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A phylogenetic analysis of *Neotoma varia* (Rodentia: Cricetidae), a rediscovered, endemic, and threatened rodent from Datil Island, Sonora, Mexico

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Abstract

The systematics of the rediscovered and threatened rodent, *Neotoma varia*, from Datil Island in the Gulf of California, was assessed using sequences from the mitochondrial cytochrome *b* gene (Cyt *b*) regarding specimens of *N. albigula* from Tiburon Island and populations on the mainland off Datil Island. *Neotoma varia* was originally described as a species and subsequently considered a subspecies, relegated to subspecific status based on morphologic characters and few specimens; no genetic analyses have been published. Bayesian inference, maximum-parsimony, maximum-likelihood, and distance optimality criteria based on 828-bp of the Cyt *b* gene from individuals representing 11 populations, converged on essentially identical tree topologies, consistent with the inclusion of *N. varia* within *N. albigula*. The population of Datil Island is related to specimens from Tiburon Island and the adjacent mainland populations showing low levels of genetic differentiation with other subspecies of *N. albigula* (0.2–1.4%). Previous morphologic analyses indicated inconstancy in characters regarding the holotype; however, *N. varia* is morphologically different in the oclusal view of the upper molars. Under these conditions, we consider *N. varia* as a subspecies of *N. albigula*. *N. a. varia* has a very specific habitat and is present only on a very small part of the island; in spite of low divergence regarding other *N. albigula* subspecies, *N. a. varia* possesses a genetic identity and needs to be considered as a critically endangered population.

Key words: *albigula*, cytochrome *b*, islands, subspecies, *varia*

Introduction

Neotoma varia Burt, has been considered an endemic rodent species of Datil Island (also known as Turner Island), west-facing the coast of Sonora, Mexico (28.7204°N, 112.2934°W). Datil Island, which is just 1.7 km south of the large Tiburon Island in the Gulf of California, has an area of 4 km^2. *Neotoma varia* was originally described as a species (Burt 1932) based on a single specimen. The characteristics used for the description were the shape of the maxillary tooth rows, pattern of third upper molar, and form of the skull. However, a morphometric analysis of the skull of three adult specimens concluded that the specimens from Datil Island could be a subspecies of *N. albigula* (Bogan 1997), rather than a different species (Hall 1981; Lawlor 1983). The taxonomic revisions of the *Neotoma albigula* species group (Hall & Genoways 1970; Edwards *et al*. 2001) did not include *N. varia* specimens; however Hall and Genoways (1970) inferred that *N. varia* and *N. albigula* genetically are more closely related than to any other species because of their resemblance. On the other hand, Edwards *et al*. (2001) only examined DNA sequence data (cytochrome *b* gene) of the populations in the mainland and *N. varia* and *N. a. seri* were not included.

The Turner Island woodrat rat *N. varia* has been named an enigmatic putative species (Bogan 1997) because of the limited number of specimens known (only four), in spite of several collection expeditions (Bogan 1997; Álvarez-Castañeda & Ortega-Rubio 2003; Álvarez-Castañeda *et al*. in press). Consequently,

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this woodrat was considered close to extinction (Álvarez-Castañeda & Ortega-Rubio 2003; Álvarez-Castañeda *et al*. 2006).

In May 2008, a team from Centro de Investigaciones Biológicas del Noroeste (CIB) and personnel of the Mexican wildlife reserve service made a survey specifically for research on the presence of *Neotoma* on Datil Island. They collected five live specimens of *Neotoma*; only one male was removed from the island. Álvarez-Castañeda *et al*. (in press) indicated that the *N. varia* specimens are only present on a very small part of the island and that the *Neotoma* population on Datil Island needs to be considered as "critically endangered." However, at present, *N. varia* is considered an endemic threatened species under Mexican law (SEMARNAT 2002).

Analyses of cytochrome *b* (Cyt *b)*, cytochrome oxidasa subunit I (COI), and subunit III (COIII) genes, in the mitochondrial DNA of rodents and other vertebrates from the Baja California Peninsula support the hypothesis that some of the island populations of many species are not different from these species on the mainland, e.g. *Ammospermophilus insularis* (Álvarez-Castañeda 2007), *Dipodomys margaritae* and *D. insularis* (Álvarez-Castañeda *et al*. 2009), *Peromyscus guardia, P. interparietalis*, and *P. dickeyi* (Hafner *et al*. 2001). Therefore, it would be very useful to understand the phylogenetic relationships of *N. varia* with the mainland forms to determine its taxonomic status and implement conservation and management recommendations.

We proposed examining the genetic relationships and tooth morphology of specimens of *Neotoma* on Datil Island with specimens of *N. albigula* from Tiburon Island and populations on the mainland off Datil Island. Bogan's analysis (1997) showed that the *N. varia* specimens were within the variation of *N. albigula* subspecies. Thus, we can expect that the genetic characters of *N. varia* will be within the genetic interval variation of *N. albigula* with a similar pattern as displayed in their morphology.

Methods

Samples. A dataset of 828 base pairs (bp) of the mitochondrial Cyt *b* gene was analyzed for 19 individuals from 11 populations, including the Datil Island specimen of *N. varia* captured in 2008 (Álvarez-Castañeda *et al*. in press) and specimens of *N. albigula* from Tiburon Island and populations on the mainland off Datil Island (Figure 1; Table 1). The specimens used in this study are deposited in the mammal collection of the Centro de Investigaciones Biológicas del Noroeste (CIB). Additional sequences of Cyt *b* from GenBank for *N. albigula* (DQ179858) and *N. leucodon* (DQ179815) were included in the analyses; their localities are unknown.

Laboratory procedures. Genomic DNA was extracted in the laboratory from liver tissues preserved in 95% ethanol using the DNeasy kit (QIAGEN, Inc., Valencia, CA). Primers MVZ05 and MVZ16 (Smith 1998) were used to amplify an ~800 bp fragment of the Cyt *b* gene. Polymerase chain reaction, purification, sequencing, and alignment protocols were performed as described in Álvarez-Castañeda *et al*. (2009). Amplified products were sequenced at Macrogen, Inc. (Seul Korea). Representative haplotypes generated for this study have been deposited in GenBank (accession numbers: HQ328506-HQ328520, Table 1).

Mitochondrial DNA analysis. Representative and non-redundant haplotypes were analyzed with software (Collapse ver. 1.1; Posada 2004). The 828-bp fragment of the Cyt *b* gene of each population was used for the phylogenetic methods. Phylogenetic analyses under the maximum-parsimony, maximum-likelihood, and distance-optimality criterion were performed using PAUP 4.0b10 (Swofford 2002). Additionally, Bayesian inference was performed using MrBayes 3.0b4 (Ronquist & Huelsenbeck 2003). The *N. leucodon* sequence was included as the ingroup because it is within the *albigula* group (Edwards *et al*. 2001). Sequences obtained from GenBank for *N. mexicana* (AF294346), *N. picta* (DQ179851), and *N. isthmica* (DQ179828) were included as an outgroup.

Maximum-parsimony analyses, with all the characters equally weighted, and heuristic searches with 1,000 random addition sequences were conducted, implementing the tree bisection reconnection (TBR) algorithm for branch swapping. For the maximum-likelihood analysis, the most appropriate evolution model for the data

was selected using Modeltest 3.06 (Posada & Crandall 1998) with Akaike Information Criterion (AIC). The GTR+I model (GTR+I) was also selected as the best-fit model of nucleotide substitution. This model was then used for maximum-likelihood searches consisting of 100 random replicates with TBR branch swapping. Under the distance criterion, phylogeny was estimated using the neighbor-joining algorithm. The support of the nodes was assessed with bootstrap analyses, including a fast heuristic procedure with 1,000 pseudo-replicates, random addition sequences. Only bootstrap values ≥50% are shown in the trees.

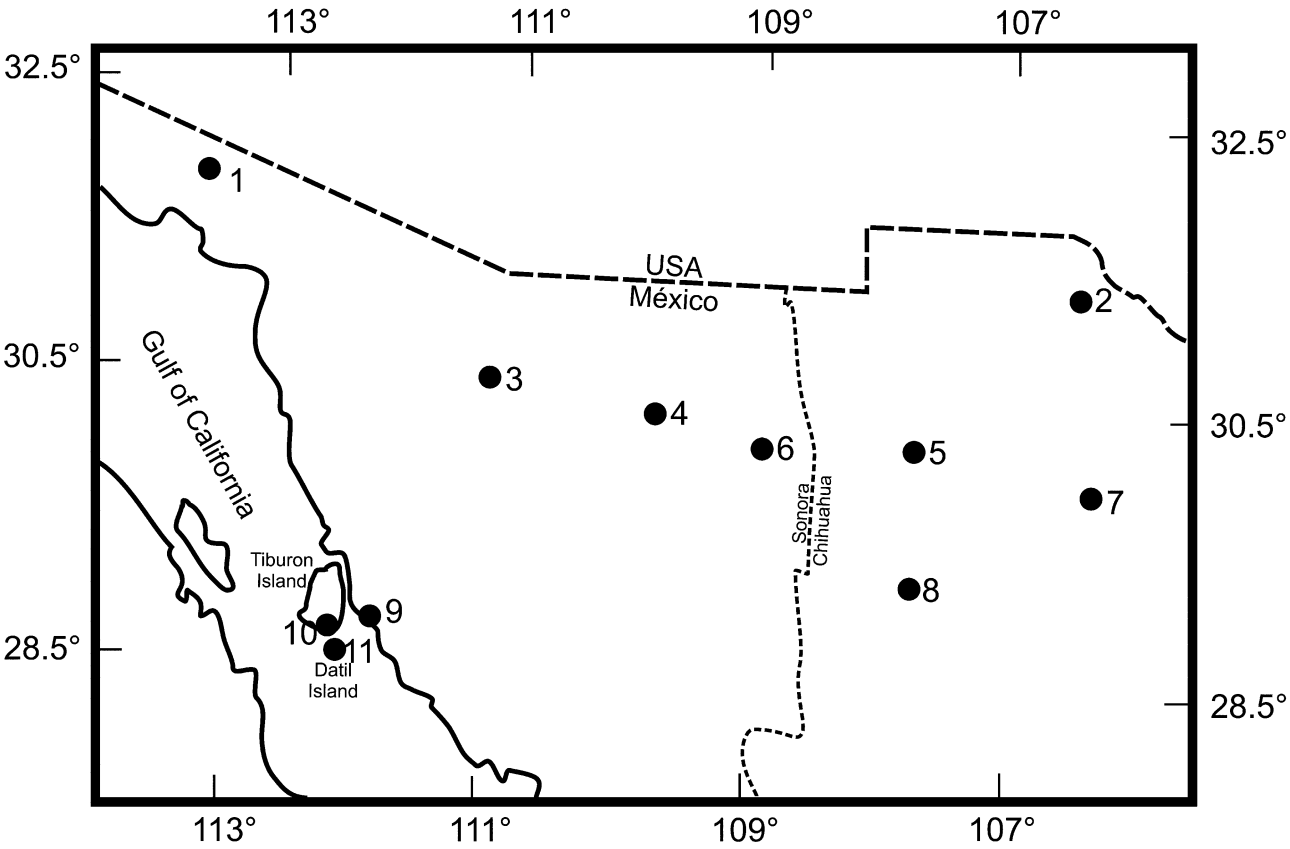

FIGURE 1. Localities for *Neotoma varia* and *N. albigula* specimens examined in this study. The numbers correspond to specific localities in Table 1.

Genetic distances were calculated using the Jukes-Cantor model. To make comparisons with the amount of percent sequence divergence values from previous studies (Bradley and Baker 2001), we used the Kimura 2-parameter (K2P) model too. Genetic distance matrix among individuals was used to generate a neighbor-joining tree and the support for nodes was assessed with bootstrap analyses.

A Bayesian phylogenetic analysis was performed using the GTR+I distance model selected by MrModeltest v2 (Nylander 2004) and by implementing the Metropolis Coupled Markov Chain Monte Carlo simulation. Four separate runs were performed, starting from a random tree with four simultaneous chains. Each run was conducted with 5 million generations and sampled at intervals of 1,000 generations. The first 500 samples of each run were discarded as burn-in, and the remaining topologies were used to calculate posterior probabilities from the 50% majority-rule consensus trees. Determination of a stationary condition was evaluated by plotting the log of the likelihood score of sample points against generation.

Morphological analyses.

To determine characters useful in differentiating among species and subspecies, we analyzed the morphological pattern of the upper molars among the specimens of *N. varia* (n = 1) and specimens of the four subspecies of *N. albigula* geographically closest to Datil Island: *N. a. albigula* from central mainland Sonora (n = 60), *N. a. melanura* from the southern part of the coast in Sonora (n = 5), *N. a. seri* from Tiburon Island (n = 57), and *N. a. venusta* from the northern part of the coast in Sonora (n = 11).

TABLE 1. Localities (n = 11) of individuals analyzed. Nomenclature follows Álvarez-Castañeda and Cortés-Calva (1999). The first columns indicate the locality (L), haplotype number (H), and state (ST). All latitudes (Lat) are north and longitudes (Long) are west. *data will be sent to GenBank during the evaluation of the manuscript.

L	H	ST	Locality	Nomenclature	Lat.	Long.	Specimen voucher (CIB)	GenBank
1	H1	Son	Tanque Papagos, El Pinacate	*N. a. sheldoni*	31.9314	-113.6042	4536, 4538	HQ3 8506
1	H2	Son	Tanque Papagos, El Pinacate	*N. a. sheldoni*	31.9314	-113.6042	4558	HQ328507
2	H3	Chih	1 km E Samalayuca	*N. a. albigula*	31.3309	-106.4691	14432	HQ328508
3	H4	Son	Santa Ana	*N. a. venusta*	30.5828	-111.1575	4539, 4540	HQ328509
3	H5	Son	Santa Ana	*N. a. venusta*	30.5828	-111.1575	4542	HQ328510
4	H6	Son	16 km E Chinapa	*N. a. albigula*	30.4224	-109.8588	14443	HQ328511
4	H7	Son	16 km E Chinapa	*N. a. albigula*	30.4224	-109.8588	14444	HQ328512
5	H8	Chih	10 km S, 7 km E Casas Grandes	*N. a. albigula*	30.2581	-107.7672	4546	HQ328513
6	H7	Son	2 km NW La Huachinera	*N. a. albigula*	30.2171	-108.9742	14445	HQ328514
7	H9	Chih	11 km NE El Sueco	*N. a. albigula*	29.9726	-106.3252	14458	HQ328515
8	H8	Chih	30 km S Gómez Farías	*N. a. albigula*	29.3094	-107.7847	4551	HQ328516
9	H10	Son	Bahía Kino	*N. a. melanura*	28.8609	-111.9863	14467, 14468	HQ328517
10	H11	Son	Isla Tiburón, W punta monumento	*N. a. seri*	28.7539	-112.3028	1574	HQ328518
10	H12	Son	Isla Tiburón, W punta monumento	*N. a. seri*	28.7539	-112.3028	1572, 1576	HQ328519
11	H13	Son	Isla Datil	*N. varia*	28.7286	-112.2961	14472	HQ328520
	A			*N. albigula*				DQ179858
	B			*N. leucodon*				DQ179815
	Outgroup							
	C			*N. mexicana*				AF294346
	D			*N. picta*				DQ179851
	E			*N. isthmica*				DQ179828

Results

Genetic variation

We obtained sequence data for 828-bp of the Cyt *b* gene from 19 individuals from 11 localities, including representative samples of *Neotoma varia*, *N. albigula seri*, *N. a. melanura*, *N. a. venusta*, *N. a. sheldoni*, and *N. a. albigula* (Table 1). Average base composition was A = 31.9%, C = 28.5%, G = 12.7%, and T = 26.7; with 19 (2.2%) polymorphic sites, four of them were non-synonymous (with aminoacid replacement); 14 (1.6%) transitions, 5 (0.6%) transversions, and 13 unique haplotypes identified (Table 1). Gene diversity (*h*) was 0.9649 ± 0.0242, nucleotide diversity was 0.0065 ± 0.0036, and the mean number of pairwise differences was 5.4269 ± 2.7346.

Phylogenetic analysis

The maximum-parsimony analysis yielded 144 trees of equal length (length = 252, CI = 0.813, RI = 0.797), and a majority-rule consensus tree was constructed. The best-fit model of nucleotide substitution was the GTR+I (A = 0.315, C = 0.294, G = 0.135, and T = 0.255, –lnL = 2474.61, K = 9, and the proportion of invariable sites = 0.6201). The maximum-likelihood analysis with the GTR+I evolution model produced one tree (score = 2305.11, Figure 2a). Both trees have similar topology (MP tree not shown). Neighbor-joining analysis showed similar results to those obtained under the maximum-likelihood criteria and maximum-parsimony (Figure 2b). Nevertheless, in this tree we observed that the substitution sites are very few among haplotypes in the ingroup. Additionally, Bayesian inference (4 replicates) converged on identical tree topologies (Figure 2c). These trees showed *Neotoma varia* within *N. albigula*, all samples in a monophyletic group (100% support). *Neotoma albigula* showed four mayor branches united in a polytomy. The only sole subspecies recovered as monophyletic is *N. a. sheldoni.* The haplotypes representing the other subspecies are scattered throughout the remaining branches. The subclade in which is *varia* (H12) has 100% support, but with a common polytomy with haplotypes from Tiburon Island (*N. a. seri*, H13) and another from mainland (*N. a. venusta*, H4).

The genetic distances obtained by using the Jukes-Cantor model and Kimura 2 parameters are shown in Table 2. The percentage of sequence divergence between the *varia* haplotype and the Tiburon Island haplotypes was lower (0.2–0.4%) than for the others subspecies and localities (Table 2). Nevertheless, genetic distances were low among all populations and subspecies. The genetic distance between *varia* and the nearest mainland population (*N. a. melanura*) is 0.7%, whereas, divergence between *varia* and the geographically farthest *albigula* subspecies is 0.9%.

The specimen of Datil Island does not have the diagnostic characteristics used to describe *N. varia* (Burt 1932; Bogan 1997); however, it is morphologically different in the oclusal view of the upper molars of the four subspecies of *N. albigula* that were examined.

Discussion

There is low genetic divergence in mitochondrial DNA among the examined specimens. Only 2.2% of the dataset of 828 bp of Cyt *b* are polymorphic sites, 0.6% are transversions. This is reflected in the close relationships among subspecies in the phylogenetic trees. The maximum-parsimony, maximum-likelihood, distance-optimality, and Bayesian inference analyses converged on essentially identical tree topologies. All analyses were consistent in placing the Datil Island specimen (*N. varia*) within *N. albigula*, in agreement with Bogan (1997). Moreover, all analyses placed this Datil Island sample in a branch with Tiburon Island (*N. a. seri*) and Santa Ana (*N. a. venusta*, more than 200 km inland in Sonora) haplotypes; at the same time, this branch is related to Bahía Kino (*N. a. melanura*) and Chinapa (*N. a. albigula*) haplotypes. If *N. varia* were a different species, we might expect that it would be as different as *N. leucodon*, a species previously considered as a subspecies of *N. albigula* (Edwards *et al.* 2001). Our results showed the opposite.

TABLE 2. Genetic distances (%) among representative haplotypes (828-bp) of *Neotoma varia, N. albigula, N. leucodon*, and outgroup specimens. The upper-right portion of the matrix was generated with the Jukes-Cantor model of evolution. Distances in the lower-left matrix were generated with the Kimura 2 parameter model to compare traditional estimates of genetic distance in small mammals. The *N. albigula* samples (subspecies) are arranged from the geographically closest to Datil Island.

Taxa	Haplotype (or sample)	H13	H12	H11	H10	H4	H5	H2	H1	H6	H7	H8	H9	H3	A	B	C	D	E
N. varia	H13	**0**	0.2	0.4	0.7	0.6	0.8	0.9	1.2	0.9	0.7	0.7	0.8	0.9	1.4	11.3	14.0	13.6	14.6
N. a. seri	H12	0.2	**0**	0.4	0.4	0.3	0.8	0.9	1.2	0.7	0.7	0.7	0.8	0.9	1.4	11.3	13.9	13.6	14.4
	H11	0.5	0.5	**0**	0.2	0.8	0.3	0.4	0.7	0.4	0.2	0.2	0.3	0.4	0.9	10.9	13.6	13.1	14.1
N. a. melanura	H10	0.7	0.5	0.2	**0**	0.8	0.6	0.7	0.9	0.4	0.4	0.4	0.6	0.7	1.1	11.2	13.4	13.1	14.0
N. a. venusta	H4	0.6	0.3	0.8	0.8	**0**	1.2	1.3	1.5	0.6	0.8	1.1	1.2	1.3	1.8	11.7	13.9	13.9	14.4
	H5	0.8	0.8	0.3	0.6	1.2	**0**	0.3	0.6	0.8	0.3	0.1	0.2	0.1	0.4	10.7	13.4	13.0	14.0
N. a. sheldoni	H2	0.9	1.0	0.5	0.7	1.3	0.3	**0**	0.2	0.9	0.4	0.2	0.3	0.4	0.8	10.6	13.3	12.9	14.2
	H1	1.2	1.2	0.7	1.0	1.6	0.6	0.2	**0**	1.2	0.7	0.4	0.6	0.7	1.1	10.9	13.5	13.0	14.3
N. a. albigula	H6	1.0	0.7	0.5	0.5	0.6	0.8	1.0	1.2	**0**	0.4	0.7	0.8	0.9	1.4	11.4	13.8	13.6	14.4
	H7	0.7	0.7	0.2	0.5	0.8	0.3	0.5	0.7	0.5	**0**	0.2	0.3	0.4	0.9	10.9	13.4	13.0	14.0
	H8	0.7	0.7	0.2	0.5	1.1	0.1	0.2	0.5	0.7	0.2	**0**	0.1	0.2	0.7	10.6	13.3	12.8	13.8
	H9	0.8	0.8	0.3	0.6	1.2	0.2	0.3	0.6	0.8	0.3	0.1	**0**	0.3	0.8	10.4	13.1	12.7	13.7
	H3	1.0	1.0	0.5	0.7	1.3	0.1	0.5	0.7	1.0	0.5	0.2	0.3	**0**	0.6	10.9	13.3	13.1	13.9
N. albigula ssp	A	1.5	1.5	1.0	1.1	1.8	0.5	0.8	1.1	1.5	1.0	0.6	0.8	0.6	**0**	11.1	14.1	13.4	14.1
N. leucodon	B	11.5	11.5	11.0	11.4	11.9	10.9	10.8	11.2	11.6	11.0	10.8	10.6	11.1	11.3	**0**	10.7	11.6	12.1
Outgroup																			
N. isthmica	C	14.3	14.1	13.9	13.7	14.1	13.7	13.6	13.7	14.1	13.7	13.6	13.4	13.6	13.7	11.8	**0**	10.0	7.7
N. mexicana	D	13.8	13.8	13.4	13.4	14.1	13.3	13.1	13.3	13.8	13.2	13.1	12.9	13.4	14.5	12.4	10.4	**0**	9.9
N. picta	E	14.9	14.7	14.5	14.3	14.7	14.3	14.5	14.7	14.8	14.3	14.2	14.0	14.2	14.5	10.9	10.3	7.9	**0**

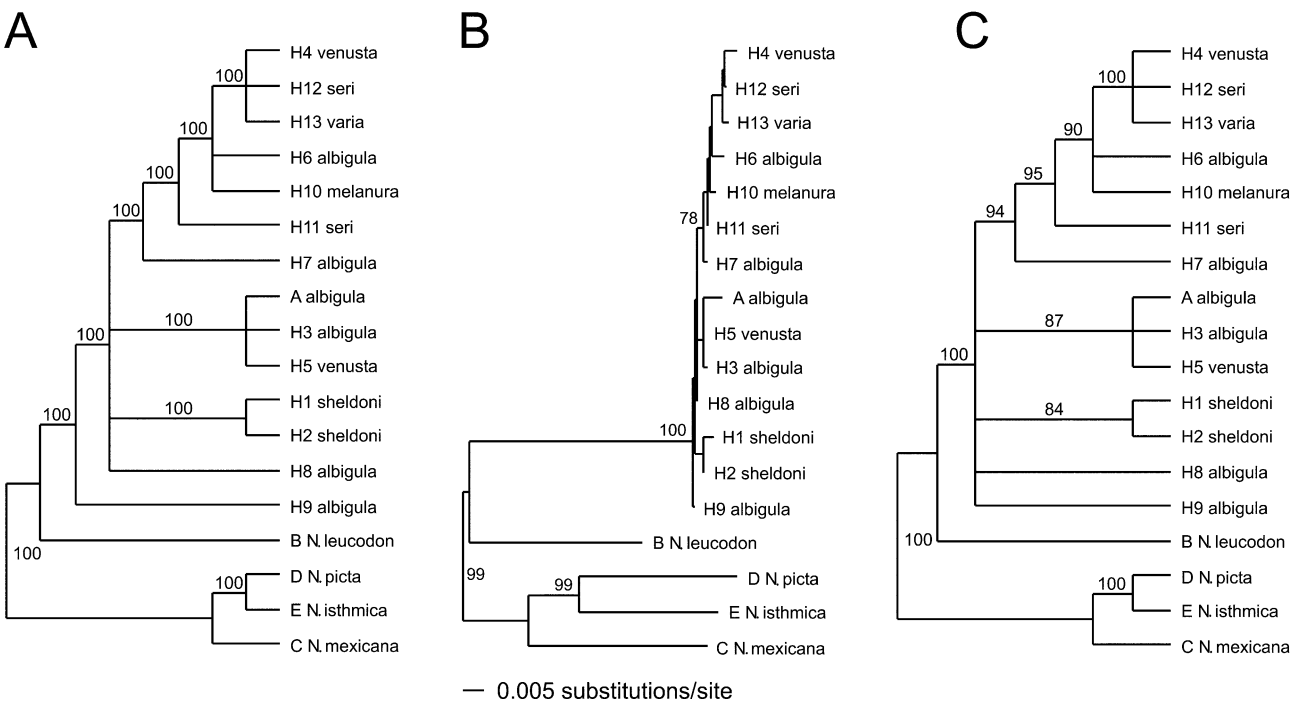

FIGURE 2. Phylogenetic trees of the 828-bp fragment of the Cyt *b* gene generated from the analyzed *Neotoma* specimens. The numbers of haplotypes and subspecies at the tip of each branch follow Table 1. The trees were constructed using the 50% majority rule consensus algorithm (except in maximum-likelihood). Values in the nodes are branch support for the analyses. The *N. leucodon* sequence was included as the ingroup and *N. picta*, *N. isthmica*, and *N. mexicana* as the outgroup. (A) Maximum-likelihood tree using the GTR+I model; (B) neighbor-joining tree, genetic distances were calculated using the Jukes-Cantor model, percentage differences per node are in table 2. (C) Bayesian inference performed using the GTR+I model.

The neighbor-joining tree showed short branches indicating low genetic divergence for *N. albigula* subspecies, <1% in almost all cases. The genetic similarity between the specimens from Datil and Tiburon Island (average divergence 0.3%) in addition to the geological evidence that when Datil Island was part of Tiburon Island (Gastil *et al.* 1983; Carreño & Helenes 2002). Suggest that *N. varia* came from the Tiburon Island population in the Pleistocene-Holocene. Therefore, Tiburon Island population and *N. a. melanura* in mailand have an average divergence 0.3%. Tiburon Island appears to have been separated from the mainland about 5,000–10,000 years ago (Wilcox 1978; Lawlor 1983; Carreño & Helenes 2002).

The molar analysis showed that all the specimens of the different subspecies of *N. albigula* do not show high variation. The only specimen of *N. varia* had differences in the second and third molars. In *N. varia*, the anterior metaloph of the second upper molar is in contact with the posterior metaloph of the first molar, creating an appearance of a single metaloph with two parts. However, the diagnostic characteristic of the tooth pattern of *N. varia* is to have the last upper molar with two lobes, with one reentrant angle (Burt 1932), instead Datil Island specimen have of three lobes with two external reentrant angles that all *N. albigula* specimens have; however, the anterior lobe of the third molar is smaller in relation to the other specimens of *N. albigula* (Figure 3).

The skull characteristics that are related and could be used to differentiate *N. varia* from *N. albigula* are "…relatively heavier rostrum with incisors more incurved; broader nasals at posterior termination, shorter palatal bridge, lighter pterygoids and more rounded, less prominent coronoid process" (Burt 1932). Bogan (1997) failed to discern such differences with the specimens he examined. The specimens we studied do not fit all these characteristics; however, they are different in their skull morphology from the 57 specimens of *N. a. seri* from Tiburon Island.

Neotoma albigula is a widespread and variable species (Hall 1981) with morphological distinctness for each subspecies. In the case of *varia*, the specimen was morphologically different in the occlusal view of the

upper molars compared to specimens of the subspecies of *N. albigula*. The specimen has a different tooth pattern on the second and third molars. However, previous morphologic analyses indicated inconstancy in characters regarding holotype (Bogan 1997); and showed that the *N. varia* specimens were within the overlapping variation of *N. albigula* subspecies (Bogan 1997). Our results also showed that the genetic characters of *N. varia* are within the range of genetic variation of *N. albigula*, which reflects the similar pattern displayed in their morphology. Under the condition of differences in the molar pattern, but small morphological variation and lower genetic differences, we considered *varia* as a subspecies of *N. albigula*, as Bogan (1997) previously recommended.

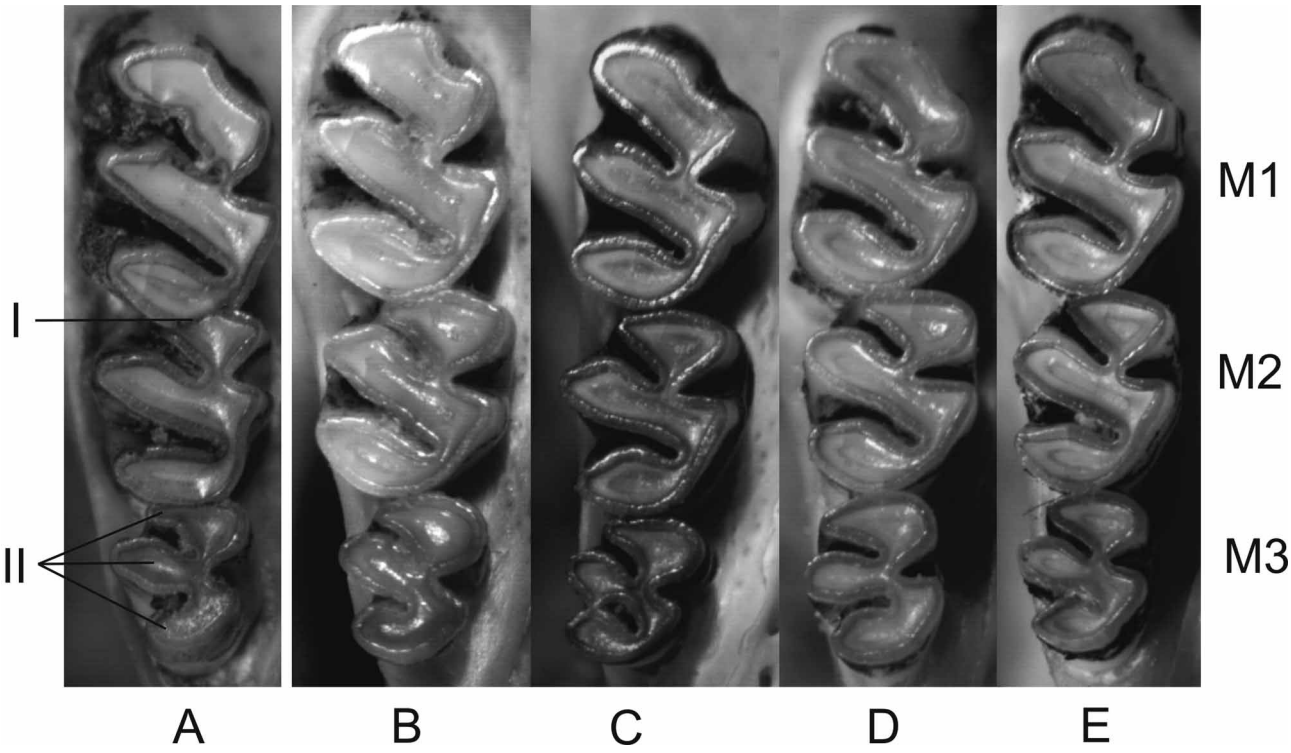

FIGURE 3. Oclusal view of the upper molars: (A) *N. varia*, (B) *N. albigula albigula*, (C) *N. a. melanura*, (D) *N. a. seri*, and (E) *N. a. venusta*. M1 is the first molar, M2 the second molar, and M3 the third molar. (I) Is the contact area between the lobes of the fist molar and the second. (II) Is the presence of three lobes in the third molar.

During the years, at least eight expeditions failed to find *N. a. varia* specimens on Datil Island (Bogan 1997; Álvarez-Castañeda & Ortega-Rubio 2003; Álvarez-Castañeda *et al*. in press). From the time of its initial description (Burt 1932) until the spring of 2008, only four complete specimens and one jaw were known. Nevertheless, some fresh signs and skull fragments had been observed on the island (Bogan 1997; Álvarez-Castañeda *et al*. in press). With this paucity of information, no aspect of biology of the population has been studied. Throughout this time, and based on the small size of the island and the pattern of mammal extinctions on other small islands, this woodrat was considered close to extinction (Álvarez-Castañeda & Ortega-Rubio 2003; Álvarez-Castañeda *et al*. 2006). As a full species (from 1994 until 1996), *N. varia*, was considered as "threatened" by the Mexican government (SEMARNAT 1994) and the International Union for Conservation of Nature (IUCN). However, in 2008, it was treated as co-specific with the white-throated woodrat *N. albigula* and included on the red list of IUCN as "least concern" (Álvarez-Castañeda *et al*. 2008). At present, it remains an endemic threatened species under Mexican law (SEMARNAT 2002).

Álvarez-Castañeda *et al*. (in press) indicated that the *varia* specimens are only present on a very small northern part of the island, from the middle to the top of a canyon with a very specific habitat containing C*ylindropuntia* sp. and *Mammillaria* sp. associated with small groups of *Jatropha* sp. They concluded that the *Neotoma* population on Datil Island needs to be considered "critically endangered" following IUCN criteria (IUCN 2008). *Neotoma albigula varia* has a very restricted distribution on the island; although our results

showed low divergence regarding other *N. albigula* subspecies and *N. a. varia* keeps its genetic identity. Therefore, we agree that it needs to be considered as a critically endangered population.

Further collections and studies must be carried out on Datil Island to learn more about this population, its biological aspects, reproduction, ecology, and current threats to mitigate the dangers and assure its survival on the island in the future. Since 1978, Datil Island has been part of the protected area "Islas del Golfo de California" (SEMARNAT 2000), which is a start to protection.

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