Honduras was included. A total of 59 samples of the genus *Scotinomys* were analysed (Appendix 7).

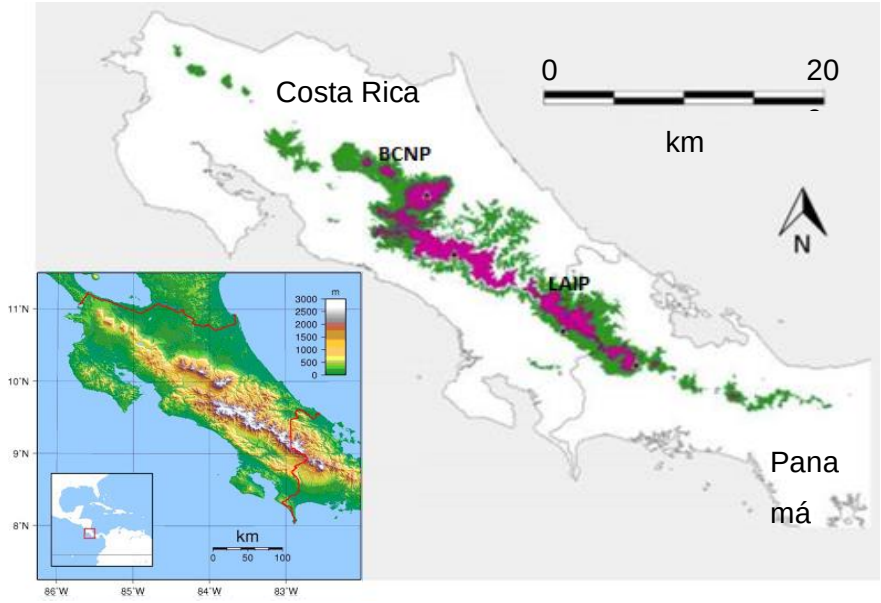


Figure 3.5. Distribution *of S. xerampelinus* (pink) and *S. teguina* (green) species in Cordillera de Talamanca (modified from Pino, 2015). LAIP: La Amistad International Park. BCNP: Braulio Carrillo National Park. The left square shows Cordillera de Talamanca elevation (obtained from https://www.mapsland.com/)

DNA was extracted using the Spin Column EasySpin kit, following the manufacturer's instructions, and eluted in 50 µL. The quality and concentration of the extracted DNA was evaluated by gel electrophoresis (0.8% agarose gel stained with GelRed™). The samples with high concentration of DNA were diluted with ultrapure water.

Amplification and sequencing

Cytb (1140 bp) was amplified in all samples, while for *COI* (653 bp) and *RAG1* (1158 bp), specimens were selected to represent species and regions (12 and 30 samples respectively for the two genes). *Cytb* and *RAG1* were sequenced using a Sanger sequencing approach whereas *COI* was sequenced using a high throughput sequencing approach, based on a double-indexing pipeline with two step PCR.

For *cytb* and *RAG1*, the polymerase chain reaction (PCR) reactions contained a total volume of 10 µL, composed by 0.4 µL of each primer (L14727-SP and H15915-SP for *cytb* (Jaarola *et al.*, 2002) and S70 and S73 for *RAG1* (Steppan *et al.*, 2004)) from a 10 µM solution, 5 µL of Qiagen© PCR Multiplex Kit Master Mix (Qiagen, Hilden, Germany), 3.2 µL of pure water, and 1 µL of extracted DNA. PCR was performed in a Biorad T100 Thermal Cycler using the following thermal conditions: initial denaturation at 95°C for 15 min, 35 cycles of 45 s at 95°C for denaturation, annealing for 45 s at 52°C

for *cytb* and 30 s at 55°C for *RAG1*, and 1 min at 72°C, and a final extension at 60°C for 5 min. Subsequently, electrophoresis (2% agarose gel stained with GelRed™) was used to examine the quality and quantity of the PCR products. The PCR products were purified with ExoSAP-IT® PCR clean-up Kit (GE, Healthcare, Piscataway, NJ, USA) to remove primers and unincorporated reagents in the PCR reaction. Next, with a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA), the sequencing reaction was performed for both strands (forward and reverse) and, in some situations for cytb, with two internal primers (CBH3 (Palumbi, 1996) and F1 (Whiting et al 2003). Thereafter, PCR products were sequenced on a 3130XL automated sequencer (Applied Biosystems, USA).

The amplification for *COI* was performed in two different overlapping fragments with the primer pairs fwhF1 and III_C_R (Vamos *et al.*, 2017., Shokralla *et al.*, 2015) and BF2 and BR2 (Elbrecht *et al.*, 2017). The PCR reactions were composed of 5 µL of Qiagen© PCR Multiplex Kit Master Mix (Qiagen, Hilden, Germany), 3.4 µL of pure water, 0.3 µL of each primer from a 10 µM solution and 1 µL of DNA extraction product in a total volume of 10 µL. The primer pair fwhF1/III_C_R of *COI* followed the next PCR thermal conditions: initial denaturation for 15 min at 95°C, then 35 cycles of 30 s at 95ºC, 90 s at 45ºC for annealing and 45 s at 72ºC, and 10 min at 60ºC for final extension. For the BF2/BR2 primer pair, the PCR conditions were: denaturation for 15 min at 95°C, succeeded by 40 cycles of 30 s at 95°C, 45 s at 51°C and 45 s at 72°C, and lastly an extension of 10 min at 60°C. Afterwards, electrophoresis was used (2% agarose gel stained with GelRed™) to visually examine the quality and quantity of the PCR products. In order to remove unincorporated nucleotides and remaining primer dimers not used in the PCR reaction, the products obtained were purified with 0.8 µL Beckman Coulter™ Agencourt AMPure XP magnetic beads per 1 µL PCR product followed by two times 30 s of ethanol cleaning, and a final immersion of purified DNA in 10nM Tris at pH 8.5. Subsequently, for attaching the adaptor and an individual identifier (barcodes) to each sample, an indexing PCR was performed. This second PCR reaction was prepared using 5 µL of KAPA ReadyMix, 1 µL of compatible Illumina index primers in each pool, 2 µL of H_2O and 2 µL of DNA. The PCR conditions were as follow: initial denaturation at 95ºC for 3 min, then 10 cycles at 95ºC for 30 s, annealing at 55ºC for 30 s and 72ºC for 30 s, and a final extension at 72ºC for 5 min. To verify that the index PCR was successful, the PCR products were examined by electrophoresis (2% agarose gel stained with GelRed™). Then, with AMPure XP magnetic beads, a second purification was

performed, and samples were quantified with NanoDrop 1000 (Thermo Scientific). The index PCR products were normalized to 15 nM concentration and pooled together in two pools, one for each *COI* fragment amplified. These pools were quantified using qPCR (KAPA Library Quant Kit qPCR Mix, Bio-Rad iCycler). Then, they were pooled together, diluted to 4 nM, and sequenced by MiSeq V2 Kit (2x250 cycles, Illumina).

Data analysis

For the *COI* marker, we used the OBITools software package to process the sequences obtained. The forward and reverse reads were overlapped and the sequences alignments with a score lower than 50 were discarded. Then the sequences were separated by sample and dereplicated into unique sequences per sample. Finally, for discarding possible PCR and sequencing errors, the unique sequences with less than five reads and less than 200bp were removed. The sequences obtained for both fragments for each sample were then assembled to obtain the 658bp sequence for *COI*, using Geneious© Bioinformatics Software (version 8.0.5).

The forward and reverse *cytb* and *RAG1* sequences were aligned to respective reference *Scotinomys* sequences from NCBI GenBank and verified manually using Geneious© Bioinformatics Software (version 8.0.5). All the *cytb* (3), *COI* (55) and *RAG1* (2) sequences of *Scotinomys* available in GenBank were retrieved and aligned with our previous sequences (Appendix 7). *RAG1* sequences were then phased into haplotypes using the software PHASE as implemented in DnaSP v5 program (Librado *et al.*, 2009) with 1,000 iterations and 1,000 burn-in iterations. DNAsp was also used to determine *cytb* and *COI* haplotypes.

Phylogenetic networks were built for *RAG1* using the median-joining algorithm (Bandelt *et al.*, 1999) implemented in PopArt (POPulation Analysis with Reticulate Trees, Leigh *et al.* 2015) software to depict the evolutionary relationship among haplotypes. In order to analyze the phylogenetic relationships among *cytb* and *COI* genes, the generation of Bayesian inferences and phylogenetic trees were performed with MrBayes 3.2 program (Ronquist *et al.* 2012) with a 2-partiton (first and second position linked., third position separated) HKI (+Γ) substitution model, as recommended for protein coding sequence data (Shapiro *et al.* 2006) and using *Peromyscus mexicanus* and *Reithrodontomys creper* as outgroups. Bayesian posterior probabilities were estimated from two runs with five chains of 1 million generations for *cytb* and 2 million generations for *COI*, with a sampling frequency that provided a total of 10 000 samples for each run, excluding 10% burn-in.

To evaluate intraspecific genetic diversity, different indices such as nucleotide (Pi) and haplotype diversity (Hd) and polymorphic sites (S) were estimated using DnaSP v5 (Librado *et al.* 2009). The divergence between the clades found was also estimated, namely the average distance between elements of the two groups (Dxy) and the average distance between elements of the two groups, corrected for the within group distances (Da).

3.2.4 Results

For *cytb* we obtained a total of 62 sequences and 23 haplotypes, 67 sequences and 21 haplotypes for *COI* and finally, for *RAG1* gene, 32 sequences and 20 haplotypes, including newly obtained sequences and those from GenBank.

The Bayesian inference tree from *cytb* retrieves divergent clades with an intermediate to high posterior probability (>0.75) (Fig 3.6.a). The *S. xerampelinus* and *S. teguina* main groups are monophyletic (posterior probabilities under 0.95), yet showing genetic subdivisions whithin each species. Within *S. teguina*, there are three strongly supported clades from Honduras and Costa Rica (CR). Within CR, the two clades detected are congruent with the geographic distribution of the samples. For *S. xerampelinus*, the phylogeny suggests two sister clades, corresponding to the two different areas of origin of the samples within CR (LAIP-VS and Central CR). The *Scotinomys teguina* (BCNP) population has the highest intraspecific diversity (nucleotide diversity: 0.257 %, haplotype diversity: 0.933: Table 3.4). The *S. teguina* (LAIP-PT) and *S. xerampelinus* Central CR populations had the lowest diversities (nucleotide diversity: 0.087 % and 0 %; haplotype diversity: 0.589 and 0). Considering all populations within each species, *S. teguina* shows a higher nucleotide diversity (2.479 %), than *S. xerampelinus* (1.307 %, Table 3.4), which is congruent with the number of clades detected within each species (Fig. 3.6.a). The highest interspecific divergences were between the *S. teguina* and *S. xerampelinus* clades (7.02 to 9.12 %, Table 3.5). Within *S. teguina*, the lowest divergence is found between the sequence retrieved from GenBank (without information on origin) and the sequence from Honduras (3.6 %) followed by the clades from BCNP and LAIP-PT (4.88 %). The two *S. xerampelinus* clades diverge by 5.36 % (Table 3.5).

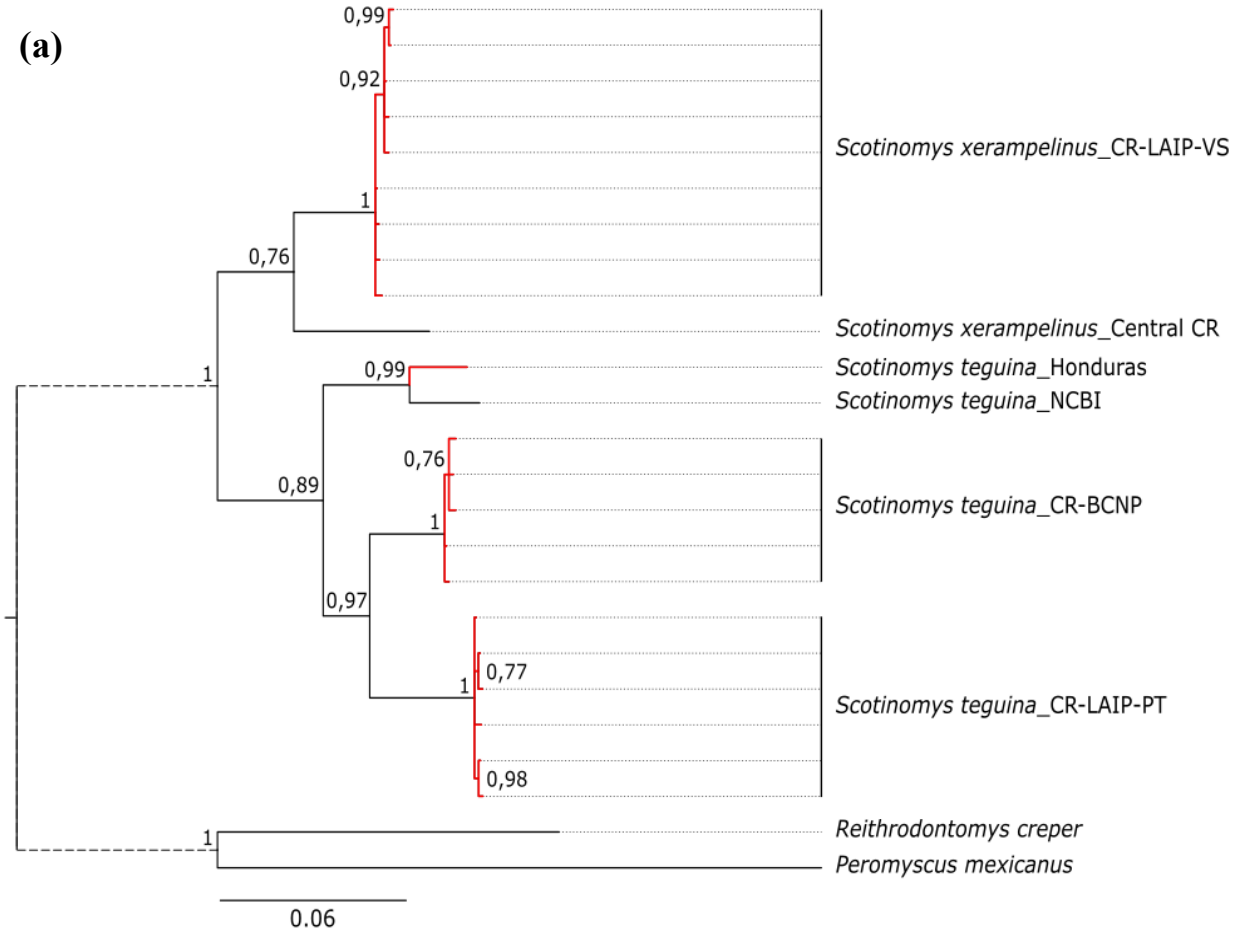
The phylogenetic tree obtained for *COI* represents haplotypes from all the Mesoamerican countries. Despite uncertainty associated with low posterior probability values, in general *S. teguina* appears as paraphyletic, as the central and northern

countries (Mexico, Honduras, Guatemala, El Salvador and Nicaragua) form clades that are separated from the *S. teguina* and *S. xerampelinus* clades from southern countries (CR and Panama) (Fig 3.6.b). In CR within *S. teguina*, two moderately supported clades were obtained, one that corresponds to northern-central CR and includes BCNP, Central CR and Northern CR, and the other clade that corresponds to only one haplotype, from southern CR and Panamá. *S. xerampelinus* is a monophyletic group in CR composed of two well supported clades (pp=1): LAIP-VS and the Central-CR (Fig 3.6.b). Most of the clades found using *COI* are congruent with geography, but some sequences from Guatemala and El Salvador, retrieved from GenBank, are highly divergent from the other sequences from the same region. Additionally, the *S. teguina* from Mexico, Central-Northern CR (including BCNP) and the LAIP-PT and Panamanian clade show low values of nucleotide diversity (Pi equals 0.102 %, 0.674 % and 0 % respectively). However, considering all Mesoamerican *S. teguina* populations grouped together, this value increases to 5.844 % (Table 3.4). In a similar way, the *S. xerampelinus* Central and LAIP-VS CR populations separately show a relatively low intraspecific diversity (0.072 % and 0.51 %) but considering the whole distribution of the species, the value increases to 1.797 %. As for *cytb*, the highest divergence rates in *COI* are between *S. teguina* and *S. xerampelinus* clades (Dxy varies from 7.39 % to 15.21 %, Table 3.6). We can differentiate two groups by their lower interspecific diversities: a *S. teguina* clade from CR (clades 6 and 7., 5.1 %) and a *S. xerampelinus* clade from CR (clades 9 and 10., 4.78 %). Within *S. teguina*, the Central Northern CR clade exhibits the smallest divergence of 5.1 % with both the Nicaraguan and the LAIP-PT and Panamanian clades. Also, the divergences with the clade grouping samples from Honduras, El Salvador and Guatemala and the *S. teguina* CR clade are slightly higher (7.14 % and 7.66 %) than with the Mexican (6.25 %) and Guatemalan (6.5 %) clades.

The haplotype network constructed from *RAG1* sequences shows two main divergent clades. One clade is composed of *S. teguina* sequences, from LAIP-PT, BCNP and a sequence from an unknown location of origin recovered from GenBank and, one *S. xerampelinus* central CR sequence retrieved from GenBank. The other clade is composed exclusively by *S. xerampelinus* from LAIP-VS (Fig 3.6.c).

For *RAG1*, the *S. teguina* populations have a haplotype diversity of 0.826 and nucleotide diversity of 0.356 % but, once the *S. xerampelinus* sequence retrieved from NCBI is incorporated, these values are slightly augmented to 0.842 and 0.358 % respectively. Moreover, the LAIP-VS *S. xerampelinus* population has a lower number of

haplotypes (9), nucleotide diversity (0.382 %) and segregating sites (11) than when all the S*. xerampelinus* sequences (LAIP-VS and Central CR recovered from NCBI) are grouped and analyzed together (11 haplotypes, 0.513 % and 21 sites, Table 3.4). Overall, in Table 4.7, the Dxy and Da values for *RAG1* are lower than those estimated for *cytb* and *COI*. The interspecific diversity between *S. xerampelinus* LAIP-VS and the *S. teguina* populations is the highest although it decreases (from 1.04 to 0.9 %) when the *S. xerampelinus* sequence retrieved from GenBank is included within *S. xerampelinus*, as this sample shares haplotype with *S. teguina* (Fig. 3.6 a and Table 3.7).



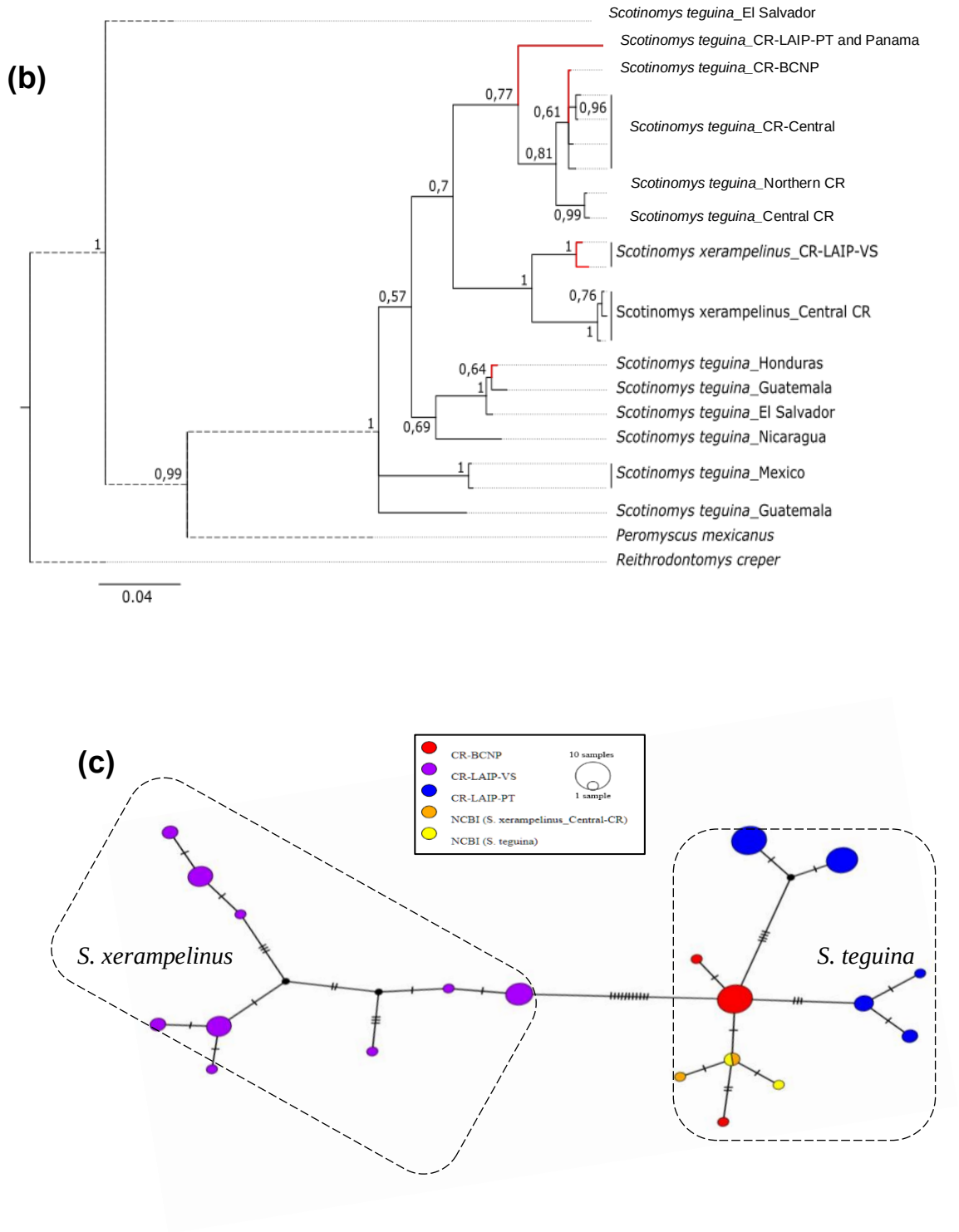


Figure 3.6. a) Bayesian inference tree for *cytb* (1140 bp). b) Bayesian inference tree for *COI* (653 bp). Red branches represent our samples. Posterior probabilities of all nodes are indicated. CR = Costa Rica., LAIP-PT = La Amistad International Park – Ranger Station Pittier (at medium altitude)., LAIP-VS = La Amistad International Park – Sector Valle del Silencio (at high altitude)., BCNP = Braulio Carrillo National Park. Outgroups: *Peromyscus mexicanus* and *Reithrodontomys creper*. c) *RAG1* (1158bp) median joining haplotype network of the genus *Scotinomys*.

Table 3.4. Genetic indices for within population diversity in the genus *Scotinomys* estimated from *cytb* (1140 bp) *COI* (653 bp) and *RAG1* (1158bp) sequences. n: number of sequences obtained, h: number of haplotypes, S: number of polymorphic sites, Hd: haplotype diversity (standard deviation in parenthesis), Pi: nucleotide diversity.

Marker	Clades	n	h	S	Hd	Pi
Cytb						
	S. teguina (NCBI)	1	-	-	-	-
	S. teguina (Honduras)	1	-	-	-	-
	S. teguina (BCNP)	6	5	7	0.933 (0.122)	0.00257
	S. teguina (LAIP-PT)	22	6	6	0.589 (0.114)	0.00087
	***S. teguina* (all populations)**	30	13	128	0.779 (0.077)	0.02479
	S. xerampelinus (Central CR)	2	1	0	0 (0)	0
	S. xerampelinus (LAIP-VS)	30	9	93	0.729 (0.077)	0.00702
	***S. xerampelinus* (all populations)**	32	10	128	0.76 (0.071)	0.01307
COI						
	S. teguina (Mexico)	4	2	1	0.667 (0.204)	0.00102
	S. teguina (Guatemala)	2	1	0	0 (0)	0
	S. teguina (El Salvador)	1	-	-	-	-
	S. teguina (Honduras, El Salvador and Guatemala)	9	3	7	0.417 (0.191)	0.00289
	S. teguina (Nicaragua)	1	-	-	-	-
	S. teguina (Central (BCNP) and Northern CR)	18	7	17	0.869 (0.042)	0.00674
	S. teguina (CR-LAIP-PT and Panama)	6	1	0	0 (0)	0
	***S. teguina* (all populations)**	41	16	158	0.928 (0.019)	0.05844
	S. xerampelinus (Central CR)	12	3	2	0.439 (0.158)	0.00072
	S. xerampelinus (CR-LAIP-VS)	3	2	5	0.667 (0.314)	0.0051
	***S. xerampelinus* (all populations)**	15	5	37	0.638 (0.129)	0.01797
RAG1						
	***S. teguina* (all populations)**	38	10	16	0.826 (0.033)	0.00356
	S. teguina (all populations and *S. xerampelinus*-NCBI)	40	11	17	0.842 (0.031)	0.00358
	S. xerampelinus (LAIP-VS)	24	9	11	0.866 (0.039)	0.00382
	***S. xerampelinus* (all populations)**	26	11	21	0.886 (0.035)	0.00513

Table 3.5. Estimates of diversity between the clades detected within the genus *Scotinomys* based in *cytb* sequences (1140 bp). The average distance between elements of the two groups (Dxy) is shown in the upper triangle and the average distance between elements of the two groups, corrected for the within group distances (Da) in the lower triangle.

	Clades	1	2	3	4	5	6	7	8
1	*S. teguina* (NCBI)	-	0.036	0.0674	0.0706	-	0.0912	0.0892	0.0893
2	*S. teguina* (Honduras)	0.036	-	0.0623	0.0689	-	0.0912	0.0856	0.0859
3	*S. teguina* (BCNP)	0.0661	0.061	-	0.0505	-	0.0883	0.075	0.0758
4	*S. teguina* (LAIP-PT)	0.0702	0.0684	0.0488	-	-	0.0896	0.0798	0.0804
5	***S. teguina* (all populations)**	-	-	-	-	-	0.0895	0.0794	0.08
6	*S. xerampelinus* (Central CR)	0.0912	0.0912	0.087	0.0892	0.0771	-	0.0571	-
7	*S. xerampelinus* (LAIP-VS)	0.0856	0.0821	0.0702	0.0759	0.0635	0.0536	-	-
8	***S. xerampelinus* (all populations)**	0.0828	0.0794	0.068	0.0735	0.0611	-	-	-

Table 3.6. Estimates of diversity between clades detected within the genus *Scotinomys* based in *COI* sequences (653 bp). The average distance between elements of the two groups (Dxy) is shown in the upper triangle and the average distance between elements of the two groups, corrected for the within group distances (Da) in the lower triangle.

	Clades	1	2	3	4	5	6	7	8	9	10	11
1	*S. teguina* (Mexico)	-	0.0620	0.1470	0.0644	0.0697	0.0791	0.0743	-	0.0900	0.0886	0.0897
2	*S. teguina* (Guatemala)	0.0615	-	0.1440	0.067	0.0965	0.0884	0.0965	-	0.0785	0.0832	0.0794
3	*S. teguina* (El Salvador)	0.1465	0.1440	-	0.15	0.1593	0.1551	0.1551	-	0.1443	0.1521	0.1459
4	*S. teguina* (Honduras, El Salvador and Guatemala)	0.0625	0.065	0.149	-	0.0727	0.0762	0.0727	-	0.0765	0.0739	0.0760
5	*S. teguina* (Nicaragua)	0.0692	0.0965	0.1593	0.0712	-	0.0544	0.0766	-	0.0859	0.0847	0.0857
6	*S. teguina* (Central (BCNP) and Northern CR)	0.0752	0.0850	0.1517	0.0714	0.0510	-	0.0544	-	0.0887	0.0818	0.0873
7	*S. teguina* (CR-LAIP-PT and Panama)	0.0738	0.0965	0.1517	0.0712	0.0766	0.0510	-	-	0.101	0.094	0.0999
8	***S. teguina* (all populations)**	-	-	-	-	-	-	-	-	0.089	0.084	0.0879
9	*S. xerampelinus* (Central CR)	0.0891	0.0781	0.1440	0.0747	0.0855	0.0850	0.1008	0.0592	-	0.0507	-
10	*S. xerampelinus* (CR-LAIP-VS)	0.0855	0.0807	0.1496	0.0699	0.0822	0.0758	0.0919	0.0526	0.0478	-	-
11	***S. xerampelinus* (all populations)**	0.0802	0.0704	0.1369	0.0656	0.0767	0.0750	0.0909	0.0497	-	-	-

Table 3.7. Estimates of diversity between the clades detected within the genus *Scotinomys* based in *RAG1* sequences (1158 bp). The average distance between elements of the two groups (Dxy) is shown in the upper triangle and the average distance between elements of the two groups, corrected for the within group distances (Da) in the lower triangle.

	Clades	1	2	3	4
1	***S. teguina* (all populations)**	-	-	0.0141	0.0133
2	*S. teguina* (all populations and *S. xerampelinus*-NCBI)	-	-	0.0141	0.0133
3	*S. xerampelinus* (LAIP-VS)	0.0104	0.0104	-	-
4	***S. xerampelinus* (all populations)**	0.009	0.0089	-	-

3.2.5 Discussion

Although *S. teguina* and *S. xerampelinus* are not very strongly supported entities in our phylogenies, overall, our study indicates a separation between these two species for all markers used, in line with findings for other genetic markers (Pino 2015), as well as morphological (Buchanan *et al.* 1967, Hooper 1972) and acoustic data (Campbell *et al.* 2010). In fact, the divergence of the two currently recognized species in *Scotinomys* is thought to have happened during the late Pliocene (Pino 2015). The *COI* Bayesian inference trees and the interspecific genetic diversity suggest a separation between the Panamanian and CR *S. teguina* and *S. xerampelinus* populations and the other Mesoamerican countries coinciding with the Nicaraguan lowlands depression as a biogeographical barrier (Fig. 3.4). High diversities within each species were observed which is congruent with the several clades found within each species. Furthermore, our results as well as Pino's work (2015) suggest that the genetic limits are congruent with the geographical distribution of the singing mice.

A total of eight clades are recognized considering three principal features: the well to moderate support of the clades in *cytb* and *COI* phylogenetic trees, the low diversity within clades and, finally, the high divergence estimated between most of the clades. In the overall analysis, we distinguish six clades previously identified as *S. teguina*: Mexican (south of Mexico), Guatemalan, Central Mesoamerican (Honduras, Guatemala and El Salvador), Nicaraguan, Central-northern CR (including BCNP) and Southern CR (including LAIP-PT and western Panama) (Fig. 3.7). In the *COI* phylogenetic tree, we did not consider as a clade one haplotype from El Salvador because it is paraphyletic with the other *S. teguina* clades and indeed, more divergent than the outgroups. This sample is probably misidentified and there is no additional information regarding other genes analysed. Unlike Pino (2015), in our study the Honduras population is clustered within the Central Mesoamerican clade whereas he

considers it as a basal member of his Guatemala and El Salvador clade. For *S. xerampelinus*, we identified two clades: a Central CR clade and a Southern CR (LAIP-VS) clade. However, an ambiguity remains in the *RAG1* haplotype network. A *S. xerampelinus* haplotype from Central CR (retrieved from GenBank) is clustered inside the *S. teguina* haplotypes, suggesting an introgression by *S. teguina* or presumably, a misidentification of the sample, for which no mitochondrial DNA sequence is available.

Hooper (1972) differentiated morphologically four subspecies within *S. teguina* distributed around Mesoamerica and a single *S. xerampelinus* species in all CR. The subspecies *teguina* inhabits mountains of southeastern Mexico and Guatemala which could correspond to both our southern Mexican and/or our Guatemalan clade. Pino's (2015) phylogeographic studies, which used *cytb*, *CReg* (mitochondrial Control Region) and *Bfib* (Beta fibrinogen intron-7) markers, also found a clear separation between Mexican and Guatemalan clades probably caused by the Chiapas Guatemalan depression (Fig. 3.4). Even though we did not obtain strong support for a Honduran clade, perhaps due to few sequences from Honduras, our Central Mesoamerican clades can be equated to the *S. teguina rufoniger* subspecies found in Honduras and El Salvador. However, Pino (2015) has considered the *rufoniger* type as a possible species exclusively from Honduras and the *teguina* form as a clade within *S. teguina* composed of Guatemalan and El Salvadoran animals. Additionally, Pino (2015) identified a small Nicaraguan taxon and associated it with the *stenopygius* locality type described by Buchanan and Howell (1967) that apparently does not have taxonomically distinctive attributes (Hooper 1972). Regardless, our Nicaraguan lineage is basal to the well supported Central Mesoamerican clade, though the support for the lineage is weak. The Central-northern and the Southern CR clades may correspond to Hooper's subspecies *S. teguina irazu* and *S. teguina apricus*. These considerations were also strongly supported by Pino (2015) who suggested a bottleneck event in the southernmost populations of *S. teguina* and a recent expansion in northern populations (Pino 2009).

Regarding *S. xerampelinus*, Hooper (1972) could not discriminate between the Central and the Southern CR clades based on morphological characteristics. Nonetheless, Pino (2015) also detected the cryptic diversity represented by these clades, supported by both mitochondrial and nuclear gene sequences. He proposed the recognition of two species, *S. harrisi* (Goodwin 1945) as an appropriate name for the Central CR clade leaving the *S. xerampelinus* name for the Southern clade.

Interestingly, in CR the *S. teguina* and *S. xerampelinus* populations show a similar geographic pattern separating the southern CR clade from the central and northern CR clade, probably reflecting a biogeographical barrier (Fig. 3.7). This pattern has been observed in other species too, namely *Cryptotis nigrescens* (Reid and Gómez 2022), which inhabits the highlands of CR and Panama. It occurs in the north (Tilarán) and central CR at Talamanca cordillera (870-2865 m) as well as in southern CR and western Panama (820-2150 m) (Woodman and Timm, 1993., GBIF, 2019). Another species with a similar distribution is *Cryptotis gracilis* (Reid and Gómez 2022) found in Cordillera de Talamanca and Chiriquí highlands (2435-3536 m) (Woodman and Timm 1993, GBIF 2019). However, throughout the Talamanca Mountain range there is not any evident geographical barrier that could prevent gene flow between these populations (Pino et al 2011). More research is needed to clarify the origin of this geographical pattern.

Thus, the combination of *cytb*, *COI* and *RAG1* markers allowed the detection of cryptic diversity within the two currently known species of *Scotinomys*. Our samples were collected principally in CR generating a more detailed analysis in this country. However, more extensive sampling is needed especially in central and northern Mesoamerican countries (from Nicaragua to south Mexico) as well as a deeper analysis with other mitochondrial and nuclear markers, for example, like *IRBP* (interphotoreceptor retinoid binding protein) (Jansa *et al.* 2004, DeBry *et al.* 2001), in order to obtain a better representation of the distribution of the genus *Scotinomys* and to assess its phylogenetic and evolutionary history and taxonomy.

The eight clades differentiated in our study, some of which with a small distributional range, may be considered as different taxonomic and conservation units and some of them, notably the *S. teguina* from CR and other Mesoamerican clades and perhaps, *S. xerampelinus* Central and Southern CR clades, may be even good candidates to be promoted to species status, following Pino (2015). To properly define these conservation units allows the implementation of more accurate and effective conservation strategies in susceptible anthropogenic threatened environments like the high elevation cloud forest and paramo habitat of *Scotinomys.*

Figure 3.7. Geographic locations of the *Scotinomys* clades identified in Mesoamerica. *S. teguina* clades are in red. Filled square = Mexican clade., Empty square = Guatemalan clade., Filled circle = Central Mesoamerican clade., Empty circle = Nicaraguan clade., Filled star = Central-northern CR clade and empty star = Southern CR clade. *S. xerampelinus* clades are in blue. Filled circle = Central CR clade and empty circle = Southern CR clade.

3.2.6 Conclusion

This research has increased the knowledge and understanding of the genetic diversity, phylogeography and phylogenetic relationships within the genus *Scotinomys.* Eight clades were genetically differentiated, some of them not previously identified based on morphological characters or on previous molecular studies. We strongly advise the reevaluation of the taxonomy within the genus *Scotinomys* because of its high genetic and cryptic diversity revealed between these clades. The distinctiveness of the clades should be considered when planning and applying conservation strategies. Nevertheless, more extensive sampling efforts are needed particularly in central and north Mesoamerican countries in order to achieve a complete representation of the *Scotinomys* genetic diversity.

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Chapter 4: General Discussion and Conclusions

4.1 Current notions about small mammals in Costa Rica

This study represents the first systematic research effort in Costa Rica to integrate the diversity of terrestrial small mammals across multiple altitudinal zones, aiming to estimate their abundance and investigate the genetic traits associated with them. In this context, the country possesses a mountainous system that encompasses a substantial level of biodiversity of mammals sheltered mainly in approximately 32% of the territory that is protected under some category of management (Sánchez-Bolaños et al 2020). For terrestrial small mammals the findings of this study reveal considerable uncertainty regarding the taxonomy of species within this specific group, as approximately 92 species have historically been reported. However, the current investigation, based on available literature, confirms the existence of only 46 species (Goodwin 1946, International Union for Conservation of Nature [IUCN] 2021, Reid 2009, Rodríguez and Chinchilla 1996, Rodríguez-Herrera *et al.* 2014, Villalobos-Chaves *et al.* 2016). Several factors account for this substantial disparity, which can be attributed to the development of techniques required for accurate species differentiation. Consequently, errors associated with researchers' expertise and technological resources primarily influence the ability to achieve precise identifications and mitigate inconsistencies.

The thorough review conducted enabled the identification of approximately 30% of regional endemism shared among Costa Rica, Panama, and Nicaragua among small mammals (Table 3.1). The highest degree of endemism was observed in conjunction with Panama, and all the endemic species shared between these countries are geographically linked to the Cordillera de Talamanca. Consequently, the natural barrier represented by this mountain range has been recognized as both a natural bridge connecting North America and South America and a significant species filter (Kappelle 2016).

The Talamanca region and other high mountains in Costa Rica may have served as refuges for terrestrial small mammals during the last ice age, as suggested by the refuge theory. Genetic studies consistently reveal significant levels of crypticity among these species, influenced by the mountain system (Steward et al 2009, Pointing et al 2014). For example, Voss *et al.* (2021) differentiated *Marmosa nicaraguae* from *Marmosa alstoni* through genetic analysis. Previously, *M. alstoni* was considered a single species found across Nicaragua, Costa Rica, and Panama. However, it is now

recognized that *M. nicaraguae* is endemic to Nicaragua and Costa Rica, while *M. alstoni* is only found in Costa Rica and Panama. The limited availability of genetic reference information hampers accurate species identification due to cryptic speciation. The assessment conducted on the risk of extinction for these species reveals that only one species is classified as vulnerable, while most have been categorized as least concern according to the IUCN. However, when comparing these findings with the existing genetic data, it becomes apparent that the potential for endemism in Costa Rica's territory might be underestimated.

4.2 The distribution patterns of terrestrial small mammals

In Costa Rica and the rest of Central America, the research landscape surrounding terrestrial small mammals presents numerous complexities. Limited logistics and economic resources hinder the attainment of a comprehensive understanding that encompasses taxonomy and adequate genetic representation, particularly within the country's protected areas. The case of Valle del Silencio, described in this thesis, serves as a clear illustration of the information gaps arising from challenges related to accessibility and the high costs associated with fieldwork. This valley is situated in a densely wooded region, making it arduous to access, and the logistics involved in conducting sampling are exceedingly intricate. As an alternative, for similar cases, open access databases play a key role in pioneering research in locations such as this (Guedes *et al.* 2017), complementing the extensive fieldwork conducted in the present doctoral study. The literature reports the importance of incorporating practical molecular procedures into remote expedition fieldwork to obtain genetic information (Bunting et al 2014) due to the significant conservation implications for species and their habitat.

The use of open access databases facilitated the compilation of 2324 records to determine the altitudinal distribution of the 46 identified species of small mammals in Costa Rica. Initially, a total of 8485 records were downloaded, which were then filtered along with the field data from the present investigation and information provided by the National Museum of Costa Rica and the Museum of Zoology of the National University of Costa Rica. Most of the data were obtained from historical records sourced from the Global Biodiversity Information Facility (GBIF: https://www.gbif.org). The final arrangement of the species was categorized into three altitudinal gradients: low altitude zones (0-1218 m.a.s.l), intermediate zones (1218-2436 m.a.s.l), and high altitude zones (2436-3654 m.a.s.l). The distribution pattern of the species exhibits a non-uniform trend, with several species exclusively associated with each gradient. There is a slight tendency

towards a higher number of species at intermediate elevations. The exclusivity of species within the highland areas is noteworthy, as practically all species found at those altitudes are unique to the conditions there, resulting in a high level of endemism.

The inclusion of biotemperature as an environmental variable in the species arrangement matrix for altitudinal distribution reveals significant differences between the extreme altitude belts and the intermediate ones. This pattern highlights an intermediate distribution characterized by a higher abundance of species. Consequently, the intermediate elevations appear to provide more favorable conditions, supporting a greater diversity of small mammal species. For instance, the fieldwork for the current investigation primarily focused on two phytogeographic units characterized by extreme conditions along elevation gradients: the slopes of the Caribbean coast of Talamanca and the slopes of the Pacific coast of Talamanca. Despite their proximity, these regions exhibit markedly distinct climatic regimes, attributed to the influence of two distinct oceanic slopes (Zamora 2016). Within this area, a significant proportion of the collected samples display substantial levels of genetic divergence, possibly attributable to species' adaptive and evolutionary processes in response to the diverse environmental conditions encountered.

The Holdridge system (1967) defines life zones based not only on altitude and biotemperature, but also on precipitation and evapotranspiration as variables that directly influence species distribution. In the case of Costa Rica, 12 life zones are established according to the tropical profile characteristics. Among these life zones, those with intermediate values for all variables, such as the Wet Forest Lower Premontane and Wet Forest Premontane, concentrate the highest number of records for small mammals in terms of both species and individuals. The analysis distinctly separates life zones with extreme values of precipitation and biotemperature, such as the Rain Forest Subalpine, Wet Forest Montane, Moist Forest Low Montane, and Dry Forest Tropical, which could be considered inhospitable for hosting small mammals. Other life zones also harbor significant numbers of species and individuals, such as the Rain Forest Montane, Rain Forest Lower Montane, Moist Forest Premontane, Rain Forest Premontane, Moist Forest Tropical, and Wet Forest Tropical. The degree of concentration varies depending on the values of the variables but without reaching extremes. Building upon the previous assessment, further in-depth analysis should be conducted on the effects of intermediate conditions for all variable ranges, as they define favorable conditions for small mammals, while extreme conditions have an antagonistic effect on these species. However, even

species adapted to extreme conditions can be highly sensitive to environmental variation, particularly those caused by anthropogenic effects such as deforestation or climate change.

The variation in species distribution and its relationship with the ecosystems they inhabit can be partially explained by the topography of the country. The fragmentation of Costa Rica by mountain ranges has been identified as a significant factor contributing to the diversification of terrestrial small mammals in the region (Bagley and Johnson 2014). This phenomenon can be attributed to the Mid-Domain Effect, which describes the increasing overlap of species ranges towards the center of a shared geographic domain (Letten et al 2013). Terrestrial small mammals' distribution is influenced by geometric boundary constraints as well as the sizes and midpoints of species' ranges (Pei et al 2022). Previous studies have demonstrated predictable changes in habitat types and the number of terrestrial small mammal species with increasing latitude and elevation, often characterized by a symmetrical peak at mid-elevation known as the Mid-Domain Effect (Colwell et al 2004). The present research also suggests a peak in diversity at middle elevations, which may be influenced by changes in climatic variables along the elevation gradient. However, high diversity is also observed in lowland areas, which could be attributed to oversampling and easier accessibility.

The diversity of terrestrial small mammals in tropical mountains may depend on specific precipitation patterns. As observed in other studies, abundance and species richness tend to decline as elevation increases (Upedo et al 2023). The distribution of records based on altitude categories further confirms the prevalence of species with larger geographical ranges in the Premontane category, influencing the species composition at both the highest elevations and in the lowlands. This pattern of elevation ranges and intermediate distribution aligns with previous assessments of the Mid-Domain Effect theory, which has been validated in Costa Rica (McCain 2004, Colwell et al 2016). Climatic conditions, particularly precipitation, play a crucial role in shaping the distribution patterns of terrestrial small mammals, with intermediate levels of precipitation associated with higher species richness. Decreased precipitation has a more significant negative impact on richness and abundance compared to other factors that have been considered.

The species richness of terrestrial small mammals has been recognized as a reliable indicator of habitat modification due to its correlation with site productivity and climatic zones (Mena and Medellin 2017). Small mammals play a significant role in

predator-prey systems and contribute to important functions such as seed dispersal. The research findings reveal that certain species are restricted to specific elevational ranges, indicating their adaptive responses to local environmental conditions. The data clearly demonstrates that highland and lowland species exhibit high exclusivity to their respective zones, while intermediate elevations conform to a mix of species distribution, including some endemic species along this gradient. Furthermore, the geographical range size of small mammals, particularly those with limited climatic suitability, underscores their vulnerability and restricted dispersal capacity. These characteristics make them valuable tools for assessing habitat quality and understanding the delicate balance between habitat and species (Caceres *et al.* 2011, Gebrezgiher *et al.* 2022).

In this context, the present study also provides evidence suggesting that small mammals may contribute to the accelerated recovery of forested areas that have undergone significant anthropogenic interventions. A comparison of species distribution patterns during the deforestation period and the protected areas period reveals a positive impact of protected areas on the habitat suitability for terrestrial small mammals, particularly at intermediate elevations. The implementation of strict forest use policies in protected areas over the past three decades appears to have created favorable conditions for multiple species of terrestrial small mammals at intermediate elevations. This observation supports the concept of the Mid-Domain Effect described before for non-volant small mammals. Additionally, changes in species' elevation ranges between the two periods may also be influenced by responses to climate change. While some mammals exhibit negative responses such as local extirpation or range contraction, others demonstrate positive responses with range expansion and increased population sizes (McCain and King 2014).

The analysis carried out for Valle del Silencio, considered a pristine community with minimal alteration, focused on the species composition of terrestrial small mammals. Among the species identified, two, namely *R. creper* and *P. nudipes*, were found to be dominant. However, there is limited knowledge about their ecological roles and their impact on the ecosystem (Reid 2009). While the taxonomy of *P. nudipes* is currently under study (Bradley et al 2016), little is known about the natural history of *R. creper*. Moreover, *R. creper* is an endemic species with a narrow distribution range, which poses challenges for investigating its biological aspects. The coexistence of these two species in the area is intriguing, particularly considering their adaptation to the harsh conditions of high-altitude mountain ecosystems. The dominance of these species in the Valle del

Silencio community suggests a natural ecological relationship, possibly attributed to minimal human activity in the area. However, it is crucial to understand the role of other species in the ecosystem and their contributions to the overall community composition. The delicate balance between the dominant species and other taxa likely depends on resource availability, and any alterations to the environmental conditions could potentially impact the dynamics and structure of the community. Further research is necessary to explore these relationships and their implications.

Microhabitats at ground level in montane oak forests, such as those found in Valle del Silencio are of utmost importance as they serve as crucial refuges for terrestrial small mammals. These microhabitats provide abundant food sources, protection, and cover, which are essential for the survival and well-being of organisms, including small mammals. Preserving these ground level microhabitats becomes imperative for the long-term conservation of these delicate ecosystems. The dynamics of temperature at this level also plays a significant role in the utilization of these microhabitats, as species like those in the *Peromyscus* genus exhibit a preference for suitable microclimates that help mitigate the temperature fluctuations common in highland areas (Hayward *et al.* 2022). Investigating the interactions among species such as *Reithrodontomys*, *Peromyscus*, and *Scotinomys* in high-altitude regions is crucial for understanding their natural history and ecological dynamics. However, limited resources and challenges associated with accessing these areas pose obstacles for conducting comprehensive research.

4.3 Genetic Conservation and Taxonomy of Terrestrial Small Mammals

Taxonomic classification plays a crucial role in distinguishing rare species and implementing effective conservation strategies, particularly for specimens classified as *Reithrodontomys* spp. in this study. These specimens may represent cryptic species that can be better resolved through the application of molecular techniques. Accurate taxonomic identification is essential for understanding the unique characteristics, ecological requirements, and conservation needs of each species within the *Reithrodontomys* genus. Molecular techniques can provide valuable insights to assess genetic diversity, population structure, and evolutionary relationships within this group, aiding in the identification of distinct species and informing targeted conservation measures (Allendorf *et al.* 2022). Therefore, the integration of molecular approaches into

taxonomic studies is vital for enhancing our understanding of cryptic species and facilitating their effective conservation.

The present study has made substantial contributions to the fields of taxonomy and conservation genetics of small mammals in Costa Rica. By providing new genetic insights and resolving inconsistencies, this research enhances understanding of the unique biodiversity and evolutionary dynamics of small mammal populations in the country. These findings have important implications for conservation efforts, as they inform targeted strategies for the preservation and management of endemic species and their respective habitats. The study revealed divergent mitochondrial lineages, some of which were supported by nuclear data, emphasizing the need for taxonomic revisions and further research to accurately classify and understand the evolutionary history of these species. The rationale for employing a DNA barcoding approach to study small mammals in Costa Rica was to uncover previously undiscovered genetic diversity that could be attributed to previously undescribed major genetic forms or even new species (Hebert *et al.* 2003). This approach, which involves analyzing a broad category of organisms, such as "small mammals" that include in this case a reasonable number of specimens, using a simple genetic marker, has long been advocated (Hebert *et al.* 2003) and has proven successful in various systems (Dasmahapatra and Mallet 2006., Jones *et al.* 2021, Tsoupas *et al.* 2022). This study used the complete *cytb* sequence as the chosen barcode sequence, based on its established use in phylogeographic studies of small mammals (e.g., Ditchfield *et al.* 2000, Kotlík *et al.* 2022).

The phylogenetic analyses of mitochondrial and nuclear DNA sequences conducted in this study have provided valuable insights into the genetic diversity and evolutionary relationships among the studied genera of small mammals. The observed high genetic diversity within the genera is consistent with previous studies conducted in the region, indicating the presence of distinct lineages and the potential for cryptic speciation. The DNA barcoding approach is good to identify situations like this. Below, the cryptic diversity that this study has uncovered with *cytb* will be discussed, but it is recognised that it is only a “first step” at the description of previously undetected diversity. Those findings are a call for further studies to establish more firmly the exact nature of the cryptic subdivisions that were uncovered. *Cytb* as a sole marker is insuficient (Galtier *et al.* 2009), so further in-depth genetic studies are needed. It should be noted that while small mammals in Costa Rica have been grossly understudied, there have been several pioneering studies of genetic diversity in Costa Rica and Central America including

Arellano *et al.*'s (2005) work on *Reithrodontomys mexicanus*., the studies of Bradley *et al.* (2007) and Pérez-Consuegra and Vázquez-Domínguez (2015) on the genus *Peromyscus*; and Corley *et al.*'s (2011) work on *Nyctomys sumichrasti*.

The information provided in the present work allows a consideration of the different small mammal species in Costa Rica and their cryptic forms. Firstly, the genus *Philander* might have a second species or a significant genetic subdivision within *Philander melanurus* based on distinct *cytb* clades. But also, there is a group *Heteromys* sp., which has been identified as two distinct clades in Costa Rica using *cytb* sequencing. The genus *Peromyscus* poses challenges in identifying species, with conflicting classifications between *P. nudipes* and species like *P. nicaraguaea* and *P. mexicanus*. New sequences fall into separate clades, potentially corresponding to different species. *Melanomys caliginosus* shows that the new sequences are more closely related to *M. chrysomelas* than the previously identified *M. caliginosus*. Lastly, *Reithrodontomys* phylogeny strongly suggests the presence of new major genetic forms, potentially new species, in an already speciose genus. Further studies are needed to fully understand these findings.

These findings underscore the importance of integrating molecular, morphological, and ecological data to fully understand patterns of cryptic diversity and the underlying mechanisms driving speciation within small mammal populations. Further research in these areas will contribute to improved taxonomic classification, conservation efforts, and overall understanding of the evolutionary processes shaping biodiversity in Costa Rica. This study sheds light on the genetic diversity patterns of small mammals in Costa Rica, emphasizing the need for further research in the country's natural heritage due to the constant discovery of new species. However, the comparison of these data with museum voucher specimens is necessary to address taxonomic issues fully. Future work involves optimizing primers for amplifying *cytb* in specific genera and sequencing the *COI* gene for barcoding and biodiversity conservation purposes. Museum specimens should be used to compare and resolve taxonomic aspects, aiding in the understanding of species evolution and diversification.

4.4 Conclusions

- There is a lack of research on terrestrial small mammals in Costa Rica and in Mesoamerica in general. This means that there is a lack of information across fields such as physiology, behaviour, and genetics, among others. Insufficient

resources, both financial and in specialized facilities, have lessened research opportunities on small mammals, but the capture-recapture procedures described here provide an example of a relatively inexpensive way of getting new information. It is imperative to implement systematic studies for this group, given their intimate relationship with the surrounding environment and their potential as reliable indicators of ecosystem health.

- Microclimatic and topographic variation in Costa Rica may generate very particular local conditions suitable for certain terrestrial small mammal species, promoting evolution of local morphotypes. These morphotypes could explain the inconsistencies at the taxonomic level evident in the species datasets generated in this study. Then, endemism maybe higher than would be recorded if systematic research advances using genetic tools were used to complement morphological analysis and ecological and behavioural studies., such an approach would uncover cryptic species.
- There is a need to systematically focus on the effect of topographical and climatic variability on species and their ecosystems to clarify the basis of the high species diversity in the country, since it has not yet been properly evaluated. Results support the Mid-domain effect, with a peak of richness and abundance at intermediate elevations. However, there was also high richness and abundance in the lowlands that could be caused by oversampling, taxonomical uncertainties or precipitation conditions similar to those tropical mountains, although this has not been evaluated properly yet.
- Several of the life zones under consideration appear to offer suitable conditions for most of terrestrial small mammals, but premontane and lower montane generated the highest numbers of species and records. Premontane and Lower Montane are life zones defined by intermediate values of climate conditions: biotemperature, evapotranspiration and precipitation. Definitively, precipitation looks like a key climatic factor for the distribution of terrestrial small mammals and helps explain the observed distribution of small mammals in lowlands and mid altitudes.
- Research in high altitude forests, such as the Valle del Silencio, is extremely limited, despite the vulnerability of these ecosystems to the impacts of climate change. Consequently, there is a critical need for research on wildlife in montane forests, to provide data to justify the protection of these ecosystems and the preservation of their biodiversity. The institutions responsible for safeguarding

these resources must facilitate a coordinated response to investigate and conserve these highly fragile ecosystems.

- It is crucial to prioritize training programs provided by authorities in the country and region to ensure proper fieldwork practices that prioritize the well-being of researchers, participants, and the small mammals that are captured. Given the high recapture rates for the protocols that we developed, it can be inferred that they are adequate for most purposes. However, adjustments should be made as appropriate to improve the experience and minimize risks for organisms during capture, handling, and subsequent release. Strict vaccination protocols should be enforced to prevent the spread of known diseases. Additionally, stringent cleanliness measures must be implemented to minimize the risk of transmission of both known and unknown diseases.
- The Valle del Silencio provides an ecosystem for stable populations of small mammals, with the species concerned existing in a delicate ecological balance with their surroundings. Alterations to this ecosystem would disturb this equilibrium. The prevalence of oak forests in this area provides diverse microhabitats for terrestrial small mammals. These microhabitats hold great potential as natural laboratories for studying the impacts of climate change on temperature-sensitive species, including those belonging to the genus *Peromyscus*. Adequate research in this context is crucial for understanding the effects of climate change on small mammals in montane systems.
- Terrestrial small mammals in Costa Rica are good indicators of habitat alterations and responses to current climate change. However, they have not been used systematically to evaluate the recovery of ecosystems by the protected areas policy. In this sense, small mammals could have a considerably positive influence on forest recovery in a way that has not yet been appreciated. Again, there is a tendency for species considered between the periods from deforestation to protected areas to converge on the mid-range elevation, consistent with the Mid-domain effect.
- The present study highlights existing information gaps on small mammals, not only with regards to species and their ecosystems, but also to the lack of capabilities to forge new research directions. The absence of protocols for field studies is one basis of our lack of understanding of small mammals in Costa Rica, which we hope to have helped rectify here. More resources are also needed. It is crucial to promote funding for research within the region to create favorable

conditions for research by fostering collaboration among diverse private and public institutions. Such initiatives would facilitate the acquisition of resources necessary for comprehensive studies aimed at bridging the knowledge gaps in this field.

- The application of current molecular techniques to study cryptic species in high altitude areas of Costa Rica is essential. Complementing existing taxonomy with molecular approaches can help clarify species identification and prevent inconsistencies. This approach should be prioritized to enhance our understanding of the species present in these and other ecosystems in the country.
- The DNA barcoding approach towards the small mammals of Costa Rica using *cytb* has newly revealed or supported a number of possible instances of new species or major genetic forms, in *Philander melanurus*, *Melanomys chysomelas*, *Heteromys*, *Peromyscus* and *Reithrodontomys*. The results for *Scotinomys* and our phylogenetic reconstructions based on GenBank sequences of *Marmosa mexicana*, *Metachirus nudicaudatus*, *Nyctomys sumichrasti* and *Transandinomys talamancae* suggest that there may be cryptic species or major genetic forms within those taxa. Clearly this work with *cytb* needs to be supplemented with further sampling, studies of other genetic markers, morphometrics and other studies to confirm the existence of new species. DNA barcoding is a quick approach to find new species – it has been very successful when considering the small mammals of Costa Rica. It is particularly for understudied species-rich higher taxa that DNA barcoding might be expected to work well – and our results support that.
- This research has increased the knowledge and understanding of the genetic diversity, phylogeography and phylogenetic relationships within the genus *Scotinomys.* Eight clades were genetically differentiated, some of them not previously identified based on morphological characters or on previous molecular studies. We strongly advise the reevaluation of the taxonomy within the genus *Scotinomys* because of its high genetic and cryptic diversity revealed between these clades. The distinctiveness of the clades should be considered when planning and applying conservation strategies. Nevertheless, more extensive sampling efforts are needed particularly in central and north Mesoamerican countries in order to achieve a complete representation of the *Scotinomys* genetic diversity.

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Appendices

Appendix 1. List of terrestrial small mammals historically registered for Costa Rica based on the recent past lists for this group in the country.

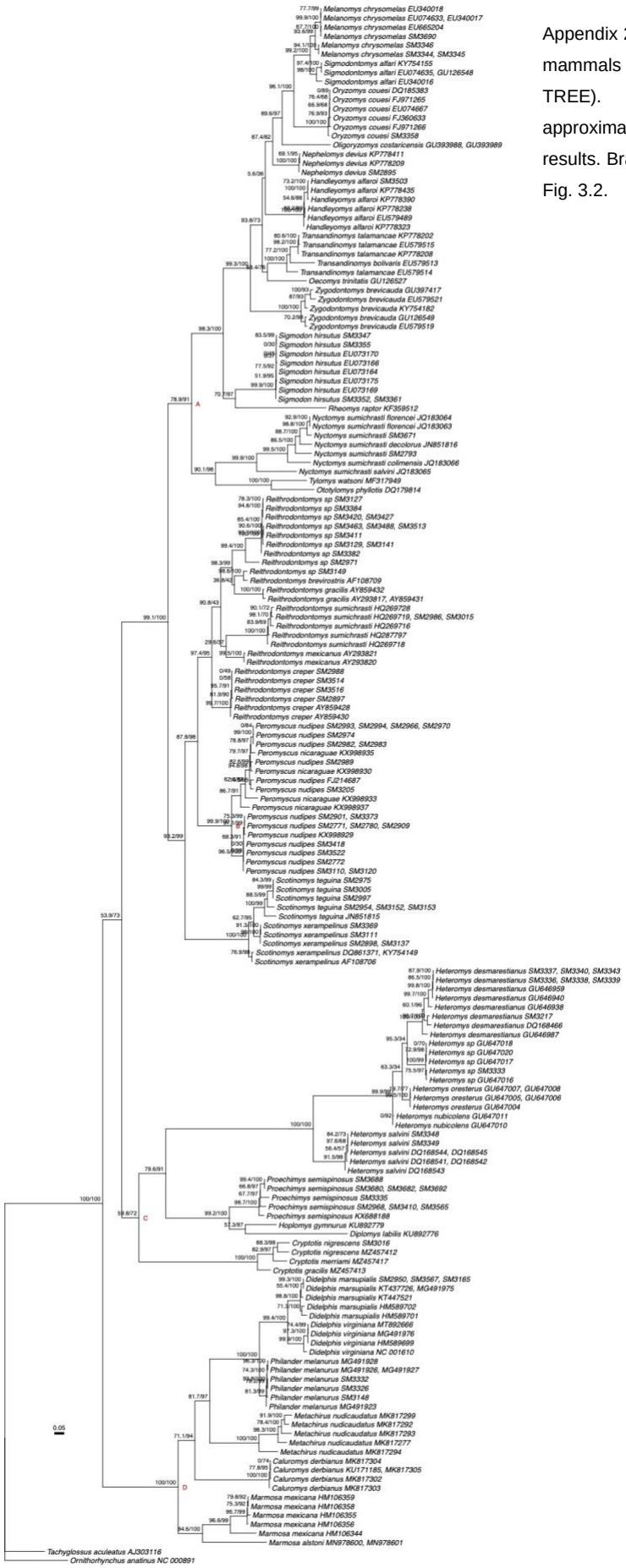
Small mammals taxonomy	Reference*							Consideration
	Rf.1	Rf.2	Rf.3	Rf.4	Rf.5	Rf.6	Rf.7	
CLASS MAMMALIA								
Order Didelphimorphia								
Family Didelphidae								
Subfamily Caluromyinae								
Caluromys derbianus	✓	✓	✓	✓	–	✓	✓	Common/confirmed
Subfamily Didelphinae								
Tribe Marmosini								
Marmosa alstoni	✓	✓	–	✓	–	✓	✓	Common/confirmed
Marmosa mexicana	✓	✓	✓	✓	–	✓	✓	Common/confirmed
Marmosa murina	✓	–	–	–	–	–	✓	Mistaken/discarded
Marmosa zeledoni	✓	–	✓	✓	–	–	✓	Rare/confirmed
Tribe Metachirini								
Metachirus nudicaudatus	✓	✓	✓	✓	–	✓	✓	Rare/confirmed
Tribe Didelphini								
Micoureus alstoni	–	–	✓	–	–	–	✓	Change/discarded
Philander opossum	✓	✓	✓	✓	–	✓	✓	Common/confirmed
Order Soricomorpha								
Family Soricidae								
Subfamily Soricinae								
Tribe Blarinini								
Blarina brevicauda	–	–	–	–	–	–	✓	Mistaken/discarded
Cryptotis gracilis	✓	✓	✓	✓	–	✓	✓	Common/confirmed
Cryptotis jacksoni	✓	✓	–	–	–	–	–	Mistaken/discarded
Cryptotis merriami	–	✓	✓	✓	–	✓	✓	Rare/confirmed
Cryptotis monteverdensis	–	–	–	–	–	–	✓	Mistaken/discarded
Cryptotis nigrescens	✓	✓	✓	✓	–	✓	✓	Common/confirmed
Cryptotis orophila	✓	–	–	✓	–	✓	✓	Common/confirmed
Cryptotis parva	–	✓	✓	–	–	–	✓	Change/discarded
Cryptotis pomel	✓	–	–	–	–	–	–	Mistaken/discarded
Order Rodentia								
Family Heteromyidae								
Subfamily Heteromyinae								
Heteromys desmarestianus	✓	✓	✓	✓	✓	✓	✓	Common/confirmed
Heteromys gaumeri	–	–	–	–	–	–	✓	Mistaken/discarded
Heteromys nubicolens	–	–	✓	✓	✓	–	✓	Common/confirmed
Heteromys oresterus	✓	✓	✓	✓	✓	✓	✓	Common/confirmed
Heteromys salvini	–	–	–	–	–	✓	–	Revision/discarded
Liomys salvini	✓	✓	✓	✓	✓	–	✓	Common/confirmed
Family Cricetidae								

Subfamily Sigmodontinae								
Tribe Ichthyomyini								
Rheomys hartmanni	✓	–	–	–	–	–	–	Change/discarded
Rheomys raptor	✓	✓	✓	✓	✓	✓	✓	Rare/confirmed
Rheomys underwoodi	✓	✓	✓	✓	✓	✓	✓	Rare/confirmed
Tribe Oryzomyini								
Nectomys alfari	✓	–	–	–	–	–	✓	Change/discarded
Nectomys dimidiatus	✓	–	–	–	–	–	–	Mistaken/discarded
Tribe Sigmodontini								
Sigmodon hirsutus	✓	✓	✓	✓	✓	✓	✓	Common/confirmed
Sigmodon hispidus	✓	✓	✓	✓	–	✓	✓	Change/discarded
Tribe Oryzomyini								
Handleyomys alfaroi	–	–	✓	✓	✓	✓	✓	Common/confirmed
Handleyomys rostratus	–	–	–	–	–	✓	–	Rare/ discarded*
Melanomys caliginosus	–	–	✓	✓	–	✓	✓	Change/discarded
Melanomys chrysomelas	–	–	–	–	✓	–	–	Change/ confirmed
Nephelomys albigularis	–	–	–	–	–	–	✓	Change/discarded
Nephelomys devius	–	–	✓	✓	✓	✓	✓	Common/confirmed
Oecomys trinitatis	–	–	✓	✓	✓	✓	✓	Rare/confirmed
Oligoryzomys fulvescens	✓	–	✓	✓	✓	✓	✓	Common/confirmed
Oligoryzomys vegetus	✓	–	✓	✓	✓	✓	✓	Common/confirmed
Oryzomys aphrastus	✓	✓	–	–	–	–	–	Change/discarded
Oryzomys bombycinus	✓	✓	–	–	–	–	–	Change/discarded
Oryzomys albigularis	–	✓	–	–	–	–	✓	Change/discarded
Oryzomys alfari	–	✓	–	–	–	–	–	Change/discarded
Oryzomys alfaroi	✓	✓	–	–	–	–	✓	Change/discarded
Oryzomys caliginosus	–	✓	–	–	–	–	–	Change/discarded
Oryzomys capito	–	–	–	–	–	–	✓	Mistaken/discarded
Oryzomys chrysomelas	✓	–	–	–	–	–	–	Change/discarded
Oryzomys concolor	–	✓	–	–	–	–	–	Mistaken/discarded
Oryzomys costaricensis	✓	–	–	–	–	–	–	Change/discarded
Oryzomys couesi	✓	✓	✓	✓	✓	✓	✓	Common/confirmed
Oryzomys devius	✓	–	–	–	–	–	–	Change/discarded
Oryzomys fulvescens	–	✓	–	–	–	–	–	Change/discarded
Oryzomys palustris	–	–	–	–	–	–	✓	Mistaken/discarded
Oryzomys talamancae	✓	✓	–	–	–	–	–	Change/discarded
Oryzomys tectus	✓	–	–	–	–	–	–	Change/discarded
Sigmodontomys alfari	–	–	✓	✓	✓	✓	–	Rare/confirmed
Sigmodontomys aphrastus	–	–	✓	–	–	–	–	Change/discarded
Tanyuromys aphrastus	–	–	–	✓	✓	✓	–	Rare/confirmed
Transandinomys bolivaris	–	–	✓	✓	✓	✓	✓	Common/confirmed
Transandinomys talamancae	–	✓	✓	✓	✓	✓	✓	Common/confirmed
Zygodontomys brevicauda	–	✓	✓	✓	✓	✓	–	Common/confirmed
Zygodontomys cherriei	✓	–	–	–	–	–	–	Change/discarded

Subfamily Tylomyinae								
Nyctomys sumichrasti	✓	✓	✓	✓	✓	✓	✓	Rare/confirmed
Ototylomys phyllotis	✓	✓	✓	✓	✓	✓	✓	Common/confirmed
Tylomys watsoni	–	–	✓	✓	✓	–	✓	Common/confirmed
Tylomys panamensis	–	–	–	–	–	–	✓	Mistaken/discarded
Tribe Baiomyini								
Scotinomys harrisi	✓	–	–	–	–	–	–	Change/discarded
Scotinomys longipilosus	✓	–	–	–	–	–	–	Change/discarded
Scotinomys teguina	✓	✓	✓	✓	✓	✓	✓	Common/confirmed
Scotinomys xerampelinus	✓	✓	✓	✓	✓	✓	✓	Common/confirmed
Subfamily Neotominae								
Tribe Neotomini								
Neotoma chrysomelas	–	–	–	–	–	–	✓	Mistaken/discarded
Tribe Peromyscini								
Peromyscus favidus	✓	–	–	–	–	–	–	Mistaken/discarded
Peromyscus mexicanus	✓	✓	✓	✓	–	✓	✓	Change/discarded
Peromyscus nudipes	✓	✓	–	–	✓	–	✓	Common/confirmed
Tribe Reithrodontomyini								
Reithrodontomys australis	✓	–	–	–	–	–	–	Change/discarded
Reithrodontomys brevirostris	✓	✓	✓	✓	✓	✓	✓	Common/confirmed
Reithrodontomys cherrii	–	–	–	–	✓	–	–	Recent/ discarded
Reithrodontomys creper	✓	✓	✓	✓	✓	✓	✓	Common/confirmed
Reithrodontomys garichensis	–	–	–	–	✓	✓	–	Recent/ discarded
Reithrodontomys gracilis	–	✓	✓	✓	✓	✓	✓	Common/confirmed
Reithrodontomys harrisi	✓	–	–	–	–	–	–	Recent/ discarded
Reithrodontomys mexicanus	✓	✓	✓	✓	–	✓	✓	Common/confirmed
Reithrodontomys musseri	–	–	–	–	✓	–	✓	Rare/confirmed
Reithrodontomys paradoxus	–	✓	✓	✓	✓	✓	✓	Rare/confirmed
Reithrodontomys rodriguezi	✓	✓	✓	✓	✓	✓	✓	Common/confirmed
Reithrodontomys sumichrasti	–	✓	✓	✓	✓	✓	✓	Common/confirmed
Family Echimyidae								
Subfamily Eumysopinae								
Tribe Myocastorini								
Hoplomys gymnurus	✓	✓	✓	✓	✓	✓	✓	Rare/confirmed
Proechimys allen	✓	–	–	–	–	–	–	Mistaken/discarded
Proechimys cayennensis	✓	–	–	–	–	–	–	Mistaken/discarded
Proechimys semispinosus	–	✓	✓	✓	✓	✓	✓	Common/confirmed
Subfamily Echimyinae								
Diplomys labilis	–	–	–	–	✓	–	–	Recent/ discarded
Order Carnivora								
Family Mustelidae								
Subfamily Mustelinae								
Mustela frenata	✓	✓	✓	✓	✓	✓	✓	Common/confirmed

The list above was created based on the follow references:
Rf.1. - Goodwin, G. 1946. Mammals of Costa Rica. Bulletin of the American Museum of Natural History, vol: 87 (5): 275-243. / Rf.2. - Rodríguez, J and Chinchilla, F. 1996. Lista de mamíferos de Costa Rica. Revista Biología Tropical, Vol. 44:877-890. / Rf.3 - Reid, F. 2009. A Field Guide to the: Mammals of Central America and Southeast Mexico. Second Edition. Oxford University Press. 246 pgs. / Rf.4. - Rodríguez, B., Ramírez, J., Villalobos, D and Sánchez, S. (2014). Actualización de la lista de especies de mamíferos vivientes de Costa Rica. Maztozoología Neotropical. Vol.21: 275-289. / Rf.5. - Villalobos, D., Ramírez, J., Chacón, E., Pineda, W and Rodríguez, B. (2016). Clave para la identificación de los roedores de Costa Rica. First Edition. School of Biology, University of Costa Rica. 37pgs. / Rf.6. - IUCN 2018. The IUCN Red List of Threatened Species. Version 2018-1. <http://www.iucnredlist.org>. Downloaded on 22 st, october 2018. Permalink: http://oldredlist.iucnredlist.org/search/link/5bcde08f-5ce0e1fb / Rf.7. - The Global Biodiversity Information Facility. 2018. < https://www.gbif.org>. Downloaded on 21 st, october 2018. Permalink: https://doi.org/10.15468/dl.kk3jpe
Check mark indicates that species was included in the reference.

Condition in the list refers to two evaluations for each species. First, either how often the species appears in the references considered (common or rare) or whether they have a special taxonomic status: mistaken, changed in revision or recent change. Second, condition to select the species for the rest of the present analysis: confirmed or discarded.



Appendix 2. Complete cytochrome b phylogeny for small mammals in Costa Rica. Maximum likelihood tree (IQ-TREE). Branch support: ultrafast bootstrap approximation/SH-like approximate likelihood ratio test results. Branches labeled A – D (in red) are highlighted in Fig. 3.2.

Appendix 3. New small mammal samples collected from Costa Rica and used to sequence cytochrome *b*.

Specimen code	Species	Province	Sampling site	LONGITUDE (CR05/CRTM05)	LATITUDE (CR05/CRTM05)
SM.3016	*Cryptotis nigrescens*	Heredia	BCNP	486058.40	1120117.42
SM.2950	*Didelphis marsupialis*	Puntarenas	LAIP-EA	608961.43	998549.80
SM.3165	*Didelphis marsupialis*	Puntarenas	Sansi	614544.099	997929.675
SM.3567	*Didelphis marsupialis*	Puntarenas	Sansi	604236.62	991198.84
SM.3503	*Handleyomys alfaroi*	Puntarenas	LAIP-PT	614544.099	997929.675
SM.3217	*Heteromys desmarestianus*	Honduras	Cusuco N.P	47755.56	1718173.43
SM.3336	*Heteromys desmarestianus*	Puntarenas	MANP	602876.95	990870.68
SM.3337	*Heteromys desmarestianus*	Puntarenas	MANP	6028773.88	990869.01
SM.3338	*Heteromys desmarestianus*	Puntarenas	MANP	602877.01	990871.03
SM.3339	*Heteromys desmarestianus*	Puntarenas	MANP	602869.58	990871.99
SM.3340	*Heteromys desmarestianus*	Puntarenas	MANP	602665.27	991166.90
SM.3343	*Heteromys desmarestianus*	Puntarenas	MANP	602855.94	990873.28
SM.3348	*Heteromys salvini*	Guanacaste	SRNP	323005.57	1199056.18
SM.3349	*Heteromys salvini*	Guanacaste	SRNP	323006.33	1199025.18
SM.3333	*Heteromys* sp	Limón	AFRS	546140.42	1154332.56
SM.3344	*Melanomys chrysomelas*	Puntarenas	MANP	602871.02	990869.44
SM.3345	*Melanomys chrysomelas*	Puntarenas	MANP	602876.90	990865.33
SM.3346	*Melanomys chrysomelas*	Puntarenas	MANP	602872.22	990868.33
SM.3690	*Melanomys chrysomelas*	Puntarenas	MANP	602876.95	990870.68
SM.2895	*Nephelomys devius*	Limón	LAIP-VS	614101.64	1007628.28
SM.2793	*Nyctomys sumichrasti*	Heredia	S. Verde	492582.70	1155615.07
SM.3671	*Nyctomys sumichrasti*	Heredia	S. Verde	492669.61	1155594.63
SM.3358	*Oryzomys couesi*	Puntarenas	LAIP-PT	614504.67	997944.86
SM.2771	*Peromyscus nudipes*	Limón	LAIP-VS	614105.10	1007651.07
SM.2772	*Peromyscus nudipes*	Limón	LAIP-VS	614105.30	1007657.50
SM.2780	*Peromyscus nudipes*	Limón	LAIP-VS	614104.48	1007634.04
SM.2901	*Peromyscus nudipes*	Limón	LAIP-VS	614075.32	1007645.35
SM.2909	*Peromyscus nudipes*	Limón	LAIP-VS	614079.67	1007637.34
SM.2974	*Peromyscus nudipes*	Heredia	BCNP	486264.79	1120205.98
SM.3110	*Peromyscus nudipes*	Limón	LAIP-VS	614108.40	1007648.10
SM.3120	*Peromyscus nudipes*	Limón	LAIP-VS	614104.48	1007634.04
SM.3373	*Peromyscus nudipes*	Limón	LAIP-VS	614125.58	100766.94
SM.3418	*Peromyscus nudipes*	Limón	LAIP-VS	614085.54	1007645.56
SM.3522	*Peromyscus nudipes*	Limón	LAIP-VS	614126.73	1007676.36
SM.2966	*Peromyscus nudipes*	Puntarenas	LAIP-PT	614556.31	997928.825
SM.2970	*Peromyscus nudipes*	Heredia	BCNP	486258.70	1120212.60
SM.2982	*Peromyscus nudipes*	Heredia	BCNP	486265.59	1120170.50
SM.2983	*Peromyscus nudipes*	Heredia	BCNP	486253.67	1120216.31
SM.2989	*Peromyscus nudipes*	Heredia	BCNP	486153.85	1120099.28

Specimen code	Species	Province	Sampling site	LONGITUDE (CR05/CRTM05)	LATITUDE (CR05/CRTM05)
SM.2993	*Peromyscus nudipes*	Heredia	BCNP	486212.33	1120204.65
SM.2994	*Peromyscus nudipes*	Heredia	BCNP	486209.14	1120218.22
SM.3205	*Peromyscus nudipes*	Honduras	Cusuco N.P.	47755.56	1718173.43
SM.3148	*Philander melanurus*	Puntarenas	LAIP-EA	609026.68	998567.17
SM.3326	*Philander melanurus*	Limón	CERS	554728.67	1165163.33
SM.3332	*Philander melanurus*	Limón	AFRS	546140.42	1154332.56
SM.2968	*Proechimys semispinosus*	Puntarenas	Sansi	604086.52	991099.08
SM.3335	*Proechimys semispinosus*	Puntarenas	MANP	602868.33	990867.15
SM.3410	*Proechimys semispinosus*	Puntarenas	Sansi	604200.26	991212.91
SM.3565	*Proechimys semispinosus*	Puntarenas	Sansi	604207.19	991105.89
SM.3680	*Proechimys semispinosus*	Heredia	S. Verde	492316.77	1155529.81
SM.3682	*Proechimys semispinosus*	Heredia	S. Verde	492308.55	1155516.38
SM.3688	*Proechimys semispinosus*	Heredia	S. Verde	492353.17	1155504.12
SM.3692	*Proechimys semispinosus*	Heredia	S. Verde	492367.87	1155568.32
SM.2785	*Reithrodontomys creper*	Limón	LAIP-VS	614105.10	1007651.07
SM.2897	*Reithrodontomys creper*	Limón	LAIP-VS	614070.75	1007551.98
SM.2899	*Reithrodontomys creper*	Limón	LAIP-VS	614058.39	1007684.12
SM.2905	*Reithrodontomys creper*	Limón	LAIP-VS	614077.82	1007653.76
SM.2907	*Reithrodontomys creper*	Limón	LAIP-VS	614082.47	1007643.26
SM.2981	*Reithrodontomys creper*	Heredia	BCNP	486129.49	1120104.57
SM.2988	*Reithrodontomys creper*	Heredia	BCNP	486270.35	1120189.04
SM.2996	*Reithrodontomys creper*	Heredia	BCNP	486296.83	1120213.13
SM.2999	*Reithrodontomys creper*	Heredia	BCNP	486091.23	1120121.39
SM.3011	*Reithrodontomys creper*	Heredia	BCNP	486296.83	1120213.13
SM.3014	*Reithrodontomys creper*	Heredia	BCNP	486258.70	1120212.60
SM.3121	*Reithrodontomys creper*	Limón	LAIP-VS	614137.87	1007646.52
SM.3363	*Reithrodontomys creper*	Limón	LAIP-VS	614077.82	1007653.76
SM.3377	*Reithrodontomys creper*	Limón	LAIP-VS	614091.17	1007676.47
SM.3413	*Reithrodontomys creper*	Limón	LAIP-VS	614174.33	1007697.84
SM.3465	*Reithrodontomys creper*	Limón	LAIP-VS	614101.64	1007628.28
SM.3486	*Reithrodontomys creper*	Limón	LAIP-VS	614089.86	1007672.38
SM.3514	*Reithrodontomys creper*	Limón	LAIP-VS	614093.56	1007648.16
SM.3516	*Reithrodontomys creper*	Limón	LAIP-VS	614104.48	1007634.04
SM.3546	*Reithrodontomys creper*	Limón	LAIP-VS	614075.66	1007525.89
SM.2781	*Reithrodontomys* sp	Limón	LAIP-VS	614105.69	1007636.03
SM.2782	*Reithrodontomys* sp	Limón	LAIP-VS	614084.60	1007590.07
SM.2969	*Reithrodontomys* sp	Heredia	BCNP	486152.92	1120072.93
SM.2971	*Reithrodontomys* sp	Heredia	BCNP	486129.49	1120104.57
SM.2973	*Reithrodontomys* sp	Heredia	BCNP	486270.35	1120189.04
SM.2977	*Reithrodontomys* sp	Heredia	BCNP	486077.46	1120070.28
SM.2979	*Reithrodontomys* sp	Heredia	BCNP	486296.83	1120213.13

Specimen code	Species	Province	Sampling site	LONGITUDE (CR05/CRTM05)	LATITUDE (CR05/CRTM05)
SM.2980	*Reithrodontomys* sp	Heredia	BCNP	486297.89	1120195.39
SM.2903	*Reithrodontomys* sp	Limón	LAIP-VS	614081.79	1007652.22
SM.2928	*Reithrodontomys* sp	Limón	LAIP-VS	614104.48	1007634.04
SM.3118	*Reithrodontomys* sp	Limón	LAIP-VS	614060.81	1007683.69
SM.3123	*Reithrodontomys* sp	Limón	LAIP-VS	614092.61	1007679.08
SM.3127	*Reithrodontomys* sp	Limón	LAIP-VS	614162.84	1007656.44
SM.3129	*Reithrodontomys* sp	Limón	LAIP-VS	614090.16	1007645.17
SM.3141	*Reithrodontomys* sp	Limón	LAIP-VS	614058.39	1007684.12
SM.3143	*Reithrodontomys* sp	Puntarenas	LAIP-EA	609050.16	998582.88
SM.3149	*Reithrodontomys* sp	Puntarenas	LAIP-EA	608808.26	998631.90
SM.3150	*Reithrodontomys* sp	Puntarenas	LAIP-PT	614537.66	998034.42
SM.3367	*Reithrodontomys* sp	Limón	LAIP-VS	614071.49	1007636.55
SM.3380	*Reithrodontomys* sp	Limón	LAIP-VS	614077.82	1007653.76
SM.3382	*Reithrodontomys* sp	Limón	LAIP-VS	614175.11	1007655.14
SM.3384	*Reithrodontomys* sp	Limón	LAIP-VS	614079.07	1007680.31
SM.3394	*Reithrodontomys* sp	Puntarenas	LAIP-PT	614498.55	997899.349
SM.3411	*Reithrodontomys* sp	Limón	LAIP-VS	614124.34	1007649.25
SM.3412	*Reithrodontomys* sp	Limón	LAIP-VS	614089.86	1007672.38
SM.3417	*Reithrodontomys* sp	Limón	LAIP-VS	614058.39	1007684.12
SM.3420	*Reithrodontomys* sp	Limón	LAIP-VS	614185.52	1007666.35
SM.3427	*Reithrodontomys* sp	Puntarenas	LAIP-EA	609209.66	998584.29
SM.3460	*Reithrodontomys* sp	Limón	LAIP-VS	614121.57	1007650.17
SM.3462	*Reithrodontomys* sp	Limón	LAIP-VS	614077.82	1007653.76
SM.3463	*Reithrodontomys* sp	Limón	LAIP-VS	614079.04	1007649.67
SM.3470	*Reithrodontomys* sp	Limón	LAIP-VS	614104.48	1007634.04
SM.3471	*Reithrodontomys* sp	Limón	LAIP-VS	614105.30	1007657.49
SM.3473	*Reithrodontomys* sp	Limón	LAIP-VS	614098.61	1007611.68
SM.3488	*Reithrodontomys* sp	Limón	LAIP-VS	614084.60	1007590.07
SM.3511	*Reithrodontomys* sp	Limón	LAIP-VS	614114.23	1007646.45
SM.3512	*Reithrodontomys* sp	Limón	LAIP-VS	614098.61	1007611.68
SM.3513	*Reithrodontomys* sp	Limón	LAIP-VS	614049.43	1007471.73
SM.3535	*Reithrodontomys* sp	Limón	LAIP-VS	614108.36	1007661.37
SM.3550	*Reithrodontomys* sp	Limón	LAIP-VS	614082.47	1007643.26
SM.3559	*Reithrodontomys* sp	Puntarenas	LAIP-PT	614477.33	997898.96
SM.3574	*Reithrodontomys* sp	Puntarenas	LAIP-PT	613860.82	997812.59
SM.2986	*Reithrodontomys sumichrasti*	Heredia	BCNP	486250.76	1120221.08
SM.3015	*Reithrodontomys sumichrasti*	Heredia	BCNP	486142.21	1120192.32
SM.2954	*Scotinomys teguina*	Puntarenas	LAIP-PT	613864.85	997826.54
SM.2975	*Scotinomys teguina*	Heredia	BCNP	486259.23	1120163.09
SM.2997	*Scotinomys teguina*	Heredia	BCNP	486178.06	1120156.68
SM.3005	*Scotinomys teguina*	Heredia	BCNP	486296.83	1120213.13

Specimen code	Species	Province	Sampling site	LONGITUDE (CR05/CRTM05)	LATITUDE (CR05/CRTM05)
SM.3152	*Scotinomys teguina*	Puntarenas	LAIP-PT	614561.81	997925.97
SM.3153	*Scotinomys teguina*	Puntarenas	LAIP-PT	613829.13	997743.92
SM.2898	*Scotinomys xerampelinus*	Limón	LAIP-VS	614101.99	1007620.98
SM.3111	*Scotinomys xerampelinus*	Limón	LAIP-VS	614080.38	1007645.03
SM.3137	*Scotinomys xerampelinus*	Limón	LAIP-VS	614082.60	1007673.79
SM.3369	*Scotinomys xerampelinus*	Limón	LAIP-VS	614072.76	1007657.17
SM.3347	*Sigmodon hirsutus*	Guanacaste	SRNP	323353.21	1198378.37
SM.3352	*Sigmodon hirsutus*	Guanacaste	SRNP	323367.73	1198380.61
SM.3355	*Sigmodon hirsutus*	Guanacaste	SRNP	323505.78	1198061.19
SM.3361	*Sigmodon hirsutus*	Guanacaste	SRNP	323044.71	1198991.63

Appendix 4: Cytochrome *b* sequences retrieved from INSDC for all-species analysis

Accession number	Species	Country	Locality	Reference
KU171185	*Caluromys derbianus*	Panamá	Ngäbe-Buglé	Diaz-Nieto *et al.* 2016
MK817302	*Caluromys derbianus*	Costa Rica	Heredia	Voss *et al.* 2019
MK817303	*Caluromys derbianus*	Ecuador	Cotopaxi, Otonga	Voss *et al.* 2019
MK817304	*Caluromys derbianus*	Panamá	Bocas del Toro	Voss *et al.* 2019
MK817305	*Caluromys derbianus*	Panamá	Ngäbe-Buglé	Voss *et al.* 2019
MZ457413	*Cryptotis gracilis*	Costa Rica	Cartago	He *et al.* 2021
MZ457417	*Cryptotis merriami*	Guatemala	Zacapa	He *et al.* 2021
MZ457412	*Cryptotis nigrescens*	Costa Rica	Puntarenas	He *et al.* 2021
HM589701	*Didelphis marsupialis*	Mexico	Veracruz	Arcangeli and Cervantes 2010
HM589702	*Didelphis marsupialis*	Mexico	Tabasco	Arcangeli and Cervantes 2010
KT437726	*Didelphis marsupialis*	Panamá	Ngäbe-Buglé	Diaz-Nieto *et al.* 2016
KT447521	*Didelphis marsupialis*	Brazil	São Paulo	Carnieli *et al.* 2016
MG491975	*Didelphis marsupialis*	Panamá	Ngäbe-Buglé, Kusapín	Voss *et al.* 2018
HM589699	*Didelphis virginiana*	Mexico	Mexico	Arcangeli and Cervantes 2010
MG491976	*Didelphis virginiana*	Mexico	Yucatán	Voss *et al.* 2018
MT892666	*Didelphis virginiana*	Mexico	Campeche	Dias *et al.* 2021
NC001610	*Didelphis virginiana*	USA	California	Janke *et al.* 1994
KU892776	*Diplomys labilis*	Panamá	San Blas	Fabre *et al.* 2017
EU579489	*Handleyomys alfaroi*	Nicaragua	Matagalpa	Hanson 2008
KP778238	*Handleyomys alfaroi*	Honduras	Comayagua	Almendra *et al.* 2018
KP778323	*Handleyomys alfaroi*	Guatemala	San Marcos	Almendra *et al.* 2018
KP778390	*Handleyomys alfaroi*	Costa Rica	Puntarenas	Almendra *et al.* 2018
KP778435	*Handleyomys alfaroi*	Panamá	Chiriqui	Almendra *et al.* 2018
DQ168466	*Heteromys desmarestianus*	Honduras	Atlantida	Rogers and Vance 2005
GU646938	*Heteromys desmarestianus*	Costa Rica	Alajuela	Rogers and González 2010
GU646940	*Heteromys desmarestianus*	Costa Rica	Cartago	Rogers and González 2010
GU646959	*Heteromys desmarestianus*	Costa Rica	Puntarenas	Rogers and González 2010
GU646987	*Heteromys desmarestianus*	Mexico	Chiapas	Rogers and González 2010
GU647010	*Heteromys nubicolens*	Costa Rica	Guanacaste	Rogers and González 2010
GU647011	*Heteromys nubicolens*	Costa Rica	Puntarenas	Rogers and González 2010
GU647004	*Heteromys oresterus*	Costa Rica	Cartago	Rogers and González 2010
GU647005	*Heteromys oresterus*	Costa Rica	San José	Rogers and González 2010
GU647006	*Heteromys oresterus*	Costa Rica	San José	Rogers and González 2010
GU647007	*Heteromys oresterus*	Costa Rica	San José	Rogers and González 2010
GU647008	*Heteromys oresterus*	Costa Rica	San José	Rogers and González 2010
DQ168541	*Heteromys salvini**	Costa Rica	Puntarenas	Rogers and Vance 2005
DQ168542	*Heteromys salvini**	Costa Rica	Puntarenas	Rogers and Vance 2005
DQ168543	*Heteromys salvini**	Honduras	Valle Province	Rogers and Vance 2005

Accession number	Species	Country	Locality	Reference
DQ168544	*Heteromys salvini**	Costa Rica	Guanacaste	Rogers and Vance 2005
DQ168545	*Heteromys salvini**	Costa Rica	Guanacaste	Rogers and Vance 2005
GU647016	*Heteromys* sp	Costa Rica	Alajuela	Rogers and González 2010
GU647017	*Heteromys* sp	Costa Rica	Limón	Rogers and González 2010
GU647018	*Heteromys* sp	Costa Rica	Limón	Rogers and González 2010
GU647020	*Heteromys* sp	Costa Rica	Limón	Rogers and González 2010
KU892779	*Hoplomys gymnurus*	Panamá	Campana	Fabre *et al.* 2017
MN978600	*Marmosa alstoni*	Panamá	Bocas del Toro	Voss *et al.* 2020
MN978601	*Marmosa alstoni*	Panamá	Bocas del Toro	Voss *et al.* 2020
HM106344	*Marmosa mexicana*	Guatemala	Zacapa	Gutiérrez *et al.* 2010
HM106355	*Marmosa mexicana*	Guatemala	El Progresso	Gutiérrez *et al.* 2010
HM106356	*Marmosa mexicana*	Guatemala	Petén	Gutiérrez *et al.* 2010
HM106358	*Marmosa mexicana*	Mexico	Campeche	Gutiérrez *et al.* 2010
HM106359	*Marmosa mexicana*	Mexico	Campeche	Gutiérrez *et al.* 2010
EU074633	*Melanomys chrysomelas*	Nicaragua	Atlantico Norte	Hanson *et al.* 2010
EU340017	*Melanomys chrysomelas*	Nicaragua	Atlantico Norte	Hanson and Bradley 2008
EU340018	*Melanomys chrysomelas*	Nicaragua	Atlantico Norte	Hanson and Bradley 2008
EU665204	*Melanomys chrysomelas*	Costa Rica	Heredia	Hanson and Bradley 2008
MK817277	*Metachirus nudicaudatus*	Peru	Cusco, Paucartambo	Voss *et al.* 2019
MK817292	*Metachirus nudicaudatus*	Panamá	Campana	Voss *et al.* 2019
MK817293	*Metachirus nudicaudatus*	Ecuador	Orellana	Voss *et al.* 2019
MK817294	*Metachirus nudicaudatus*	Guyana	Potaro-Siparuni	Voss *et al.* 2019
MK817299	*Metachirus nudicaudatus*	Panamá	Ngäbe-Buglé	Voss *et al.* 2019
KP778209	*Nephelomys devius*	Costa Rica	Puntarenas	Almendra *et al.* 2018
KP778411	*Nephelomys devius*	Costa Rica	Cartago	Almendra *et al.* 2018
JN851816	*Nyctomys sumichrasti*	Guatemala	Petén	Corley *et al.* 2011
JQ183063	*Nyctomys sumichrasti*	El Salvador	La Paz	Corley *et al.* 2011
JQ183064	*Nyctomys sumichrasti*	El Salvador	La Paz	Corley *et al.* 2011
JQ183065	*Nyctomys sumichrasti*	Mexico	Chiapas	Corley *et al.* 2011
JQ183066	*Nyctomys sumichrasti*	Mexico	Jalisco	Corley *et al.* 2011
GU126527	*Oecomys trinitatis*	Peru	Loreto	Percequillo *et al.* 2011
GU393988	*Oligoryzomys costaricensis*	Panamá	Gamboa	Hanson *et al.* 2011
GU393989	*Oligoryzomys costaricensis*	Panamá	Gamboa	Hanson *et al.* 2011
DQ185383	*Oryzomys couesi*	Honduras	Olancho, Catacamas	Milazzo *et al.* 2006
EU074667	*Oryzomys couesi*	Honduras	Atlantida	Hanson *et al.* 2010
FJ360633	*Oryzomys couesi*	Guatemala	Baja Verapaz	Hanson *et al.* 2010
FJ971265	*Oryzomys couesi*	Nicaragua	Granada	Hanson *et al.* 2010
FJ971266	*Oryzomys couesi*	Guatemala	El Peten	Hanson *et al.* 2010

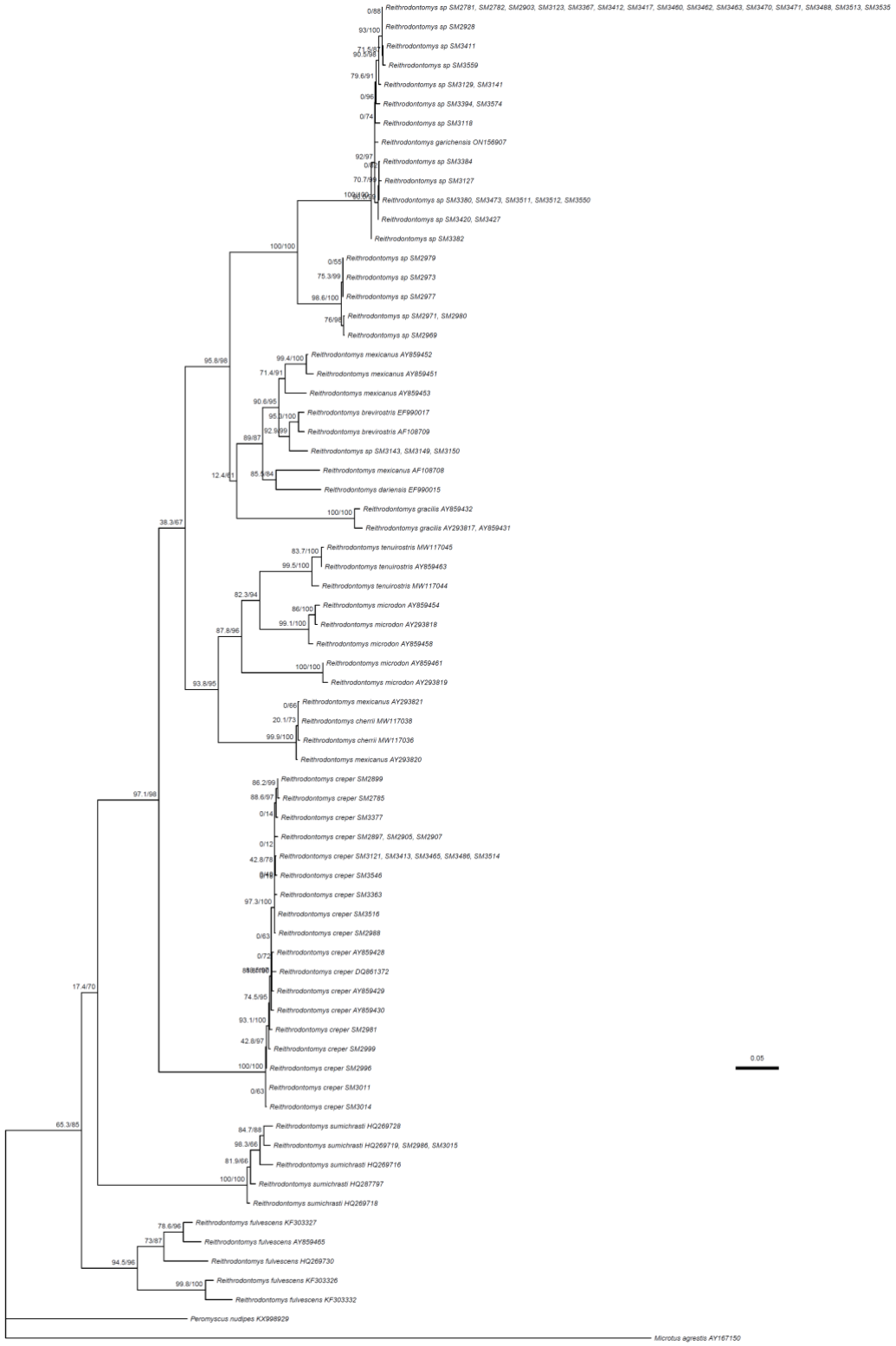
Accession number	Species	Country	Locality	Reference
DQ179814	*Ototylomys phyllotis*	Honduras	Atlantida	Matocq *et al.* 2007
KX998930	*Peromyscus nicaraguae*	Costa Rica	Puntarenas	Bradley *et al.* 2016
KX998933	*Peromyscus nicaraguae*	Nicaragua	Matagalpa	Bradley *et al.* 2016
KX998935	*Peromyscus nicaraguae*	Honduras	Colón	Bradley *et al.* 2016
KX998937	*Peromyscus nicaraguae*	Nicaragua	Jinotega	Bradley *et al.* 2016
FJ214687	*Peromyscus nudipes*	Nicaragua	Madriz	Bradley *et al.* 2016
KX998929	*Peromyscus nudipes*	Panamá	Chiriquí	Bradley *et al.* 2016
MG491923	*Philander melanurus*	Colombia	Caldas	Voss *et al.* 2018
MG491926	*Philander melanurus*	Panamá	Bocas del Toro	Voss *et al.* 2018
MG491927	*Philander melanurus*	Panamá	Bocas del Toro	Voss *et al.* 2018
MG491928	*Philander melanurus*	Panamá	Panamá	Voss *et al.* 2018
KX688188	*Proechimys semispinosus*	Colombia	Cauca	Ferreira da Silva *et al.* unpub.
AF108709	*Reithrodontomys brevirostris*	Costa Rica	Cartago	Smith and Patton 1999., Gardner and Carleton 2009
AY859428	*Reithrodontomys creper*	Costa Rica	Cartago	Arellano *et al.* 2005
AY859430	*Reithrodontomys creper*	Costa Rica	Heredia	Arellano *et al.* 2005
AY293817	*Reithrodontomys gracilis*	Mexico	Yucatán	Bradley *et al.* 2004
AY859431	*Reithrodontomys gracilis*	Mexico	Yucatán	Arellano *et al.* 2005
AY859432	*Reithrodontomys gracilis*	Mexico	Campeche	Arellano *et al.* 2005
AY293820	*Reithrodontomys mexicanus†*	Costa Rica	San José	Arellano *et al.* 2005
AY293821	*Reithrodontomys mexicanus†*	Costa Rica	San José	Arellano *et al.* 2005., Bradley *et al.* 2004
HQ269716	*Reithrodontomys sumichrasti*	Guatemala	Chimaltenango	Hardy *et al.* 2013
HQ269718	*Reithrodontomys sumichrasti*	Nicaragua	Estelí	Hardy *et al.* 2013
HQ269719	*Reithrodontomys sumichrasti*	Costa Rica	Cartago	Hardy *et al.* 2013
HQ269728	*Reithrodontomys sumichrasti*	Panamá	Chiriquí	Hardy *et al.* 2013
HQ287797	*Reithrodontomys sumichrasti*	Honduras	Intibuca	Hardy *et al.* 2013
KF359512	*Rheomys raptor*	Costa Rica	Puntarenas	Hanson *et al.* 2015
JN851815	*Scotinomys teguina*	Honduras	Comayagua	Corley *et al.* 2011
AF108706	*Scotinomys xerampelinus*	Costa Rica	Cartago	Smith and Patton 1999
DQ861371	*Scotinomys xerampelinus*	Costa Rica	Cartago	Rogers *et al.* 2007
KY754149	*Scotinomys xerampelinus*	Costa Rica	Cartago	Steppan and Schenk 2017
AY517528	*Sigmodon hirsutus*	Honduras	Francisco Morazan	Bradley *et al.* 2008
EU073164	*Sigmodon hirsutus*	Nicaragua	Nueva Segovia	Bradley *et al.* 2008
EU073166	*Sigmodon hirsutus*	Nicaragua	Boaco	Bradley *et al.* 2008
EU073169	*Sigmodon hirsutus*	Nicaragua	Matagalpa	Bradley *et al.* 2008
EU073170	*Sigmodon hirsutus*	Honduras	Cortez	Bradley *et al.* 2008
EU073175	*Sigmodon hirsutus*	Honduras	Colon	Bradley *et al.* 2008

Accession number	Species	Country	Locality	Reference
EU078398	*Sigmodon hirsutus*	Mexico	Chiapas	Bradley *et al.* 2008
EU074635	*Sigmodontomys alfari*	Panamá	Bocas del Toro	Hanson and Bradley 2008
EU340016	*Sigmodontomys alfari*	Ecuador	Esmeraldas	Hanson and Bradley 2008
GU126548	*Sigmodontomys alfari*	Panamá	Bocas del Toro	Percequillo *et al.* 2011
KY754155	*Sigmodontomys alfari*	Panamá	Bocas del Toro	Steppan and Schenk 2017
EU579513	*Transandinomys bolivaris*	Ecuador	Esmeraldas	Hanson 2008
EU579514	*Transandinomys talamancae*	Ecuador	El Oro	Hanson 2008
EU579515	*Transandinomys talamancae*	Panamá	Bocas del Toro	Hanson 2008
KP778202	*Transandinomys talamancae*	Panamá	Darien	Almendra *et al.* 2018
KP778208	*Transandinomys talamancae*	Panamá	Darien	Almendra *et al.* 2018
MF317949	*Tylomys watsoni*	Costa Rica	Puntarenas	Porter *et al.* 2017
EU579519	*Zygodontomys brevicauda*	Venezuela	Portugessa	Hanson and Bradley unpub.
EU579521	*Zygodontomys brevicauda*	Venezuela	Sucre	Hanson and Bradley unpub.
GU126549	*Zygodontomys brevicauda*	Venezuela	Sucre	Percequillo *et al.* 2011
GU397417	*Zygodontomys brevicauda*	Panamá	Isla Coiba	González *et al.* 2010
KY754182	*Zygodontomys brevicauda*	Bolivia	San Ignacio Yuruani	Steppan and Schenk 2017
NC000891	*Ornithorhynchus anatinus*	Australia		Janke *et al.* 1996
AJ303116	*Tachyglossus aculeatus*	Australia		Janke *et al.* 2002

* At the time of collection of these sequences, this species was known as *Liomys salvini*, but it is now been renamed *Heteromys salvini*. † *Reithrodontomys mexicanus* is no longer considered part of the fauna of Costa Rica (Ramírez-Fernández *et al.* 2023). However, at the time of collection of these sequences it was, and we have retained the authors' designation.

Appendix 5. Cytochrome *b* sequences retrieved from INSDC for *Reithrodontomys* analysis.

Accession number	Species	Country	Locality	Reference
AF108709	*Reithrodontomys brevirostris*	Costa Rica	Cartago	Smith and Patton 1999., Gardner and Carleton 2009
AY859428	*Reithrodontomys creper*	Costa Rica	Cartago	Arellano *et al.* 2005
AY859429	*Reithrodontomys creper*	Costa Rica	Heredia	Arellano *et al.* 2005
AY859430	*Reithrodontomys creper*	Costa Rica	Heredia	Arellano *et al.* 2005
DQ861372	*Reithrodontomys creper*	Costa Rica	Cartago	Rogers *et al.* 2007
AY859465	*Reithrodontomys fulvescens*	Mexico	Chiapas	Arellano *et al.* 2005
HQ269730	*Reithrodontomys fulvescens*	Mexico	Oaxaca	Hardy *et al.* 2013
KF303326	*Reithrodontomys fulvescens*	Mexico	Puebla	Gonzalez-Cozatl *et al.* unpub.
KF303327	*Reithrodontomys fulvescens*	Mexico	Oaxaca	Gonzalez-Cozatl *et al.* unpub.
KF303332	*Reithrodontomys fulvescens*	Mexico	Durango	Gonzalez-Cozatl *et al.* unpub.
AY293817	*Reithrodontomys gracilis*	Mexico	Yucatán	Bradley *et al.* 2004
AY859431	*Reithrodontomys gracilis*	Mexico	Yucatán	Arellano *et al.* 2005
AY859432	*Reithrodontomys gracilis*	Mexico	Campeche	Arellano *et al.* 2005
AF108708	*Reithrodontomys mexicanus*	Columbia	Risaralda	Smith and Patton 1999
AY293820	*Reithrodontomys mexicanus*	Costa Rica	San José	Arellano *et al.* 2005
AY293821	*Reithrodontomys mexicanus*	Costa Rica	San José	Arellano *et al.* 2005., Bradley *et al.* 2004
AY859451	*Reithrodontomys mexicanus*	Guatemala	Baja Verapaz	Arellano *et al.* 2005
AY859452	*Reithrodontomys mexicanus*	Guatemala	Zacapa	Arellano *et al.* 2005
AY859453	*Reithrodontomys mexicanus*	El Salvador	Santa Ana	Arellano *et al.* 2005
AY293818	*Reithrodontomys microdon*	Mexico	Chiapas	Arellano *et al.* 2005
AY293819	*Reithrodontomys microdon*	Mexico	Oaxaca	Arellano *et al.* 2005
AY859454	*Reithrodontomys microdon*	Mexico	Chiapas	Arellano *et al.* 2005
AY859458	*Reithrodontomys microdon*	Guatemala	Huehuetenango	Arellano *et al.* 2005
AY859461	*Reithrodontomys microdon*	Mexico	Oaxaca	Arellano *et al.* 2005
HQ269716	*Reithrodontomys sumichrasti*	Guatemala	Chimaltenango	Hardy *et al.* 2013
HQ269718	*Reithrodontomys sumichrasti*	Nicaragua	Estelí	Hardy *et al.* 2013
HQ269719	*Reithrodontomys sumichrasti*	Costa Rica	Cartago	Hardy *et al.* 2013
HQ269728	*Reithrodontomys sumichrasti*	Panamá	Chiriquí	Hardy *et al.* 2013
HQ287797	*Reithrodontomys sumichrasti*	Honduras	Intibuca	Hardy *et al.* 2013
AY859463	*Reithrodontomys tenuirostris*	Mexico	Chiapas	Arellano *et al.* 2005
MW117044	*Reithrodontomys tenuirostris*	Mexico	Chiapas	Martínez-Borrego *et al.* 2022
MW117045	*Reithrodontomys tenuirostris*	Mexico	Chiapas	Martínez-Borrego *et al.* 2022
KX998929	*Peromyscus nudipes*	Panamá		Bradley *et al.* 2016
AY167150	*Microtus agrestis*	UK		Jaarola and Searle 2002



Appendix 6. *Reithrodontomys* phylogeny using IQ TREE, including short sequences of otherwise unrepresented or poorly represented species (*R. brevirostris*, *R. cherrii*, *R. dariensis*, *R. garichensis*). The new sequences are as follows: EF990017 (Costa Rica, Alajuela) and EF990015 (Panamá, Danen): Miller and Engstrom 2008., MW117036 and MW117038 (Costa Rica, San José) and MW117044 and MW117045 (Mexico, Chiapas): Martínez-Borrego *et al.* 2022., AY859463 and AY859465 (Mexico, Chiapas): Arellano *et al.* 2005., HQ269730 (Mexico, Oaxaca): Hardy *et al.* 2013., KF303326 (Mexico, Puebla), KF303327 (Mexico, Oaxaca) and KF303332 (Mexico, Durango): Gonzalez-Cozatl *et al.* unpub.

Appendix 7. Sample identification of *Scotinomys teguina* and *Scotinomys xerampelinus* by locality and markers considered.

				Cytb	***COI***	***RAG1***
Species	**Sample/ accession**	**Locality**	**Country**	**Accession no.**	**Accession no.**	**Accession no.**
Scotinomys teguina	SM.2972	Braulio Carrillo National Park	Costa Rica	X	X	X
Scotinomys teguina	SM.2975	Braulio Carrillo National Park	Costa Rica	X	X	X
Scotinomys teguina	SM.2976	Braulio Carrillo National Park	Costa Rica	X	X	X
Scotinomys teguina	SM.2984	Braulio Carrillo National Park	Costa Rica	X		X
Scotinomys teguina	SM.2997	Braulio Carrillo National Park	Costa Rica	X		X
Scotinomys teguina	SM.3005	Braulio Carrillo National Park	Costa Rica	X		X
Scotinomys teguina	SM.2954	La Amistad International Park - Ranger Station Pittier	Costa Rica	X	X	X
Scotinomys teguina	SM.2963	La Amistad International Park - Ranger Station Pittier	Costa Rica	X	X	X
Scotinomys teguina	SM.3152	La Amistad International Park - Ranger Station Pittier	Costa Rica	X	X	X
Scotinomys teguina	SM.3153	La Amistad International Park - Ranger Station Pittier	Costa Rica	X	X	X
Scotinomys teguina	SM.3156	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		X
Scotinomys teguina	SM.3159	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		
Scotinomys teguina	SM.3397	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		
Scotinomys teguina	SM.3403	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		
Scotinomys teguina	SM.3432	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		
Scotinomys teguina	SM.3433	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		
Scotinomys teguina	SM.3434	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		X
Scotinomys teguina	SM.3437	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		X

Scotinomys teguina	SM.3441	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		X
Scotinomys teguina	SM.3443	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		X
Scotinomys teguina	SM.3448	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		
Scotinomys teguina	SM.3449	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		
Scotinomys teguina	SM.3452	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		
Scotinomys teguina	SM.3555	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		
Scotinomys teguina	SM.3561	La Amistad International Park - Ranger Station Pittier	Costa Rica	X	X	X
Scotinomys teguina	SM.3562	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		
Scotinomys teguina	SM.3572	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		X
Scotinomys teguina	SM.3576	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		X
Scotinomys teguina	SM.3580	La Amistad International Park - Ranger Station Pittier	Costa Rica	X		
Scotinomys teguina	SM.3219		Honduras	X	X	
Scotinomys teguina	JN851815	National Park Cerro Azul Meámbar, Comayagua	Honduras	X		
Scotinomys teguina	JF444451	Iztaru, Cerros de La Carpintera, Cartago	Costa Rica		X	
Scotinomys teguina	JF444452	Iztaru, Cerros de La Carpintera, Cartago	Costa Rica		X	
Scotinomys teguina	JF444453	Iztaru, Cerros de La Carpintera, Cartago	Costa Rica		X	
Scotinomys teguina	JF444454	Iztaru, Cerros de La Carpintera, Cartago	Costa Rica		X	
Scotinomys teguina	JF444455	Iztaru, Cerros de La Carpintera, Cartago	Costa Rica		X	
Scotinomys teguina	JF444456	Iztaru, Cerros de La Carpintera, Cartago	Costa Rica		X	
Scotinomys teguina	JF444457	Iztaru, Cerros de La Carpintera, Cartago	Costa Rica		X	

Scotinomys teguina	JF444458	Iztaru, Cerros de La Carpintera, Cartago	Costa Rica	X
Scotinomys teguina	JF444459	Iztaru, Cerros de La Carpintera, Cartago	Costa Rica	X
Scotinomys teguina	JF459549	Iztaru, Cerros de La Carpintera, Cartago	Costa Rica	X
Scotinomys teguina	JF459551	Iztaru, Cerros de La Carpintera, Cartago	Costa Rica	X
Scotinomys teguina	JF459554	Santa Cruz, Cartago	Costa Rica	X
Scotinomys teguina	JF459557	6.5 Km NNE Of Capellades, Cartago	Costa Rica	X
Scotinomys teguina	JF444741	Santa Ana, National Park Montecristo	El Salvador	X
Scotinomys teguina	JF444742	Santa Ana, National Park Montecristo	El Salvador	X
Scotinomys teguina	JF444743	Santa Ana, National Park Montecristo	El Salvador	X
Scotinomys teguina	JF444744	Santa Ana, National Park Montecristo	El Salvador	X
Scotinomys teguina	JF444745	Santa Ana, National Park Montecristo	El Salvador	X
Scotinomys teguina	JF444746	Santa Ana, National Park Montecristo	El Salvador	X
Scotinomys teguina	JF444747	Santa Ana, National Park Montecristo	El Salvador	X
Scotinomys teguina	JF444750	Santa Ana, National Park Montecristo	El Salvador	X
Scotinomys teguina	JF444748	Zacapa, 2 Km N of San Lorenzo, Sierra De Las Minas	Guatemala	X
Scotinomys teguina	JF444749	Zacapa, 2 Km N of San Lorenzo, Sierra De Las Minas	Guatemala	X
Scotinomys teguina	JF444751	Baja Verapaz, 5 Km E of Puruhla	Guatemala	X
Scotinomys teguina	JF446274	Chiapas, 12 Km N of Berriozabal	México	X
Scotinomys teguina	JF446275	Chiapas, 12 Km N of Berriozabal	México	X
Scotinomys teguina	JF446276	Chiapas, 12 Km N of Berriozabal	México	X

Scotinomys teguina	JF446277	Chiapas, 12 Km N of Berriozabal	México		X		
Scotinomys teguina	JF459544	Cerro La Muerte, San Gerardo De Dota, San Jose	Costa Rica		X		
Scotinomys teguina	JF459545	Puntarenas, Monte Verde Biological Station	Costa Rica		X		
Scotinomys teguina	JF459547	Esteli	Nicaragua		X		
Scotinomys teguina	JF459558	Chiriqui, Ojo De Agua, 2 Km N of Santa Clara	Panama		X		
Scotinomys teguina	KC953578						X
Scotinomys xerampelinus	SM.2775	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.2778	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.2779	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.2786	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.2790	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.2898	La Amistad International Park - Valle del Silencio	Costa Rica	X	X		X
Scotinomys xerampelinus	SM.2902	La Amistad International Park - Valle del Silencio	Costa Rica	X			X
Scotinomys xerampelinus	SM.2908	La Amistad International Park - Valle del Silencio	Costa Rica	X			X
Scotinomys xerampelinus	SM.2910	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.2920	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.2922	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.2931	La Amistad International Park - Valle del Silencio	Costa Rica	X			X
Scotinomys xerampelinus	SM.2937	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.2941	La Amistad International Park - Valle del Silencio	Costa Rica	X			

Scotinomys xerampelinus	SM.3111	La Amistad International Park - Valle del Silencio	Costa Rica	X		X
Scotinomys xerampelinus	SM.3126	La Amistad International Park - Valle del Silencio	Costa Rica	X		
Scotinomys xerampelinus	SM.3137	La Amistad International Park - Valle del Silencio	Costa Rica	X		
Scotinomys xerampelinus	SM.3366	La Amistad International Park - Valle del Silencio	Costa Rica	X		X
Scotinomys xerampelinus	SM.3369	La Amistad International Park - Valle del Silencio	Costa Rica	X	X	X
Scotinomys xerampelinus	SM.3376	La Amistad International Park - Valle del Silencio	Costa Rica	X		X
Scotinomys xerampelinus	SM.3379	La Amistad International Park - Valle del Silencio	Costa Rica	X		
Scotinomys xerampelinus	SM.3386	La Amistad International Park - Valle del Silencio	Costa Rica	X	X	X
Scotinomys xerampelinus	SM.3389	La Amistad International Park - Valle del Silencio	Costa Rica	X		
Scotinomys xerampelinus	SM.3391	La Amistad International Park - Valle del Silencio	Costa Rica	X		
Scotinomys xerampelinus	SM.3414	La Amistad International Park - Valle del Silencio	Costa Rica	X		
Scotinomys xerampelinus	SM.3421	La Amistad International Park - Valle del Silencio	Costa Rica	X		X
Scotinomys xerampelinus	SM.3424	La Amistad International Park - Valle del Silencio	Costa Rica	X		
Scotinomys xerampelinus	SM.3477	La Amistad International Park - Valle del Silencio	Costa Rica	X		X
Scotinomys xerampelinus	SM.3485	La Amistad International Park - Valle del Silencio	Costa Rica	X		
Scotinomys xerampelinus	SM.3493	La Amistad International Park - Valle del Silencio	Costa Rica	X		
Scotinomys xerampelinus	SM.3482	La Amistad International Park - Valle del Silencio	Costa Rica	X		
Scotinomys xerampelinus	SM.3483	La Amistad International Park - Valle del Silencio	Costa Rica	X		
Scotinomys xerampelinus	SM.3484	La Amistad International Park - Valle del Silencio	Costa Rica	X		

Scotinomys xerampelinus	SM.3485	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.3486	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.3487	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.3488	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.3489	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.3490	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.3491	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.3492	La Amistad International Park - Valle del Silencio	Costa Rica	X			
Scotinomys xerampelinus	SM.3493	La Amistad International Park - Valle del Silencio	Costa Rica	X			X
Scotinomys xerampelinus	DQ861371	National Park Volcán Irazú, Cartago	Costa Rica	X			
Scotinomys xerampelinus	KY754149	National Park Volcán Irazú, Cartago	Costa Rica	X			
Scotinomys xerampelinus	JF444460	National Park Volcán Irazú, Cartago	Costa Rica	NA	X		
Scotinomys xerampelinus	JF444461	National Park Volcán Irazú, Cartago	Costa Rica	NA	X		
Scotinomys xerampelinus	JF444462	National Park Volcán Irazú, Cartago	Costa Rica	NA	X		
Scotinomys xerampelinus	JF444463	National Park Volcán Irazú, Cartago	Costa Rica	NA	X		
Scotinomys xerampelinus	JF444464	National Park Volcán Irazú, Cartago	Costa Rica	NA	X		
Scotinomys xerampelinus	JF444465	National Park Volcán Irazú, Cartago	Costa Rica	NA	X		
Scotinomys xerampelinus	JF459560	National Park Volcán Irazú, Cartago	Costa Rica	NA	X		
Scotinomys xerampelinus	JF459561	National Park Volcán Irazú, Cartago	Costa Rica	NA	X		

Scotinomys xerampelinus	JF459562	National Park Volcán Irazú, Cartago	Costa Rica	NA	X	
Scotinomys xerampelinus	JF459563	National Park Volcán Irazú, Cartago	Costa Rica	NA	X	
Scotinomys xerampelinus	JF459564	National Park Volcán Irazú, Cartago	Costa Rica	NA	X	
Scotinomys xerampelinus	JF459565	National Park Volcán Irazú, Cartago	Costa Rica	NA	X	
Scotinomys xerampelinus	MF097942	San Jose Province, 1 rd km SW Poas	Costa Rica	NA		X