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Insights into the evolutionary and demographic history of the extant endemic rodents of the Galápagos Islands

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Evolutionary radiations stemming from colonization of archipelagos provide valuable insights into mechanisms and modes of speciation. For this reason, the fauna inhabiting the Galápagos Islands has been the focus of numerous emblematic ecological and evolutionary studies. However, studies focused on rodents have been scarce. Rice rats radiated *in situ* into at least six endemic species: *Aegialomys galapagoensis*, *Nesoryzomys narboroughi*, *N. swarthi*, *N. fernandinae*, *N. indefessus*, and *N. darwini*. Only the first four species remain extant on the archipelago. These species are considered vulnerable, mainly due to human activities and invasive species. Despite their interesting evolutionary history, questions surrounding phylogenetic relationships, colonization events, genetic diversity and demography of populations remain unresolved. We used the D-loop region of mtDNA to infer phylogenetic relationships, colonization events, date divergences, and conduct population genetic analyses of the four extant endemic species inhabiting the Galápagos Islands. We found that all species were monophyletic. *A. galapagoensis* is sister to *A. xanthaeolus* from the continent, and both of them are the sister clade of the genus *Nesoryzomys*. Our results also showed that there were two colonization events to the islands. The first event was the arrival of the ancestor of *Nesoryzomys* during the Pliocene, when divergences between genera occurred. The second was *Aegialomys* during middle Pleistocene, when species diversification began. Populations on each island show high genetic diversity and most show signals of recent expansion. However, future studies are needed to accurately assess the conservation status of these populations. We suggest ongoing monitoring of these vulnerable endemic species, including ecological and population genetic studies. In addition, future studies using genome-wide molecular markers and additional species from the continent, as well as sampling extinct species from the islands, will improve our knowledge about the origin and relationships of the endemic rodents of the Galápagos Islands.

Las radiaciones evolutivas que ocurren en los archipiélagos, posterior a su colonización, proporcionan información relevante sobre las formas y mecanismos de especiación. Por tal motivo, la fauna que habita en las Islas Galápagos ha sido objeto de numerosos y emblemáticos estudios. A pesar de lo anterior, los estudios enfocados a roedores han sido escasos. Las ratas arroceras han radiado *in situ* en al menos seis especies endémicas: *Aegialomys galapagoensis*, *Nesoryzomys narboroughi*, *N. swarthi*, *N. fernandinae*, *N. indefessus* y *N. darwini*. A la fecha, solo las primeras cuatro especies aún se distribuyen en el archipiélago. Dichas especies se encuentran clasificadas como vulnerables, debido principalmente a las actividades humanas y las especies invasoras. A pesar de la interesante historia evolutiva que presentan estas especies, las interrogantes sobre sus relaciones filogenéticas, los eventos de colonización de las islas y la diversidad genética e historia demográfica de sus poblaciones, siguen sin resolverse. Dado lo anterior, usamos la región D-loop del DNA mitocondrial para inferir las relaciones filogenéticas, los eventos de colonización, los tiempos de divergencia y analizar el estado genético poblacional de las cuatro especies endémicas existentes en las Islas Galápagos. Encontramos que todas las especies representan grupos monofiléticos, que *A. galapagoensis* es la especie hermana de *A. xanthaeolus* del continente, y que estas dos especies son el grupo hermano del género *Nesoryzomys*. Nuestros resultados indican dos eventos de colonización en las islas. El primer evento muestra la llegada del ancestro de *Nesoryzomys* durante el Plioceno, siendo contemporáneo a la divergencia entre los dos géneros. El segundo evento de colonización ocurrió a mediados del Pleistoceno, cuando *Aegialomys* invadió el archipiélago y los eventos de diversificación de las especies comenzaron. En general, las poblaciones en las islas muestran una diversidad genética alta y una señal de expansión reciente. A pesar de ello, se requiere de más estudios para evaluar con precisión el estado de conservación de las poblaciones. Sugerimos que las poblaciones de estos roedores endémicos y vulnerables sean monitoreadas, realizando estudios ecológicos y genéticos. Adicionalmente, estudios futuros que utilicen marcadores moleculares distribuidos a través del genoma completo y que incluyan a las especies extintas en las islas y a más especies del continente, mejorarían el conocimiento sobre el origen y las relaciones de los roedores endémicos de las Islas Galápagos.

Key words: *Aegialomys*; colonization; diversification; *Nesoryzomys*; populations; speciation.

Introduction

Remote oceanic islands and archipelagos are biologically simpler than continental regions and therefore provide ideal geographical and historical settings for the study of colonization, adaptation, speciation, and diversification of species. Islands have long been recognized as natural models for the study of evolutionary processes (Parent *et al.* 2008; Losos 2010; Rodrigues and Diniz-Filho 2016; Román-Palacios and Wiens 2018). Compared to continental regions, islands are more ideal places to observe and interpret patterns of evolution due to their geographic isolation, small size, fewer numbers of species, and a high degree of endemism. The development of ecological and evolutionary systems can be directly observed on volcanic islands as we are now better able to date the timing of their emergence above the ocean surface as blank slates for colonization and the timing of subsequent evolutionary diversification (Losos and Ricklefs 2009; Hendriks *et al.* 2019).

The Galápagos Islands are a young oceanic and volcanic archipelago resulting from the eastward passage of the Nazca plate over a hotspot, at a rate of 59 km/My, located in the Pacific Ocean at approximately 960 km west of the coast of South America. It is composed of 13 major islands larger than 10 km^2, six smaller islands, over 40 islets with official names and many smaller unnamed islets and rocks, for a total of approximately 8,000 km^2 of land spread over 45,000 km^2 of water (Snell *et al.* 1996; Parent *et al.* 2008, Geist *et al.* 2014, Harpp *et al.* 2014). The age of the islands increases moving eastward with the oldest islands located towards the southeast of the archipelago. The present islands date from up to 3.5 to 4 million years ago (Ma) for the eastern islands of Española and San Cristóbal, respectively, to 60,000 years ago for Fernandina Island (Geist *et al.* 2014).

Despite their tropical climate, the Galápagos Islands have been the stage of surprisingly few animal diversifications compared with other Pacific tropical island groups. Among vertebrates, the absence of amphibians and the virtual absence of mammals are particularly striking and nearly unique among terrestrial island ecosystems (Parent *et al.* 2008). However, Román-Palacios and Wiens (2018) showed that the Galápagos archipelago drove faster rates of speciation and diversification in tanagers and tortoises, at least three times higher than in other related lineages of birds and tortoises inhabiting different islands.

The rodents of the Galápagos Islands are the only terrestrial mammals that have naturally colonized the islands and diversified within the archipelago. They belong to the Neotropical rice rat family Cricetidae, subfamily Sigmodontinae, tribe Oryzomyini. Two genera of rodents are currently known to be present on the islands: *Aegialomys* (Weksler *et al.* 2006) and *Nesoryzomys* (Heller 1904). There are two commonly recognized and described species in the genus *Aegialomys*: *A. xanthaeolus* the type species from the mainland and *A. galapagoensis*, formerly known from San Cristobal Island but has not been collected since their initial capture by Darwin in 1835 and is presumed extinct there (Patton and Hafner 1983; Dowler *et al.* 2000). In addition, the subspecies *A. galapagoensis bauri*, extant in Santa Fé Island, was previously considered a separate species but has been most often considered synonymous with *A. galapagoensis* (Cabrera 1961; Musser and Carleton 1993, 2005; Weksler *et al.* 2006; Weksler and Percequillo 2011; do Prado and Percequillo 2018). The genus *Nesoryzomys* comprises two extinct and three extant species. The two extinct species are *N. indefessus* from Santa Cruz and Baltra Islands and *N. darwini* from Santa Cruz Island. The extant species include *N. swarthi* from Santiago Island, and *N. narboroughi* and *N. fernandinae* from Fernandina Island (Dowler and Carroll 1996; Dowler *et al.* 2000). *N. narboroughi* has sometimes been synonymized with *N. indefessus* (Heller 1904; Musser and Carleton 1993, 2005), but in this manuscript we retain both *N. indefessus* and *N. narboroughi* as different species as recommended by Dowler (2015). The giant rice rat, *Megaoryzomys curioi*, is known only from subfossil remains from Santa Cruz Island and is not known to have a mainland representative (Patton and Hafner 1983). It is possible that its extinction occurred prior to human settlement of the archipelago.

To date, the only systematic study to include nearly all of the species in the Galápagos, both extinct and extant, is Patton and Hafner (1983). They did not analyze *N. fernandinae* (Hutterer and Hirsch 1980), which was described as a new species after their research was in press (Dowler *et al.* 2000). Based on a variety of data sets, including morphology, anatomy, protein electrophoresis and chromosome number and morphology, they suggested that: a) *Nesoryzomys* should be recognized at the generic level, nevertheless its origin is ambiguous, b) there were at least two independent colonizations of the islands, with *Nesoryzomys* representing an early arrival at 3 to 3.5 Ma, followed considerably later by *Aegialomys* as late as a few hundred to a thousand years ago, c) both *Aegialomys* taxa from the islands derived from *A. xanthaeolus* of the coastal Peruvian river valleys, d) *N. narboroughi, N. swarthi* and *N. indefessus* should be considered as races of a single species; and e) *A. galapagoensis* and *A. bauri* should be considered conspecific.

Dowler *et al.* (2000) performed one of the most recent field surveys during which they discovered a population of *N. fernandinae*; this allowed the first opportunity to describe the appearance of this species, which was previously known only from skeletal remains (Hutterer and Hirsch 1980) from Fernandina Island. They also found a viable population of *N. swarthi* on Santiago Island, which had previously been presumed extinct. These specimens represent the first endemic rodents taken on Santiago Island since the type series was collected in 1906 (Orr 1938), and a single partial skull was found in 1965 (Peterson 1966). However, recent molecular phylogenetic studies included only *A. xanthaeolus*, *N. narboroughi* and *N. swarthi*. Weksler (2003, 2006) and Weksler *et al.* (2006), using a nuclear exon and morphology, found that *N. narboroughi* and *N. swarthi* are monophyletic, and they are closely related to *A. xanthaeolus*. Pine *et al.* (2012), Leite *et al.* (2014), and Machado *et al.* (2014), using morphology and mitochondrial and nuclear genes, supported the results of Weksler (2003, 2006) and the results of Weksler *et al.* (2006).

Outside of what was learned from these studies, little is known about the endemic rodents of the Galápagos Islands; however, it is clear that they are critically threatened by invasive species and human activities. The goal of the present study was to undertake the first study including comprehensive sampling of all four extant endemic rodent species (*A. galapagoensis, N. narboroughi, N. swarthi* and *N. fernandinae*) inhabiting the archipelago, to provide a dated phylogeny, and to elucidate the population genetics status of each species. This information will help to elucidate the evolutionary history of these island taxa and synthesize information about evolution and biogeography at scales that span remote islands, archipelagoes and continents.

Methods

Sample collection. We obtained tissue samples (liver, kidney) from museum specimens deposited at Angelo State Natural History Collections (ASNHC) at Angelo State University and the Museum of Vertebrate Zoology at the University of California, Berkeley (MVZ). Additional samples were from ear biopsies from animals released at the collection site. We sampled 159 individuals of the *Aegialomys* and *Nesoryzomys* genera inhabiting the Galápagos Islands, *A. galapagoensis* (*n* = 43), *N. narboroughi* (*n* = 49), *N. swarthi* (*n* = 43), and *N. fernandinae* (*n* = 24; Figure 1, Appendix 1), and two samples of *A. xanthaeolus* from Ecuador to elucidate its relationship with *A. galapagoensis*. We used *Pseudoryzomys simplex* and *Oligoryzomys microtis* as outgroups for the phylogenetic analyses (sequences downloaded from GenBank, accession numbers AY863422.1 and AY863420.1, respectively).

DNA isolation and mitochondrial gene amplification. We extracted DNA from tissues using the DNeasy® Blood and Tissue Kit (QIAGEN, Inc., Valencia, CA) by cutting approximately 20 µg of tissue into several small pieces and following the manufacturer's protocol. We amplified the mitochondrial control region (D-loop) by polymerase chain reaction (PCR) using the primers designed for *Oligoryzomys* spp. (González-Ittig *et al.* 2002). In rodents, the D-loop has been useful in phylogenetic analyses due to its elevated mutation rate, lack of recombination and maternal inheritance (Robins *et al.* 2014). Also, due to its high mutation rate, this marker has been used to detect signatures of population structure at a scale of just a few kilometers (Hirota *et al.* 2004; Urgoiti *et al.* 2018). The PCR reactions contained 14.85 µL DEPC H2O, 3.0 µL 10X Reaction buffer, 3.0 µL deoxynucleotide triphosphates (dNTPs, 2 mM of each), 1.5 µL each forward and reverse primer (10 µM), 3.0 µL of 0.1 % bovine serum albumin (BSA), 0.15 µL AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA), and 3 µL of DNA for a final reaction volume of 30 µL. We used 1 % agarose gels stained with ethidium bromide to visualize DNA extractions and to amplify products. Amplification parameters were as follows: initial step of 95 °C (4 min), followed by 34 cycles of denaturation at 95 °C (40 sec), annealing at 50 °C (30 sec), extension at 72 °C (90 sec), and a final extension at 72 °C (10 min). Reaction products were purified using AMPure Magnetic Beads (Agencourt Bioscience, Beverly, MA). The *Oligoryzomys* D-loop primers were used in an initial sequencing run. More specific internal forward and reverse primers were designed using the sequence fragments obtained from this initial run. The internal primers GIF (5' - CCACTACCAGCACCCAAAGCTG - 3') and GIREV (5'- GGTTGTGTTGATTAATGATCC - 3') were used in all sequencing reactions. Five microliters of cleaned PCR product were added to 4µL of ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) and 1.0 µL of 1.6 µM internal primer in each sequencing reaction (GIF and GIFREV). Sequencing reaction conditions were: 96 °C (1 min), 45 cycles of 96 °C (30 sec), 58 °C for GIF or 52 °C for GIREV (15 sec), 60 °C (4 min), followed by a final holding step of 4 °C. All sequencing reactions were performed using GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA). Final sequencing products were purified using Sephadex G-50 powder then dried in a vacuum centrifuge and stored at - 20 °C. Sequences were re-hydrated with the addition of 5 µL of HiDi Formamide with 0.1 mM EDTA, denatured at 95 °C (3 min) and sequenced with capillary action electrophoresis using SCE 9610 Genetic Analysis System (SpectruMedix, State College, PA).

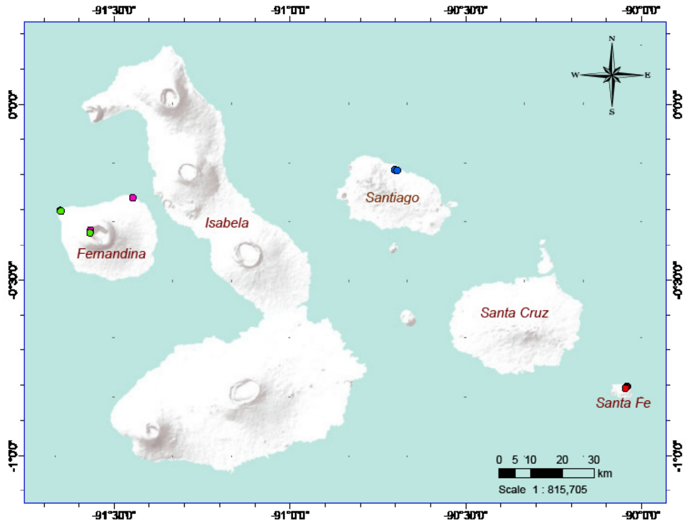

Figure 1. Sampling localities of *A. galapagoensis* (red dots; *n* = 43), *N. narboroughi* (pink dots; *n* = 49), *N. swarthi* (blue dots; *n* = 43) and *N. fernandinae* (green dots; *n* = 24) in the Galápagos Islands.

Phylogenetic analyses and divergence times estimation. We cleaned and edited sequences using Geneious® 11.1.4 (https://www.geneious.com), and performed multiple sequence alignment using ClustalW v.2.1 (Larkin *et al.* 2007) implemented in Geneious. The best evolutionary model of nucleotide substitution was estimated in jModelTest 2.1.1 (Guindon and Gascuel 2003; Darriba *et al.* 2012) using the Akaike information criterion. A Bayesian Inference (BI) analysis was performed in MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), run for 20 million generations sampling every 1,000 generations. Output parameters were visualized using Tracer v1.7.1 (Rambaut *et al.* 2018) to check for convergence between runs, and the first 25 % of the trees were discarded as burn-in.

We used BEAST v2.5.2 (Bouckaert *et al.* 2014) to estimate molecular dates of divergences under an uncorrelated lognormal relaxed molecular clock model. The time to the most recent common ancestor for the main lineages was obtained using Bayesian Markov chain Monte Carlo

(MCMC) searches. We sampled trees and divergence dates for all nodes every 10,000 iterations for 50,000,000 generations. These analyses implemented the Yule speciation processes model and the randomly generated starting tree as priors. We used three calibration points. The first calibration was based on a biogeographical event: the origin of the Galápagos archipelago at 5 Ma (Geist *et al.* 2014). Machado *et al.* (2014) found that the lineage leading to the endemic genus *Nesoryzomys* derived from an ancestor shared with the clade composed of *Melanomys*, *Sigmodontomys* and *Aegialomys* and split around 1.49 (95 % HPD : 0.26 to 3.23) Ma. We used the split between *Nesoryzomys* and *Aegialomys* as a second calibration point. The third calibration point was based on the split of the lineage leading to *Pseudoryzomys* around 2.58 (95 % HPD : 0.43 to 5.38) Ma (Machado *et al.* 2014). We checked convergence statistics for effective sample sizes using Tracer v1.7.1 (Rambaut *et al.* 2018). We used TreeAnnotator v2.5.2 (available in the BEAST package) to get a consensus tree with node height distribution after elimination of 25 % of trees as burn-in. We visualized MrBayes and Beast results using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

We performed BI and Beast analyses including all samples per species (trees not shown). We chose to perform the phylogenetic and dating analyses using representative samples of each species to reduce the saturation effects. Populations-level analyses included all the samples.

Population analyses. We conducted population genetic analyses separately for each clade determined with the BI analysis, with the exception of *A. xanthaeolus* because of the small sample size. The number of haplotypes (*H*), nucleotide diversity (π) (Nei 1987), haplotypic diversity (*h*), number of polymorphic segregating sites (*S*), singletons (S_1), parsimony informative sites (PIS), and the average number of nucleotide differences (*K*) were estimated using DNAsp v6.12 (Rozas *et al.* 2017). We performed Fu's *Fs* (Fu 1997) and Tajima's *D* (Tajima 1989a) neutrality tests to evaluate whether data departed from a neutral model of evolution due to factors such as population bottleneck or sudden expansion. Statistical significance was determined using the coalescent simulator in DNAsp v6.12 (Rozas *et al.* 2017) with 1000 simulations.

We used the distribution of the number of pairwise mutational differences among individuals, or mismatch distribution, to explore demographic patterns of populations using DNAsp v6.12 (Rozas *et al.* 2017); graphical representation was made by means of the growth-decline model. Raggedness (*r*) index (Harpending 1994) and R_2 statistics of Ramos-Onsins and Rozas (2002) were calculated to analyze goodness of fit of a population expansion model using 1000 simulations in the same program. Populations at demographic equilibrium or in decline should provide a multimodal distribution of pairwise differences, whereas populations that have experienced a sudden demographic expansion should display a star-shaped phylogeny and a unimodal distribution (Tajima 1989b; Slatkin and Hudson 1991; Rogers and Harpending 1992; Harpending and Rogers 2000). However, recent changes in population size may not be detectable in mismatch distribution analyses due to threshold effects, time lags, or earlier demographic events that may mask the effects of recent events (Rogers and Harpending 1992; Harpending and Rogers 2000).

We analyzed the magnitude of historical demographic events by constructing Bayesian Skyline Plots (BSP) using BEAST v2.5.2 (Bouckaert *et al.* 2014). This analysis infers population fluctuations over time by estimating the posterior distribution of the effective population size at specific intervals along a phylogeny (Drummond and Rambaut 2007). Genealogies and model parameters were sampled every 10,000 iterations along 50,000,000 generations under a relaxed molecular clock, with 25 % of burn-in. Convergence statistics for effective sample sizes and demographic plots were visualized using Tracer v1.7.1 (Rambaut *et al.* 2018). In comparison with simple parametric and older coalescent demographic methods, the smoother estimates and sensitivity of this method, together with credibility intervals, provide a realistic population size function and enable retrieval of more details than just summary statistics (Deli *et al.* 2016).

To further investigate the genetic relationships of the haplotypes at the intraspecific level we constructed haplotype networks using the Median-Joining algorithm (Bandelt *et al.* 1999) implemented in PopART v1.7 (Leigh and Bryant 2015).

Results

Phylogenetic lineages and divergence time. We amplified an average of 643 bp of the D-loop gene from 159 individuals of the *Aegialomys* and *Nesoryzomys* genera inhabiting the Galápagos Islands (*A. galapagoensis n* = 43, *N. narboroughi n* = 49, *N. swarthi n* = 43, and *N. fernandinae n* = 24, Figure 1, Appendix 1) and two samples of *A. xathaeolus* from the mainland.

The TVM with gamma distribution (+ G) model was recognized as the best fitting model with the following parameters: base frequencies A = 0.3612, C = 0.2445, G = 0.1109, T = 0.2834; nst = 6; and rates = gamma with shape parameter (α) = 0.6020. The BI and Beast analyses including all the samples per species (trees not shown) confirmed the monophyly of each of the species. However, the relationship with the outgroups was not well resolved and the posterior probabilities were lower. This could be due to the high mutation rates that are inherent to the D - loop region which can result in genetic saturation. Distantly related taxa are often affected by saturation effects. When sequences in a multiple alignment have undergone multiple substitutions, the apparent distances largely underestimate the real genetic distances and the alignment is said to be saturated (Philippe *et al.* 2011). In phylogenetics, saturation effects result in long branch attraction, decrease of phylogenetic information, and underestimation of observed divergence times (Wilke *et al.* 2009, Philippe *et al.* 2011). We thus focused our subsequent results and conclusions on the analyses that included only representative samples of each species. The BI analyses showed a topology in which four main clades for the Galápagos species were recognized with high levels of support and one clade including the continental species (Figure 2). All of the Galápagos rodent species analyzed were monophyletic. Clade 1 corresponds to all individuals recognized as *A. xanthaeolus*, which is a sister group of Clade 2, which includes all representatives of *A. galapagoensis*. Clade 3 corresponds to all samples identified as *N. narboroughi*, which is the sister to Clade 4 and Clade 5, corresponding to *N. swarthi*, and *N. fernandinae*, respectively.

The analysis estimating the time to the most recent common ancestor (TMRCA) showed that the oldest divergence event corresponds at the split between the *Nesoryzomys and Aegialomys* genera, dated around 3.84 (95 % HPD : 2.91 – 4.88) Ma (Figure 3). The first split between a continental versus an island species, *A. xanthaeolus* and *A. galapagoensis*, occurred around 1.11 (95 % HPD : 0.37 to 2.11) Ma. Speciation within the genus *Nesoryzomys* started around 2.23 (95 % HPD : 1.32 to 3.12) Ma with the split between *N. fernandinae* and *N. swarthi* versus *N. narboroughi*, followed by the split between the two lineages composed of *N. fernandinae* and *N. swarthi* which occurred around 1.58 (95 % HPD : 0.91 to 2.42) Ma. The estimated dates of divergence for the main nodes and their highest posterior density values are shown in Table 1. According to these results, the main speciation events occurred since the early-middle Pliocene up to the Pleistocene. However, the diversification within each species started at the end of the Pleistocene around 525,500 years ago.

Demographic reconstruction. Genetic diversity and neutrality test per species are shown in Table 2. All of the endemic species of the Galápagos Islands showed high genetic diversity (*Hd* > 0.965). *N. fernandinae* has the highest number of unique haplotypes in proportion with the number of samples, and *N. swarthi* the lowest.

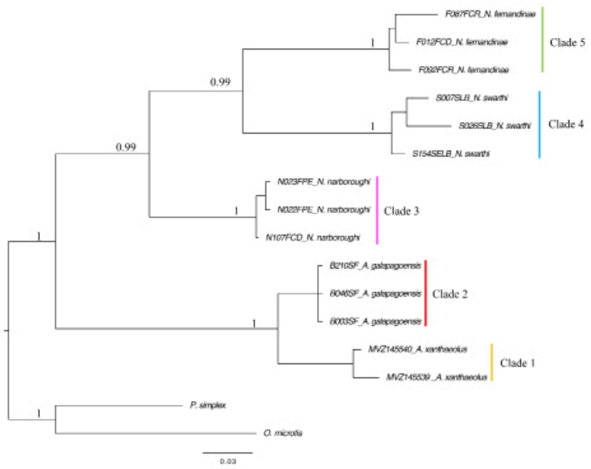

Figure 2. Phylogenetic tree of the four extant endemic rodents of the Galápagos Islands based on Bayesian Inference analysis of mtDNA D-loop sequence data. Numbers at nodes indicate support values of posterior probabilities.

Table 1. Estimated dates of divergence (time to the most recent common ancestor – TMRCA – and 95 % High Posterior Density confidence intervals – HPD – in Ma) for the extant species within the *Nesoryzomys* and *Aegialomys* genera. Clades depicted by letters correspond to those indicated in Figure 2.

Clade	TMRCA	95 % HPD
A	4.26	3.01 – 5.63
B	3.84	2.91 – 4.88
C	2.44	0.72 – 4.01
D	2.23	1.32 – 3.12
E	1.58	0.91 – 2.42
F	1.11	0.37 – 2.11
G	0.52	0.21 – 0.95
H	0.45	0.14 – 0.84
I	0.26	0.03 – 0.58
J	0.16	0.02 – 0.41
K	0.11	0.01 – 0.27

The applied neutrality test revealed significant deviations from mutation-drift equilibrium for all the species inhabiting Galápagos according to Fu´s F_s values, and only for *A. galapagoensis* using Tajima´s *D*. The negative values suggest recent population expansion events in these species (Table 2).

The statistical analyses of mismatch distribution showed unimodal distributions for *A. galapagoensis*, *N. narboroughi* and *N. fernandinae*, which also suggests a recent demographic expansion (Slatkin and Hudson 1991, Rogers and Harpending 1992) or spatial expansion (Ray *et al.* 2003, Excoffier 2004). Statistical analysis of the mismatch distribution *r* index and R_2 were significant (Figure 4).

The Bayesian skyline demographic reconstructions showed a pattern of constant size of populations through time, followed by a recent and small increase (Figure 5). *A. galapagoensis* and *N. fernandinae* show a small growth period, which started around 10,000 and 13,000 years ago, respectively. *N. narboroughi* and *N. swarthi* show a more constant trend of population increase, starting around 50,000 and 90,000 years ago, with a rapid increase starting around

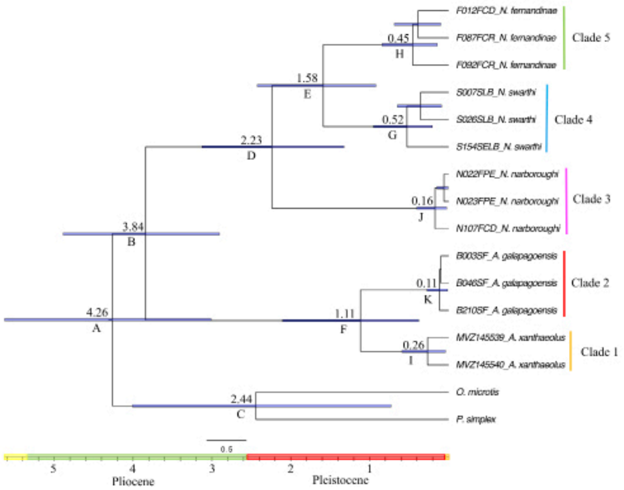

Figure 3. Dated phylogeny of the extant endemic rodents of the Galápagos Islands reconstructed from mtDNA D-loop haplotypes inferred from BEAST. The horizontal bars show the 95 % confidence intervals. Time-scale in millions of years ago. Dates and letters at nodes depict values calculated in Table 1.

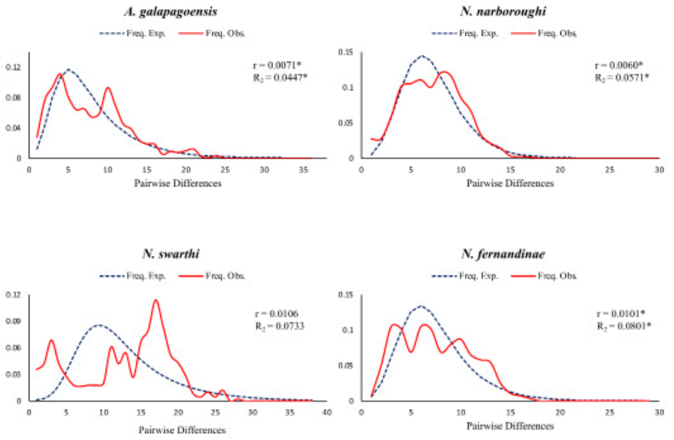

Figure 4. Mismatch distribution of pairwise differences of haplotypes for each of the extant species inhabiting the Galápagos Islands. Shown are observed (red lines) and expected (dark blue square dot lines) frequencies obtained under a model allowing for population size change. Raggedness (*r*) index and R_2 statistics values are shown. Significance is indicated with a star (*).

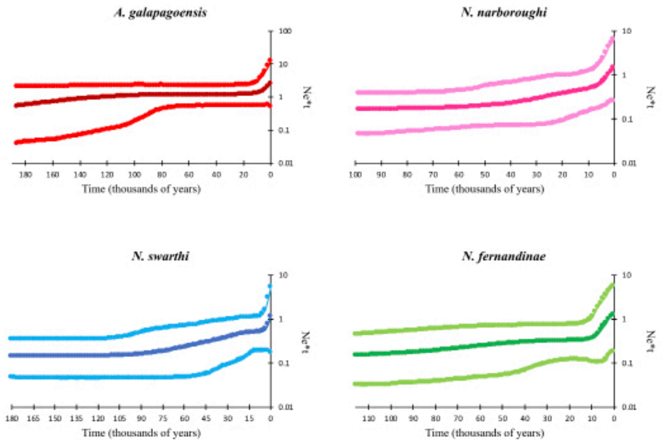

Figure 5. Skyline plots for each extant species of rodents inhabiting the Galápagos Islands. Plots show posterior median (darker lines) and 95 % Bayesian credible intervals (lighter lines on the outside) of the effective population size. The Y axis is in logarithm scale.

6,000 and 25,000 years ago, respectively. Skyline reconstruction showed that *A. galapagoensis*, *N. narboroughi*, *N. swar*thi and *N. fernandinae* have similar populations sizes.

Overall, the network analyses including all haplotypes for each species show a few abundant haplotypes, with frequencies between two and six, and numerous unique ones for all the species (Figure 6). *N. fernandinae* showed more unique haplotypes, with two being the highest frequency observed for a haplotype. The four species show long branches with haplotypes that are highly differentiated from the other haplotypes. *N. narboroughi* and *N. fernandinae*, for which samples were collected from three and two localities in Fernandina Island, respectively, do not show that the distribution of the haplotypes follows any structure or differentiation. Only *N. narboroughi* has two shared haplotypes among localities. Though *A. galapagoensis* and *N. narboroughi* do not show networks with a star-like shape, both networks show haplotypes with many connections, suggesting recent populations or with recent demographic expansion. In contrast, the networks of *N. swarthi* and *N. fernandinae* may suggest older or more stable populations.

Discussion

This is the first genetic study to include all extant endemic rodent species of the Galápagos Islands. We implemented different genetic analyses in order to elucidate the phylogenetic relationships among these rodents inhabiting the archipelago, as well as demographic history and relationships of their populations. This information is extremely important for conservation of these endemic species, given that there is very little known about their biology and ecology, and that they are considered vulnerable, mainly due to human activities and the introduction of invasive species. Furthermore, we contributed phylogenetic and demographic information as well as divergence estimates in order to form hypotheses regarding colonization of the islands and compare them with previous hypotheses.

Our phylogenetic study corroborated the monophyly of the genera *Aegialomys* and *Nesoryzomys* as Patton and Hafner (1983) and Weksler (2003, 2006), proposed. However, those authors did not include all the extant species. We also corroborated the monophyly of the continental species *A. xanthaeolus* and the island species *A. galapagoensis*, *N. naboroughi*, *N. swarthi* and *N. fernandinae*. Our calibration results suggest that the main speciation events started during the Pliocene with the split between the genus *Aegialomys* and *Nesoryzomys* (3.84 Ma, 95 % HPD:2.9 to 4.88), which agrees with the time proposed by Patton and Hafner (1983), using Nei's methods, dated around 3 to 3.5 Ma; it differs from that proposed by Machado *et al.* (2014), dated around 1.49 Ma (95 % HDP:0.26 to 3.23), and Parada *et al.* (2013) around 2.4 Ma (95 % HDP not available) during the Pleistocene. The difference between Machado *et al.*'s estimate and ours could be due to the genes used; they used IRBP and Cyt b, while we used D - loop. Also, despite the fact that our study and Machado *et al.*'s both used the

Table 2. Variability of the mtDNA D-loop sequences of *Aegialomys* and *Nesoryzomys* from Galápagos Islands. Number of polymorphic segregating sites (*S*), singletons (S_1), parsimony informative sites (PIS), haplotype diversity (*Hd*), nucleotide diversity (π), and average number of nucleotide differences (*K*). Significance is indicated with a star (*).

Species	Total basepairs	Number of samples	Number of haplotypes	*S*	S_1	PIS	*Hd*	π	*K*	Fu's F_s	Tajimas'*D*
Aegialomys galapagoensis	652	43	29	70	46	24	0.972 ∓ 0.012	0.01298∓0.0017	6.970	-14.570*	-2.18653*
Nesoryzomys narboroughi	711	49	32	48	27	21	0.973∓0.011	0.01003∓0.0006	6.179	-19.085*	-1.50303
Nesoryzomys swarthi	629	43	30	71	42	29	0.965∓ 0.016	0.02130∓ 0.0012	11.817	-8.969*	1.20756
Nesoryzomys fernandinae	578	24	22	33	15	18	0.993∓ 0.014	0.01204∓0.0015	6.467	-15.631*	-1.39461

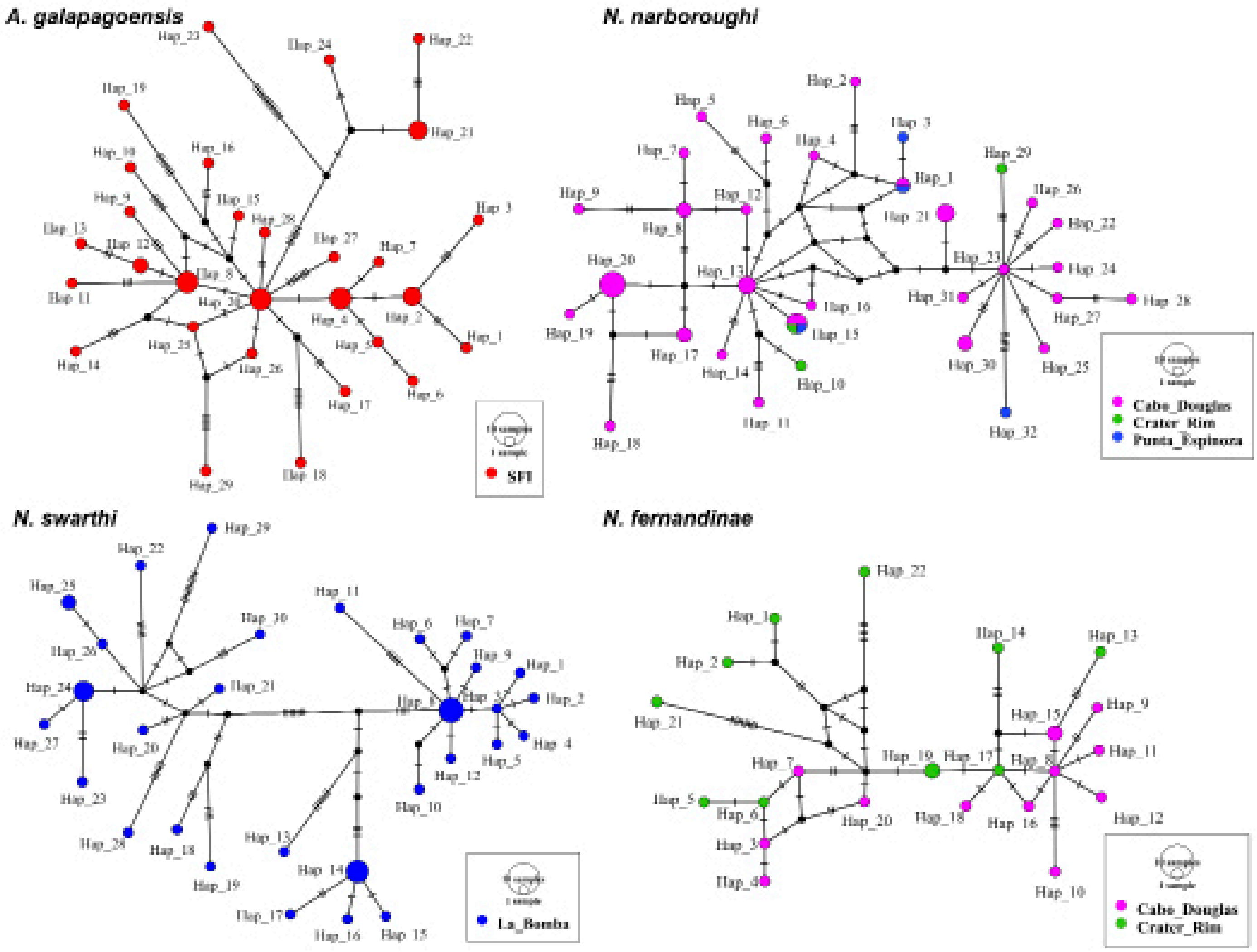

Figure 6. Median-Joining network for each species of the extant rodent of the Galápagos Islands, using mtDNA D-loop haplotypes. Circles sizes are proportional to the haplotype's frequencies in the population. The lines along the branches connecting the haplotypes show the number of substitutions.

origin of the Galápagos as a calibration point, we used the new date proposed by Geist *et al.* (2014) of 5 Ma, while Machado *et al.* used 4 Ma (Geist 1984). We also included *A. galapagoensis* from the islands, while they only used *A. xanthaeolus*. Including the two species of the genus *Aegialomys,* and specifying an older date for the origin of the archipelago, resulted in an older date for the split between these genera, which is well supported with the paleogeographic and biogeographical information. Despite this discrepancy, there is substantial overlap in the credibility intervals of these two assessments.

The second major speciation event occurred within the genus *Nesoryzomys* with the split between *N. narboroughi* versus *N. swarthi* and *N. fernandinae* dated at 2.23 Ma (95 % HDP:1.32 to 3.12) during the Pleistocene. Leite *et al.* (2014) dated the split between *N. narboroughi* and *N. swarthi* around 2 Ma (95 % HDP not available) during the Pleistocene, supporting our results. The third major event occurred at 1.58 Ma (95 % HDP:0.91 to 2.42) during the Pleistocene with the split between *N. swarthi* and *N. fernandinae*. Finally, the speciation between *A. xanthaeolus* and *A. galapagoensis* occurred at 1.11 Ma (95 % HDP: 0.37 to 2.10), also during the Pleistocene.

Rice rats are the only terrestrial mammals that naturally colonized the Galápagos and diversified within the archipelago (Clark 1984, Parent *et al.* 2008). There are some prior hypotheses about how these two genera colonized islands. Patton and Hafner (1983) suggested that *Nesoryzomys* is an old immigrant to the islands while the *Aegialomys* species is quite recent. They based their results on the high degree of morphological and biochemical (allozyme) distinctiveness of *Nesoryzomys* relative to other oryzomyines, including *Aegialomys*. This supported a more ancient origin and a single immigration to the islands, whether of pre-*Nesoryzomys* or *Nesoryzomys* form with subsequent radiation within the islands. In contrast *A. galapagoensis* is nearly morphological identical to the mainland *A. xanthaeolus*, which suggests an introduction to the islands within the last few hundred to thousand years, perhaps via pre-Columbian humans coursing the west coast of Perú (Patton 1984; Pine *et al.* 2012). Parent *et al.* (2008) suggested that colonization events on the Galápagos occurred over the last 3 to 4 Ma during the existence of the present islands, and might have happened earlier when now sunken islands were above sea level. The presence of several drowned seamounts on the Carnegie Ridge east of the Galápagos (Christie *et al.* 1992) suggests that earlier volcanic islands may have served as stepping stones for colonization by some of the terrestrial fauna. Parent *et al.* (2008) also mentioned that rice rat diversity is the result of one colonization event from the South American continent, where their close relatives inhabit, as it has been shown in other species of tortoises and lizards (*i. e.,* Caccone *et al.* 1999; Kizirian *et al.* 2004; Benavides *et al.* 2007).

Our results suggest two colonization events to the Galápagos by the extant species. The first arrival by the ancestor of *Nesoryzomys* was probably a species of *Sigmodontomys* or *Melanomys* that originated from lower montane and lowland forest habitats in South America (Harris and Macdonald 2007). This event should be dated from the early-late Pliocene boundary onwards, as proposed by Leite *et al.* (2014). This was followed by a second arrival of *Aegialomys* from coastal Perú by rafting over recent historical times to the middle Pleistocene, giving rise to the *Aegialomys* of the Galápagos (Patton 1975; Steadman 1985; Hutterer and Oromí 1993; Weksler 2003). It is well known that these species, and the most closely related ones, are excellent dispersers across salt water (Pine *et al.* 2012), making colonization easier for them.

The fauna and flora of the Galápagos Islands is principally derived from western South America, Central America, and the Caribbean (Merlen 2014). So, we assume that colonization from there to the archipelago during the Pliocene was possible because there is strong evidence (Christie *et al.* 1992; Werner *et al.* 1999) that islands have been forming over the hotspot for at least twice as long as the age of the oldest islands, and perhaps as long as 20 Ma. These ancient islands are now seamounts east of the present-day Galápagos along the Carnegie Ridge on the Nazca Plate (Christie *et al.* 1992) and northeast of the archipelago on the Cocos Plate on the Cocos Ridge (Werner *et al.* 1999). There has been a "conveyor belt" of islands produced over millions of years, providing the potential for organic colonization during that time (Merlen 2014).

Once the ancestors of *Nesoryzomys* and *Aegialomys* arrived to the Galápagos Islands, dispersal, colonization, speciation, and diversification were possible during the Pleistocene, in part, because the integrated area of the Galápagos Islands was much greater than today, land bridges existed between a few of the major islands, and many more minor islands and islets were exposed. In fact, there is a hypothesis that between 1 and 5 Ma, at least nineteen major Galápagos Islands existed but are currently submerged; these are in addition to the thirteen that exist today (Geist *et al.* 2014). Later island fragmentation has led to diversification by vicariance as well as dispersal. Dispersal was also possible because the main part of the Galápagos lies in shallow water created by the broad Galápagos platform, which formed by the accumulation of lavas (Geist *et al.* 2008).

Aegialomys galapagoensis, the Galápagos rice rat, is only found in one population on Santa Fé Island and is the latest species to have colonized the archipelago. Our results show high genetic diversity for the species and a signal of recent expansion. This finding is consistent with the fact that *A. galapagoensis* and *N. narboroughi* have been considered common by virtually all researchers visiting the islands (Clark 1980; Patton and Hafner 1983; Clark 1984; Key and Muñoz-Heredia 1994). In fact, Dowler *et al.* (2000) reported that the species was abundant and rice rats could be seen running among the lava rocks before dark. Another reason is that both Santa Fé Island and Fernandina Island are the only two islands that do not have introduced species of rats and mice (Dowler *et al.* 2000), allowing the persistence of the native species. This species is considered vulnerable by the Red List of the International Union for Conservation of Nature (IUCN 2019). We dated the events of speciation and diversification to around 1.11 Ma and 111,200 years ago, respectively; both of them occurred after the emergence of Santa Fé Island, dated at 2.9 Ma (Geist *et al.* 2014). Clark (1980) conducted an ecological study and found that *A. galapagoensis* has high survival and low reproduction relative to congeners of other geographic areas. He did not know if this strategy was specific to *A. galapagoensis* or a common feature of the Galápagos rodent life history (Harris and Macdonald 2007).

Nesoryzomys narboroughi, the large Fernandina rice rat, is only found on Fernandina Island. It is the oldest species within the extant rodents of Galápagos, with a speciation event dated at 2.23 Ma; however, its diversification is the most recent among the extant *Nesoryzomys*, starting around 169,400 years ago. This species is considered vulnerable according to the Red List (IUCN 2019); however, Dowler *et al.* (2000) verified the presence of sustainable populations, which are found from the coastline to the volcano rim. We found that the genetic diversity of this species is high, and their populations are under recent expansion. This could be due to the fact that Fernandina Island does not have introduced species of rats and mice, allowing the persistence of *N. narboroughi*.

Nesoryzomys fernandinae, the small Fernandina rice rat, is also found on Fernandina Island in sympatry with *N. narboroughi* (Hutterer and Hirsch 1980; Dowler *et al.* 2000). Its populations range from the coast up to the volcano rim. Dowler *et al.* (2000) found that this species is more abundant at high elevations along the volcano rim, where vegetation, primarily *Scalasia spp*., is most dense. Meanwhile, *N. narboroughi* is more abundant near the coast, suggesting that the species distributions are influenced by habitat (Dowler and Carroll 1996). *N. fernandinae* was considered extinct (IUCN 1996; Nowak 1999), but now its status is vulnerable according to the Red List (IUCN 2019). This species has the highest genetic diversity within the genus, and it shows a more stable population in comparison with *N. narboroughi*, which showed recent expansion. We dated the events of speciation and diversification to 1.58 Ma and 452,900 years ago, respectively. The presence of *N. narboroughi* and *N. fernandinae* on Fernandina Island suggest that volcanic activity has not occurred on an island-wide scale sufficient to destroy their populations as Merlen (2014) suggested. He also proposed that Fernandina Island is a refuge for biodiversity in Galápagos based on the formation of endemic species, the establishment of terrestrial ecosystems at several altitudes, and climate conditions.

Fernandina Island has the most active volcano and is the youngest major island in the archipelago. Its emergence is suggested to have occurred approximately 32,000 years ago with a maximum emergence around 60,000 years ago (Geist *et al.* 2014). The dates that we obtained for the speciation and diversification of *N. narboroughi* and *N. fernandinae* are older than the dates proposed for the origin of the island.

Nesoryzomys swarthi, the Santiago Galápagos mouse, is endemic to Santiago Island where it exists as a single population in the arid zone of the north-central coast (Dowler *et al.* 2000; Harris *et al.* 2006). This species coexists with *Rattus rattus* and *Mus musculus* on the island. However, the three species are only sympatric near the beach, where the densities of the invasive species are lower (Dowler *et al.* 2000; Harris *et al.* 2006). Brosset (1963) considered that *N. swarthi* was extinct based on the introduction of diseases or parasites by invasive species, and/or competition with them. At present, it is considered vulnerable according to the Red List (IUCN 2019). Our results showed that the species has relatively high genetic diversity, despite being the lowest genetic diversity among the other native rodents. Two of our analyses show a signal of recent expansion, but one of them rejected this hypothesis. We consider that more genetic analyses, including more loci, are necessary to resolve this discrepancy. It is possible that the species underwent a recent bottleneck; however, the genetic signal is not strong. A bottleneck event in the species would be consistent with the fact that it was considered extinct for decades while it went undetected until Dowler *et al.* 2000 rediscovered it. However, we do not have enough data to thoroughly test this hypothesis. The emergence of Santiago Island has been dated to a minimum of 800,000 years ago and a maximum of 1.4 Ma, which agrees with the time of speciation and diversification that we found for *N. swarthi* around 1.58 Ma and 525,500 years ago, respectively. Harris and Macdonald (2007) performed an ecological study and found that the unpredictable environment of the Galápagos arid zone has selected for a strategy of high survivorship and low reproduction in *N. swarthi* that is more typically found among desert Heteromyidae than other members of the Oryzomyini. Clark (1980) found the same result in *A. galapagoensis*. It seems that this strategy is a common feature of native rodents of the Galápagos Islands.

According to our results, we have two main conclusions. The first is that populations of the extant rodents on the Galápagos show high genetic diversity and most show a signal of recent expansion. Despite the challenges that are faced by these species by the extreme arid conditions on the islands, the impact of human activities, and the presence of invasive species, our results suggest that these species do not have genetic signatures implying that they have experienced dramatic population declines and, rather, that populations are demographically stable. However, based on previous studies this conclusion should be considered carefully. For example, the desert-adapted life histories of these species are impressive because their ancestors were not well adapted to dry environments and oryzomyine rodents of semiarid habitats often lack the physiological adaptations to xeric conditions (Best 1988; Meserve 1978; Ribeiro *et al.* 2004; Harris and Macdonald 2007). Moreover, the fact that the abundance of these endemic rodents is positively related to vegetation density suggests that the presence of these species is resource-limited (Clark 1980; Dowler and Carroll 1996; Harris and Macdonald 2007). This could explain why some native species can coexist with invasive species or coexist in sympatry with other native species if there are enough resources to minimize competition. In this sense, there is now evidence that introduced rodents may not be the sole cause of decline in native rodents in Galápagos; feral cats may be a second important factor in rodent decline and extinction (Dowler *et al.* 2000; Dexter *et al.* 2004), as well as loss of habitat and resources or the introduction of pathogens. In light of these previous findings and our results, we should continue to monitor these vulnerable species, performing more fine-scale ecological and population studies in the future.

Our second major conclusion is that the major speciation events of the four extant rice rats occurred within the archipelago during the Pleistocene. In the case of *A. galapagoensis* and *N. swarthi*, their diversification occurred on Santa Fé and Santiago Islands, respectively, and their speciation likely occurred within their current range. However, for the species inhabiting Fernandina Island, *N. narboroughi* and *N. fernandinae*, speciation and diversification likely occurred on a different island, because Fernandina Island is younger than these events. We hypothesize that after speciation on a different island, they dispersed to Fernandina Island. Isabela Island is the largest within the archipelago, and it is close to Fernandina. Its emergence is dated to around 500,000 to 800,000 years ago, and it is therefore possible that diversification of both species occurred there. However, we dated speciation events older than this emergence. Speciation of *Nesoryzomys* likely occurred on islands that emerged at least between 1.5 to 2.3 Ma. It is difficult to know where it occurred because it is recognized that volcanic islands leave little evidence of their ancient biological past, because the lava flows consume organic matter (Steadman 1986). Thus, species may have gone through events of colonization and speciation and subsequently been driven to extinction, leaving no sign (Merlen 2014). Geist *et al.* (2014) suggested that because the Galápagos archipelago is dynamic over evolutionary timescales, for any model of dispersal, colonization, speciation, and radiation involving island geography more than 20,000 years ago, the current map of the Galápagos Islands is close to irrelevant. Despite these problems, we suggest that future studies using genome-wide molecular markers and sampling of extinct species and fossils from the islands, as well as additional continental species, will add further insights into the origin and relationships of the endemic rodents of the Galápagos Islands, which remain ambiguous.

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Appendix 1

List of individuals used in this study, indicating ID (identity) manuscript, ID collection, genus, species, name of the island, locality (if it was available), sex, kind of tissue (L – Liver, K – Kidney, H – Heart, E – Ear, S – Swab, and F – Feces), and GenBank accession number data.

ID manuscript	Collection Number (ASNHC)	Genus	Species	Island	Locality	Sex	Tissue	GenBank accession numbers
B003SF_*A. galapagoensis*	ASK 4110	*Aegialomys*	*galapagoensis*	Santa Fé		M	-	MN398993
B005SF_*A. galapagoensis*	ASK 5643	*Aegialomys*	*galapagoensis*	Santa Fé		F	L	MN398994
B006SF_*A. galapagoensis*	ASK 5644	*Aegialomys*	*galapagoensis*	Santa Fé		F	L	MN398995
B036SF_*A. galapagoensis*	ASK 4106	*Aegialomys*	*galapagoensis*	Santa Fé		M	KL	MN398996
B038SF_*A. galapagoensis*	ASK 4111	*Aegialomys*	*galapagoensis*	Santa Fé		F	-	MN398997
B039SF_*A. galapagoensis*	ASK 5601	*Aegialomys*	*galapagoensis*	Santa Fé		M	L	MN398998
B041SF_*A. galapagoensis*	ASK 5603	*Aegialomys*	*galapagoensis*	Santa Fé		M	L	MN398999
B042SF_*A. galapagoensis*	ASK 5604	*Aegialomys*	*galapagoensis*	Santa Fé		F	L	MN399000
B043SF_*A. galapagoensis*	ASK 5594	*Aegialomys*	*galapagoensis*	Santa Fé		-	L	MN399001
B044SF_*A. galapagoensis*	ASK 5595	*Aegialomys*	*galapagoensis*	Santa Fé		F	L	MN399002
B046SF_*A. galapagoensis*	ASK 5597	*Aegialomys*	*galapagoensis*	Santa Fé		F	L	MN399003
B047SF_*A. galapagoensis*	ASK 5598	*Aegialomys*	*galapagoensis*	Santa Fé		-	L	MN399004
B048SF_*A. galapagoensis*	ASK 5599	*Aegialomys*	*galapagoensis*	Santa Fé		M	L	MN399005
B049SF_*A. galapagoensis*	ASK 5600	*Aegialomys*	*galapagoensis*	Santa Fé		F	L	MN399006
B050SF_*A. galapagoensis*	ASK 5593	*Aegialomys*	*galapagoensis*	Santa Fé		M	L	MN399007
B199SF_*A. galapagoensis*	SF-01	*Aegialomys*	*galapagoensis*	Santa Fé		F	ES	MN399008
B200SF_*A. galapagoensis*	SF-02	*Aegialomys*	*galapagoensis*	Santa Fé		F	EF	MN399009
B201SF_*A. galapagoensis*	SF-03	*Aegialomys*	*galapagoensis*	Santa Fé		F	EF	MN399010
B202SF_*A. galapagoensis*	SF-04	*Aegialomys*	*galapagoensis*	Santa Fé		M	EF	MN399011
B203SF_*A. galapagoensis*	SF-05	*Aegialomys*	*galapagoensis*	Santa Fé		F	EF	MN399012
B206SF_*A. galapagoensis*	SF-08	*Aegialomys*	*galapagoensis*	Santa Fé		F	EF	MN399013
B207SF_*A. galapagoensis*	SF-09	*Aegialomys*	*galapagoensis*	Santa Fé		F	EF	MN399014
B208SF_*A. galapagoensis*	SF-10	*Aegialomys*	*galapagoensis*	Santa Fé		M	EF	MN399015
B209SF_*A. galapagoensis*	SF-11	*Aegialomys*	*galapagoensis*	Santa Fé		M	EF	MN399016
B210SF_*A. galapagoensis*	SF-12	*Aegialomys*	*galapagoensis*	Santa Fé		F	EF	MN399017
B211SF_*A. galapagoensis*	SF-13	*Aegialomys*	*galapagoensis*	Santa Fé		-	F	MN399018
B215SF_*A. galapagoensis*	SF-17	*Aegialomys*	*galapagoensis*	Santa Fé		M	ES	MN399019
B217SF_*A. galapagoensis*	SF-19	*Aegialomys*	*galapagoensis*	Santa Fé		F	ES	MN399020
B218SF_*A. galapagoensis*	SF-20	*Aegialomys*	*galapagoensis*	Santa Fé		F	ES	MN399021
B219SF_*A. galapagoensis*	SF-21	*Aegialomys*	*galapagoensis*	Santa Fé		F	ES	MN399022
B220SF_*A. galapagoensis*	SF-22	*Aegialomys*	*galapagoensis*	Santa Fé		M	ES	MN399023
B221SF_*A. galapagoensis*	SF-23	*Aegialomys*	*galapagoensis*	Santa Fé		F	ES	MN399024
B222SF_*A. galapagoensis*	SF-24	*Aegialomys*	*galapagoensis*	Santa Fé		F	ES	MN399025
B223SF_*A. galapagoensis*	SF-25	*Aegialomys*	*galapagoensis*	Santa Fé		F	ES	MN399026
B224SF_*A. galapagoensis*	SF-26	*Aegialomys*	*galapagoensis*	Santa Fé		M	ES	MN399027
B225SF_*A. galapagoensis*	SF-27	*Aegialomys*	*galapagoensis*	Santa Fé		M	ES	MN399028
B226SF_*A. galapagoensis*	SF-28	*Aegialomys*	*galapagoensis*	Santa Fé		F	ES	MN399029
B227SF_*A. galapagoensis*	SF-29	*Aegialomys*	*galapagoensis*	Santa Fé		M	ES	MN399030
B231SF_*A. galapagoensis*	SF-33	*Aegialomys*	*galapagoensis*	Santa Fé		M	ES	MN399031
B232SF_*A. galapagoensis*	SF-34	*Aegialomys*	*galapagoensis*	Santa Fé		F	ES	MN399032
B234SF_*A. galapagoensis*	SF-36	*Aegialomys*	*galapagoensis*	Santa Fé		M	ES	MN399033
B235SF_*A. galapagoensis*	SF-37	*Aegialomys*	*galapagoensis*	Santa Fé		M	ES	MN399034
B236SF_*A. galapagoensis*	SF-38	*Aegialomys*	*galapagoensis*	Santa Fé		F	E	MN399035
MVZ145539_*A. xanthaeolus*	MVZ145539	*Aegialomys*	*xanthaeolus*	Ecuador		-	L	MN399060
MVZ145540_A. xanthaeolus	MVZ145540	*Aegialomys*	*xanthaeolus*	Ecuador		-	L	MN399061
N018FCD_*N. narboroughi*	ASK 5520	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	HKL	MN399062

N019FCD_*N. narboroughi*	ASK 5521	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	HKL	MN399063
N020FCD_*N. narboroughi*	ASK 5523	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	HKL	MN399064
N021FPE_*N. narboroughi*	ASK 5513	*Nesoryzomys*	*narboroughi*	Fernandina	Punta spinoza	M	HKL	MN399065
N022FPE_*N. narboroughi*	ASK 5514	*Nesoryzomys*	*narboroughi*	Fernandina	Punta spinoza	M	HKL	MN399066
N023FPE_*N. narboroughi*	ASK 5515	*Nesoryzomys*	*narboroughi*	Fernandina	Punta spinoza	F	HKL	MN399067
N024FPE_*N. narboroughi*	ASK 5517	*Nesoryzomys*	*narboroughi*	Fernandina	Punta spinoza	F	HKL	MN399068
N065F_*N. narboroughi*	ASK 7276	*Nesoryzomys*	*narboroughi*	Fernandina	Crater Rim	M	KL	MN399069
N066F_*N. narboroughi*	ASK 7282	*Nesoryzomys*	*narboroughi*	Fernandina	Crater Rim	M	KL	MN399070
N102FCR_*N. narboroughi*	FE-V08	*Nesoryzomys*	*narboroughi*	Fernandina	Crater Rim	F	EF	MN399071
N104FCD_*N. narboroughi*	FE-C02	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	ES	MN399072
N105FCD_*N. narboroughi*	FE-C03	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	ES	MN399073
N106FCD_*N. narboroughi*	FE-C04	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	ES	MN399074
N107FCD_*N. narboroughi*	FE-C05	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	ES	MN399075
N108FCD_*N. narboroughi*	FE-C06	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	ES	MN399076
N109FCD_*N. narboroughi*	FE-C07	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	ES	MN399077
N110FCD_*N. narboroughi*	FE-C08	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	ES	MN399078
N112FCD_*N. narboroughi*	FE-C11	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399079
N113FCD_*N. narboroughi*	FE-C12	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	ESF	MN399080
N114FCD_*N. narboroughi*	FE-C13	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399081
N115FCD_*N. narboroughi*	FE-C14	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399082
N116FCD_*N. narboroughi*	FE-C15	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399083
N118FCD_*N. narboroughi*	FE-C17	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	ESF	MN399084
N119FCD_*N. narboroughi*	FE-C18	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	ESF	MN399085
N120FCD_*N. narboroughi*	FE-C19	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399086
N122FCD_*N. narboroughi*	FE-C21	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399087
N124FCD_*N. narboroughi*	FE-C23	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	ESF	MN399088
N126FCD_*N. narboroughi*	FE-C26	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	ES	MN399089
N127FCD_*N. narboroughi*	FE-C27	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	ES	MN399090
N129FCD_*N. narboroughi*	FE-C30	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399091
N130FCD_*N. narboroughi*	FE-C31	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399092
N131FCD_*N. narboroughi*	FE-C33	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399093
N132FCD_*N. narboroughi*	FE-C34	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399094
N133FCD_*N. narboroughi*	FE-C35	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399095
N134FCD_*N. narboroughi*	FE-C36	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399096
N135FCD_*N. narboroughi*	FE-C37	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399097
N136FCD_*N. narboroughi*	FE-C38	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399098
N137FCD_*N. narboroughi*	FE-C39	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399099
N140FCD_*N. narboroughi*	FE-C42	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399100
N141FCD_*N. narboroughi*	FE-C43	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399101
N143FCD_*N. narboroughi*	FE-C45	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399102
N144FCD_*N. narboroughi*	FE-C46	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399103
N145FCD_*N. narboroughi*	FE-C48	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399104
N146FCD_*N. narboroughi*	FE-C49	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399105
N147FCD_*N. narboroughi*	FE-C50	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399106
N148FCD_*N. narboroughi*	FE-C51	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399107
N149FCD_*N. narboroughi*	FE-C53	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399108
N150FCD_*N. narboroughi*	FE-C54	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	M	EF	MN399109
N151FCD_*N. narboroughi*	FE-C55	*Nesoryzomys*	*narboroughi*	Fernandina	Cabo Douglas	F	EF	MN399110
S002SLB_*N. swarthi*	ASK 5509	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	HKL	MN399111
S007SLB_*N. swarthi*	ASK 4093	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	F	KL	MN399112
S009SLB_*N. swarthi*	ASK 4094	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	KL	MN399113
S025SLB_*N. swarthi*	ASK 5501	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	HKL	MN399114
S026SLB_*N. swarthi*	ASK 5502	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	F	HKL	MN399115

S028SLB_*N. swarthi*	ASK 5505	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	F	HKL	MN399116
S029SLB_*N. swarthi*	ASK 5506	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	HKL	MN399117
S030SLB_*N. swarthi*	ASK 5511	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	F	HKL	MN399118
S051SLB_*N. swarthi*	ASK 5508	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	HKL	MN399119
S060SLB_*N. swarthi*	ASK 5500	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	F	HKL	MN399120
S152SELB_*N. swarthi*	SN-B01	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	M	ES	MN399121
S154SELB_*N. swarthi*	SN-B03	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	M	ES	MN399122
S155SELB_*N. swarthi*	SN-B04	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	F	ES	MN399123
S156SELB_*N. swarthi*	SN-B05	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	M	ES	MN399124
S157SELB_*N. swarthi*	SN-B06	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	F	ES	MN399125
S158SELB_*N. swarthi*	SN-B07	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	F	ES	MN399126
S159SELB_*N. swarthi*	SN-B08	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	M	ES	MN399127
S160SELB_*N. swarthi*	SN-B09	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	M	ES	MN399128
S161SELB_*N. swarthi*	SN-B10	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	F	ES	MN399129
S162SELB_*N. swarthi*	SN-B11	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	M	ES	MN399130
S163SELB_*N. swarthi*	SN-B12	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	F	ES	MN399131
S164SELB_*N. swarthi*	SN-B13	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	F	ES	MN399132
S165SELB_*N. swarthi*	SN-B14	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	F	ES	MN399133
S166SELB_*N. swarthi*	SN-B15	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	M	ES	MN399134
S167SELB_*N. swarthi*	SN-B16	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	M	ES	MN399135
S168SELB_*N. swarthi*	SN-B17	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	M	ES	MN399136
S169SELB_*N. swarthi*	SN-B18	*Nesoryzomys*	*swarthi*	Santiago	Beach east of La Bomba	F	ES	MN399137
S170SLB_*N. swarthi*	SN-LB01	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	F	ES	MN399138
S173SLB_*N. swarthi*	SN-LB04	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	F	ES	MN399139
S174SLB_*N. swarthi*	SN-LB05	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	ES	MN399140
S175SLB_*N. swarthi*	SN-LB06	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	F	ES	MN399141
S176SLB_*N. swarthi*	SN-LB07	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	ES	MN399142
S177SLB_*N. swarthi*	SN-LB08	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	F	ES	MN399143
S179SLB_*N. swarthi*	SN-LB10	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	EF	MN399144
S181SLB_*N. swarthi*	SN-LB12	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	EF	MN399145
S183SLB_*N. swarthi*	SN-LB14	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	EF	MN399146
S184SLB_*N. swarthi*	SN-LB15	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	E	MN399147
S185SLB_*N. swarthi*	SN-LB16	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	EF	MN399148
S186SLB_*N. swarthi*	SN-LB17	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	F	EF	MN399149
S187SLB_*N. swarthi*	SN-LB18	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	F	EF	MN399150
S188SLB_*N. swarthi*	SN-LB19	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	EF	MN399151
S189SLB_*N. swarthi*	SN-LB20	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	EF	MN399152
S191SLB_*N. swarthi*	SN-LB22	*Nesoryzomys*	*swarthi*	Santiago	La Bomba	M	EF	MN399153
F010FCD_*N. fernandinae*	ASK 5567	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	F	L	MN399036
F011FCD_*N. fernandinae*	ASK 5568	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	M	L	MN399037
F012FCD_*N. fernandinae*	ASK 5569	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	M	L	MN399038
F013FCD_*N. fernandinae*	ASK 5571	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	F	L	MN399039
F014FCD_*N. fernandinae*	ASK 5572	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	M	L	MN399040
F015FCD_*N. fernandinae*	ASK 5562	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	F	L	MN399041
F016FCD_*N. fernandinae*	ASK 5563	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	F	L	MN399042
F017FCD_*N. fernandinae*	ASK 5566	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	F	L	MN399043
F083FCR_*N. fernandinae*	FE-V05	*Nesoryzomys*	*fernandinae*	Fernandina	Crater Rim	M	ESF	MN399044
F084FCR_*N. fernandinae*	FE-V06	*Nesoryzomys*	*fernandinae*	Fernandina	Crater Rim	M	ESF	MN399045
F085FCR_*N. fernandinae*	FE-V07	*Nesoryzomys*	*fernandinae*	Fernandina	Crater Rim	F	ESF	MN399046
F086FCR_*N. fernandinae*	FE-V09	*Nesoryzomys*	*fernandinae*	Fernandina	Crater Rim	M	ESF	MN399047
F087FCR_*N. fernandinae*	FE-V10	*Nesoryzomys*	*fernandinae*	Fernandina	Crater Rim	F	ESF	MN399048
F088FCR_*N. fernandinae*	FE-V11	*Nesoryzomys*	*fernandinae*	Fernandina	Crater Rim	M	ESF	MN399049
F089FCR_*N. fernandinae*	FE-V12	*Nesoryzomys*	*fernandinae*	Fernandina	Crater Rim	M	ESF	MN399050

F090FCR_*N. fernandinae*	FE-V13	*Nesoryzomys*	*fernandinae*	Fernandina	Crater Rim	M	ESF	MN399051
F092FCR_*N. fernandinae*	FE-V15	*Nesoryzomys*	*fernandinae*	Fernandina	Crater Rim	M	ESF	MN399052
F093FCR_*N. fernandinae*	FE-V16	*Nesoryzomys*	*fernandinae*	Fernandina	Crater Rim	F	ESF	MN399053
F095FCR_*N. fernandinae*	FE-V18	*Nesoryzomys*	*fernandinae*	Fernandina	Crater Rim	F	EF	MN399054
F096FCD_*N. fernandinae*	FE-C09	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	M	EF	MN399055
F097FCD_*N. fernandinae*	FE-C24	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	M	EF	MN399056
F098FCD_*N. fernandinae*	FE-C29	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	M	EF	MN399057
F099FCD_*N. fernandinae*	FE-C32	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	M	EF	MN399058
F101FCD_*N. fernandinae*	FE-C52	*Nesoryzomys*	*fernandinae*	Fernandina	Cabo Douglas	M	EF	MN399059