DNA Barcoding of Mammals in Mexico: Implications for Biodiversity

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Abstract: The Mexican Barcode of Life (MEXBOL) project started as an initiative by researchers who wanted México to join the international DNA barcoding (iBOL) collaboration to establish a genetic system of species identification and discovery. MEXBOL has three main nodes associated with different research institutions in the northern, central, and southern parts of the country. At the beginning of 2009, the laboratories of the three nodes began to operate with support from the University of Guelph in Canada where DNA barcoding began in 2003. By the end of 2011, the laboratories in México will be completely equipped and fully operational. The project is funded by the Consejo Nacional de Ciencia y Tecnología (CONACyT). In addition, the steering committee of MEXBOL supports the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO) initiative to offer grants to any university and research center for barcoding collections of Mexican specimens. We present preliminary results of projects dealing with barcoding Mexican mammals. The aims of these analyses are to evaluate the importance of the DNA barcoding (using cytochrome *c* oxidase subunit I gene; COI) for the identification of species, study of genetic diversity, and taxonomic review. Our preliminary data suggest that DNA barcodes can be used for the discovery of cryptic species and have important implications to the understanding of the mega-diversity of mammals in México.

Keywords: *Chaetodipus*, Cytochrome C oxidase subunit I, Cryptic biodiversity, Heteromyidae, *Heteromys*, MEXBOL, Species diversity.

INTRODUCTION

México is one of the mega-diverse countries of the world [1, 2]. Part of the biological richness of this region is the endemic species in many biological groups, such as plants, invertebrates, fish, and mammals. For vertebrates other than fish, 32% of the 2,500 species known are endemic. In recent years, studies using genetic data (DNA sequences) have shown the existence of Mexican cryptic species accentuating the importance of continued reassessment of cryptic biodiversity. For example, in a study analyzing DNA sequences (nuclear and mitochondrial genes) combined with morphological data of fish from Mexico's Reserva de la Biósfera del Alto Golfo de California y Delta del Río Colorado, was discovered the second fish species endemic to the river's delta, the Delta Mudsucker (*Gillichthys detrusus*). This species was erroneously placed in the synonymy of *G. mirabilis* in 1907 and had since remained unrecognized until 2011 [3]. In the endemic Mexican pocket gopher *Cratogeomys merriami* a study revealed three major genetic and morphological clades within the species. Here, *C. perotensis*, *C. fulvescens*, and *C. merriami* were recognized for central Mexico [4]. Given the foregoing, DNA barcoding represents an application to the study of biodiversity [5] and the discovery of cryptic species. In México, an organization was created to establish a genetic identification system for the plants and animals, The Mexican Barcode of Life (MEXBOL). This is linked directly to the International Barcode of Life (iBOL) project. Additionally, MEXBOL is part of a network supported directly by the Consejo Nacional de Ciencia y Tecnología (CONACyT) and received the first installment of funding in March 2009. Funds to support barcoding and a mirror site for the Barcode of Life Database (BOLD) have also been provided by the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO).

The goal of MEXBOL is to operate a National Laboratory system with three nodes situated at institutions in the northern, southern and central parts of México: 1) Centro de Investigaciones Biológicas del Noroeste (CIBNOR), 2) El Colegio de la Frontera Sur (ECOSUR); both of which are research centers of the Federal Government under CONACyT, and 3) Universidad Nacional Autónoma de

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México through the Instituto de Biología. We are aiming for a capacity of about 15,000 reactions per year at each node for the extraction of DNA, PCR amplification, and sequencing of nucleotides. When fully operational, we will seek additional support from other Mexican biorepositories to speed the inventorying of biodiversity in México, and to increase the collaboration with Mesoamerican researchers. In adition, CONABIO has financed 20 projects on DNA barcoding to support researchers at 12 Mexican institutions (see http://www.conabio.gob.mx/institucion/proyectos/doctos/pdf/selecc_CB09.pdf).

The most relevant aspects of this project are: a) establishing México in a leading international role in taxonomic identification and systematic study; b) creating facilities for the cryopreservation of tissues and DNA extracts that are associated with voucher specimens housed in public institutions; and c) establishing a bioinformatics platform to be used for the generation, storage and management of sequences and associated curatorial data that will be diseminated through the internet.

The main scientific and tecnological benefits that MEXBOL will provide are: 1) a genetic methodology for the biodiversity inventory of México; 2) a database with an algorithm for automatic identification of submitted sequences; 3) molecular taxonomic tools to aid in the description of new species; and 4) development of alliances and synergies to reduce the laboratory costs for sequencing and achieving faster identifications of the eukaryotic species of the world.

We consider DNA barcoding as one of the components of the toolkit used for taxonomic identification and as an additional character set that should be combined with morphological methods to delimit species and their evolutionary relationships. However, DNA barcodes should be associated with voucher specimens that have been identified by taxonomic experts.

Mammals have one of the best known taxonomic classifications [6]. However, some species are still not clearly understood because there is variation in their diagnostic features, such as morphology of the skull [7-9]. Molecular tools are an option that can be used in addition to morphology to help get a better understanding of the species diversity in mammals and their phylogenetic relationships [10-12]. However, some recent phylogenetic studies using molecular tools have conflicted with traditional morphologically derived phylogenies [7-9]. The development of DNA barcoding as a genetic reference system for species identification is a strong effort to bridge this technological gap by using standardized sequences with associated voucher specimens. For example, comparison of verified voucher specimens with recently collected small mammals from Suriname indicated several differences between field identifications and species determinations [11]. Based on initial analyses of DNA barcodes in México, there is not a wide use of these techniques for taxonomy, but many studies are using molecular techniques for systematic studies [7-9, 13, 14].

In México, many of the previous phylogenetic studies of mammals have been done mainly with the cytochrome *b* or the cytochrome *c* oxidase subunit III genes. However at the moment, with the implemantation of the MEXBOL project, the cytochrome *c* oxidase subunit I (COI) is now the most represented and sequenced gene among Mexican mammal species. However, we think that where possible, COI should be combined with other genes, nuclear or mitochondrial, for a robust systematic study.

Herein, we present two case studies demonstrating the utility of DNA barcoding on Mexican mammals including an investigation of species groups in *Chaetodipus arenarius* (Heteromyidae: Rodentia) from throughout its distributional range, including type localities, which was developed at the Centro de Investigaciones Biológicas del Noroeste, and an analysis of species diversity within the genus *Heteromys* (Heteromyidae: Rodentia), which was developed at El Colegio de la Frontera Sur.

The pocket mouse, *Chaetodipus arenarius*, was formally recognized as a unique species endemic to the Baja California Peninsula of México [15-19] with 12 subspecies [20] and *C. dalquesti* [17] as a junior synonym of *C. a. arenarius*. However, recent molecular and morphological analyses proposed that this taxon should be considered a species complex composed of *C. arenarius*, *C. dalquesti* and *C. siccus* [21]. Here, we focused on obtaining barcodes of the three putative species with distribution in the Baja California Peninsula to test the utility of DNA barcoding to identify species similar in morphology. The study also aimed to use COI gene sequences to hypothesize the phylogenetic relationships of the *Chaetodipus arenarius* complex.

The genus *Heteromys* has had many taxonomic changes in the last few years, including the description of several new species [22-25]. In México, three species have been recorded: 1) *H. desmarestianus* is a polytypic species with distribution restricted to the southern states of Veracruz, Tabasco, Oaxaca, Chiapas and the Yucatan Peninsula to 2,400 m above sea level [26]. Some studies indicate that *H. desmarestianus* represents a complex of several externally similar species, but with considerable variation in their karyotypes, allozyme and cranial morphology [25, 27]. This species comprises 12 subspecies, of which three are distributed in México; *H. d. desmarestianus*, *H. d. goldmani* and *H. d. temporalis* [6]. Rogers and Schmidly [27] considered *H. goldmani* (distributed in southeastern Chiapas and southwestern Guatemala to 2,000 m above sea level) to be different from *H. desmarestianus* based on cranial variation and baculum morphology. However, later allozyme analysis found no differences between the two taxa and *H. goldmani* was again considered as a subspecies of *H. desmarestianus* [28]. Nevertheless, some authorities still consider *H. goldmani* as a different species [26]. 2) *H. nelsoni*, a monotypic species, is known by only a few specimens from Cerro Mozotal, in Chiapas, and Volcán Tajumulco, in Guatemala [26]. It has been documented at high elevations (2,500 to 2,800 m above sea level [26, 29, 30]) and is not sympatric with *H. d. goldmani*, which is found at lower elevations [31]. It is listed as critically endangered by the International Union for Conservation of Nature [32], and receives special protection by the Mexican Government [33]. A third population not previously known was registered in Chiapas in January of 2009 [34]. 3) *H.*

gaumeri, a monotypic species, has a distribution restricted to deciduous forests of the Yucatan Peninsula to 100 m above sea level. This species can be very similar in color to *H. desmarestianus* in sympatric areas [26]. It may represent an ancient lineage of *Heteromys* and was removed from the *desmarestianus* group and placed in its own group [35].

MATERIALS AND METHODS

The specimens of *Chaetodipus arenarius* were DNA barcoded and analyzed with *C. baileyi*, *C. rudinoris* and *C. spinatus* as outgroups (Appendix 1). *Heteromys* was barcoded and analyzed with *Liomys pictus* and *L. salvini* as outgroups (Appendix 2). Total genomic DNA was extracted using the standard rapid salt-extraction protocol [36]. The 5' fragment of the mitochondrial COI gene (~640 bp) was amplified using the universal primer cocktail for DNA barcoding [37]. All polymerase chain reactions (PCR) included 2.2 µl ddH_2O, 1.25 µl of each primer (10 nM concentration), 0.237 µl (0.4 nM) dNTPs, 0.25 µl (3 mM) $MgCl_2$, 0.0625 µl Taq polymerase (platinum, Invitrogen, Carlsbad, CA), and 1× Taq buffer to a final volume of 12.5 µl.

Non-redundant haplotypes were identified using the Collapse ver. 1.1. [38], and TCS ver. 1.18 [39] software. The model comparison software MrModeltest ver. 2.2 [40] with the Akaike Information Criterion (AIC) was used to select the most appropriate model of nucleotide substitution. Maximum-likehood (ML), maximum-parsimony (MP), using an heuristric procedure with 10 random sequence additions and tree bisection reconnection (TBR) branch swapping, and neighbor-joining using Kimura 2-parameter model (K2P) and The General Time Reversible model (GTR) were conducted in PAUP ver. 4.0b10 software [41]. Support for nodes was assessed with bootstrap analyses of 1,000 replicates. A Bayesian inference analysis (BI) was performed using MrBayes ver. 3.0b4 software [42]. A consensus tree was generated with the 50% majority-rule algorithm. For *Chaetodipus arenarius*, a minimum spanning network, Fu's *F* test, network construction (using the parsimony algorithm), haplotype diversity, and nuclear diversity were performed with ARLEQUIN version 2.0 [43], to contrast the genetic parameters among species.

RESULTS

There were 42 unique haplotypes identified in the 640 bp fragment of COI sequenced for *Chaetodipus*. The four phylogenetic analyses converged on essentially identical tree topologies obtained in a previous study that concatenated the Cyt *b* and COIII genes [21]. There were three monophyletic clades, each with 100% support, representing *C. arenarius*, *C. siccus*, and *C. dalquesti* (Fig. **1**). The maximum parsimony analysis yielded 4,392 trees (length = 430, CI = 0.672, RI = 0.925). The maximum likelihood analysis with the GTR + I + G evolution model (A = 0.310, C = 0.223, G = 0.117, and T = 0.348), invariable sites = 0.632, and gamma distribution = 2.921) produced one tree (score = 7589.0, Fig. **1b**). The average interspecific sequence divergence (K2P) ranged from 5.8% between *C. dalquesti* and *C. siccus* to 15.8% between *C. arenarius* and *C. dalquesti* (Table **1**). The average intraspecific sequence divergence ranged from 0.6% for *C. siccus* to 2% for *C. arenarius*.

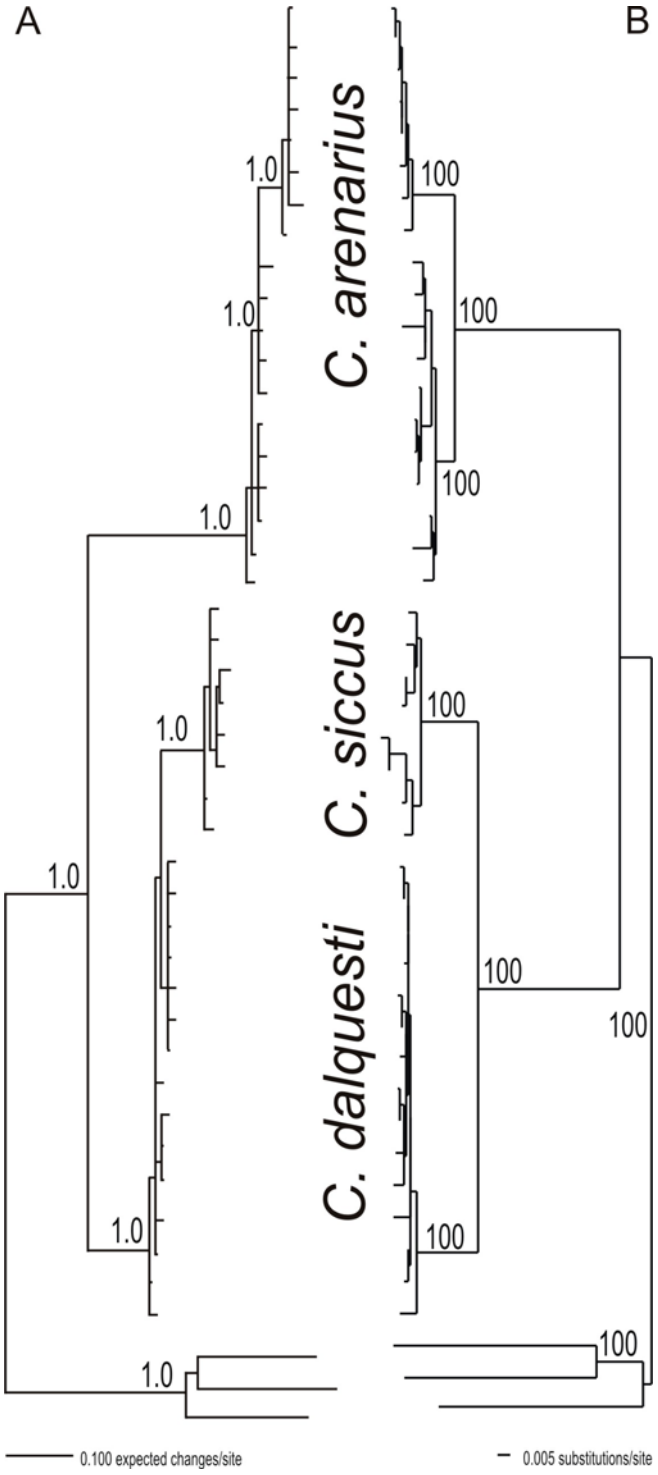

Fig. (1). Phylogenetic relationships among 42 haplotypes of the COI gene sequences of the *Chaetodipus arenarius* complex. The topology was the same for maximum parsimony, neighbor-joining, maximum likelihood, and Bayesian inference analyses. Three groups are monophyletic representing *C. arenarius*, *C. siccus*, and *C. dalquesti*. *C. baileyi*, *C. rudinoris*, and *C. spinatus* were used as outgroup. (**A**) Bayesian inference, and (**B**) neighbor-joining analyses. The values at the nodes are branch support.

Table 1. Average Genetic Distances (Minimum-maximum) between *Chaetodipus arenarius*, *C. dalquesti*, and *C. siccus*. Under the Evolutionary Model Most Appropriate for the Dataset, General Time Reversible (GTR, below Diagonal) and Kimura 2 Parameter (K2P, above Diagonal).

	1	2	3
1) *C. arenarius*	2.00 (0.15-3.92)	15.82 (14.54-17.23)	15.72 (14.64-17.23)
2) *C. dalquesti*	15.37 (14.17-16.72)	0.95 (0.15-1.55)	5.81(5.10-6.96)
3) *C. siccus*	15.27 (14.27-16.58)	5.76 (5.06-6.88)	0.63 (0-1.54)

In the minimum spanning network only one haplotype was present in two different type localities (H34; Migriño and La Paz), representing the subspecies *C. d. dalquesti* and *C. d. sublucidus*, respectively. Using maximum parsimonious connections (27 steps, $P > 0.95$), three disjointed networks were obtained: *C. arenarius*, *C. dalquesti*, and *C. siccus*. These were separated by a minimum of 79 and 32 mutational steps, respectively (Fig. **2**), well beyond the confidence limits for parsimony. The genetics parameters are presented in Table **2**.

The *C. arenarius* network (19 haplotypes) covers a large geographic area of the peninsula and includes seven type localities. Specimens of *C. a. ambiguus* (type locality Yubay, 30 mi SE Calamahué, Baja California) and *C. a. mexicalis* (type locality Los Muertos Canyon Fan, 32° 27' N, 115° 53' W, Gaskill´s Tank, near Laguna Salada, Baja California) were not collected. No haplotype was found to be widespread among the type localities. The average distance among the localities is 430 ± 242 km (120 – 880 km). There are two sub-networks representing the northern and southern Peninsula separated by the Vizcaino Desert that has more mutational steps than the minimum spanning network obtained from Cyt *b* [21], but these differences are not significant ($P < 0.05$).

The *C. dalquesti* network (15 haplotypes) covers a moderate geographic area of the southern part of the peninsula and includes three type localities. No haplotype was found to be widespread among the type localities and there was a star-like pattern indicating a recent expansion.

The *C. siccus* network (8 haplotypes) covers a small geographic area on Cerralvo Island in the southern part of the peninsula, and includes only one type locality. There were no sub-networks found but there was a star-like pattern with few mutational steps between haplotypes (Fig. **2**).

For the 640 bp COI fragment of *Heteromys*, the average base composition was A = 27.1%, C = 22.8%, G = 17.1%, and T = 32.9%. There were 162 (24.6%) polymorphic sites of 128 (19.4%) transitions, and 52 (7.9%) transversions, and 13 unique haplotypes were identified. Gene diversity (*h*; mean ± *SD*) was 0.8968 ± 0.0302, nucleotide diversity was 0.060176 ± 0.0298, and the mean number of pairwise differences was 39.5956 ± 17.6784.

The maximum parsimony analysis yielded three trees (length = 718, CI = 0.937, RI = 0.971, RC = 0.910). The maximum likelihood analysis with the GTR evolution model (Base = 0.2658 0.2386 0.1696); Nst = 6; Rmat = 8.9586 14.8996 8.7153 0.9262 32.6384; Rates = equal; Pinvar = 0; K = 8, AIC = 6123.345) produced one tree (score =

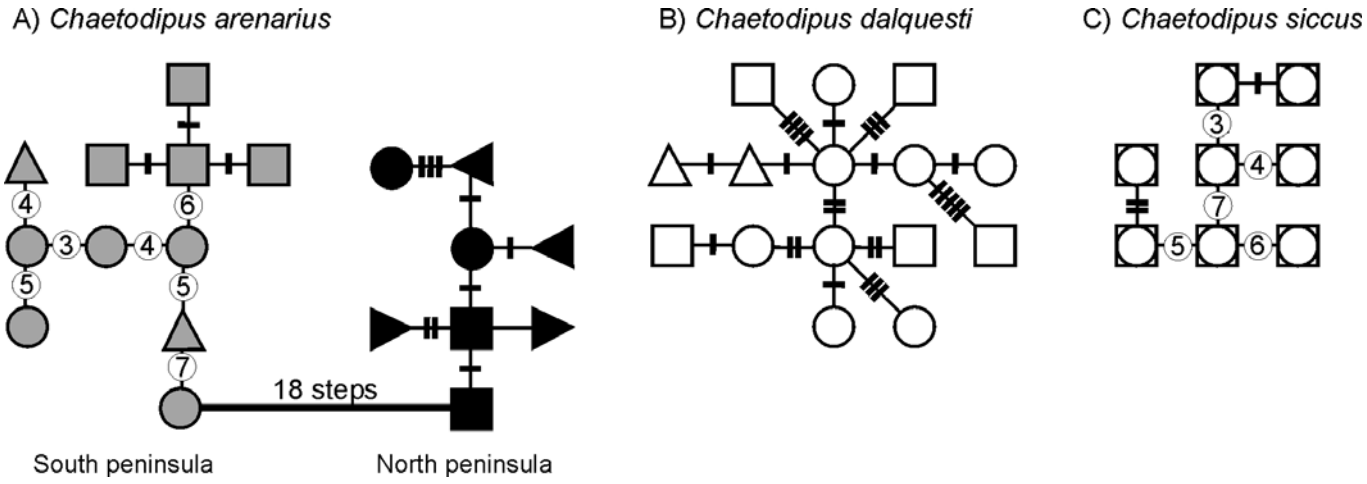

Fig. (2). The minimum spanning network for the COI data set of *Chaetodipus* from the Baja California Peninsula. Each perpendicular hash mark across the line or number within the circle between adjacent haplotypes in the network represents a single-base substitution. **A**) The minimum spanning network of *C. arenarius* has two sub-networks: northern peninsula and southern peninsula (Magdalena Island, grey triangle; San Jorge, grey circle; El Mogote, grey square; San Quintin, solid square; Guerrero Negro, solid circle; El Barril, solid right triangle; and San Felipe, solid left triangle). **B**) The minimum spanning network of *C. dalquesti*: La Paz, circle; Margarita Island, square; and Migriño, triangle. **C**) The minimum spanning network of *C. siccus.* All the specimens are from Cerralvo Island.

3053.672; Fig. **3a**). It had a similar topology to the Bayesian tree (Fig. **3b**) and also to the maximum parsimony and neighbor joining trees (not shown). All phylogenetic analyses recovered the three recognized species of *H. nelsoni*, *H. gaumeri*, and *H. desmarestianus* as deeply divergent and strongly supported clades.

Heteromys nelsoni had only two haplotypes that differed by four transitional mutations indicating relatively minimal genetic variability in this species. In *H. gaumeri*, four haplotypes were defined with a maximum of six mutations between haplotypes. *H. desmarestianus* had two subclades (Fig. **3**) representing the subspecies, *H. d. desmarestianus*

Table 2. Molecular Diversity Indices (With Standard Deviations Where Appropriate for Subspecies of *Chaetodipus* species from Localities in Baja California and Baja California Sur. Sample Size (*n*), Number of Haplotypes (*k*), Haplotypic or Nei´s Diversity (*h*), Pairwise Differences (PW), Nucleotide Diversity (π), Tajima's D, and Fu's *F* Test

subspecies	Locality	*n*	*k*	*h*	PW	π	(D)	(Fu)
Chaetodipus arenarius								
albescens	San Felipe	2	2	1.000 ± 0.50	2.00 ± 1.73	0.003 ± 0.003	0.00	0.69
albulus	Magdalena Island	9	2	0.222 ± 0.16	1.33 ± 0.91	0.002 ± 0.001	-1.73	2.71
arenarius	San Jorge	6	5	0.933 ± 0.12	6.20 ± 3.43	0.001 ± 0.006	-0.71	0.02
helleri	San Quintin	5	2	0.600 ± 0.17	0.60 ± 0.56	0.001 ± 0.001	1.22	0.63
paralios	El Barril	6	2	0.533 ± 0.17	2.13 ± 1.37	0.003 ± 0.002	1.18	3.15
ramirezpulidoi	Mogote	7	5	0.809 ± 0.12	1.04 ± 0.78	0.001 ± 0.001	-0.65	-1.39
sabulosus	Guerrero Negro	2	2	1.000 ± 0.50	4.00 ± 3.16	0.007 ± 0.006	0.00	1.38
Chaetodipus siccus								
siccus	Cerralvo Island	17	8	0.816 ± 0.08	4.92 ± 2.520	0.007 ± 0.004	-0.66	0.20
Chaetodipus dalquesti								
ammophilus	Margarita Island	8	2	0.428 ± 0.16	0.42 ± 0.42	0.001 ± 0.001	0.33	0.54
dalquesti	Migriño	5	5	0.933 ± 0.12	5.80 ± 3.23	0.008 ± 0.005	-1.07	-0.09
sublucidus	La Paz	8	8	0.955 ± 0.05	3.26 ± 1.83	0.004 ± 0.003	-0.72	-3.27

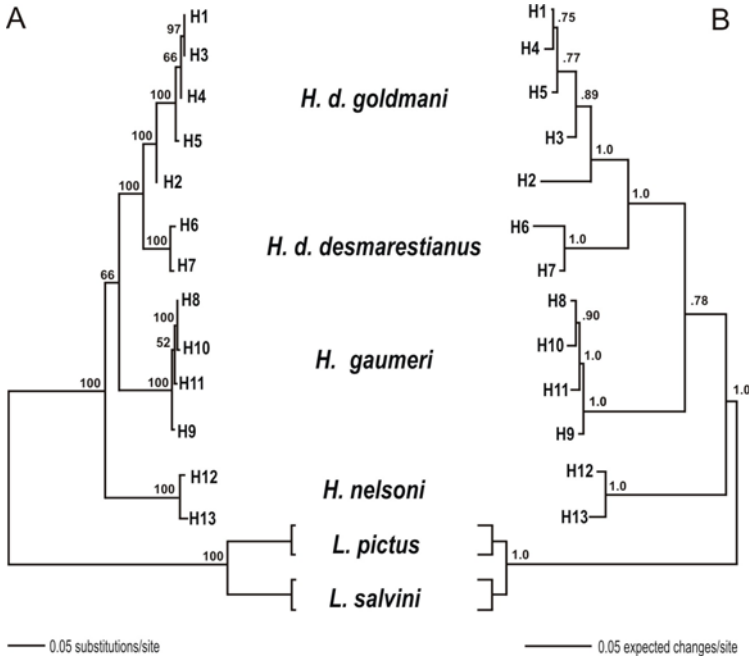

Fig. (3). Phylogenetic relationships between the species of *Heteromys* based on analysis of COI sequence variation. Branch support values are indicated on the tree. The genus *Liomys* was used as outgroup. (**A**) Maximum-likelihood and (**B**) Bayesian inference trees.

with two haplotypes, and *H. d. goldmani* in southeastern Chiapas with five haplotypes. The average genetic distance (K2P) among the species of *Heteromys* was between 13.1% and 18.6%. The distance between *H. d. desmarestianus* and *H. d. goldmani* was 8.1%, and for *H. gaumeri* and *H. nelsoni* was 18.6%. The average intraspecific divergence ranged from 0.4% for *H. nelsoni* to 1.5% for *H. d. goldmani* (Table **3**).

DISCUSSION

For *Chaetodipus* from the Baja California Peninsula, the average K2P genetic distances of COI found among clades (5.8% to 15.8%) were higher than suggested by the genetic species concept for average sister species differences based on mitochondrial cytochrome *b* gene [44]. In addition, the three groups are monophyletic following the phylogenetic species concept [45] suggesting that *C. arenarius, C. dalquesti*, and *C. siccus* warrant species recognition, as previously suggested [21]. Within *C. arenarius*, there is a phylogeographic break (3.22% sequence divergence) that corresponds to the southern and northern peninsula separated at the Vizcaino Desert, as also indicated by cytochrome *b* data [21].

For *Heteromys* from México, the molecular analysis of DNA barcodes showed three well-supported monophyletic groups with low variation within *H. nelsoni* and *H. gaumeri*, but high variation within *H. desmarestianus*. The genetic distance within *H. desmarestianus* (8.3%) was similar to the range of values based on cytochrome *b* previously reported (8.0% to 9.2%) [35]. These authors considered *H. desmarestianus* and *H. goldmani* as separate species. They are allopatrically distributed and separated by the physical barrier of the Sierra Madre de Chiapas mountain chain. Their distinction had been supported by morphologic and morphometric analyses in Chiapas, where the populations of the southeast (*H. d. goldmani*) were different from the populations of the north (*H. d. desmarestianus*) [35]. Since *H. goldmani* has also been accepted by other studies based on karyotype, allozyme, morphological, cytochrome *b*, and nuclear genes analyses [35, 46], we conclude that COI data is further support of it as a valid species. Thus, there are four species of *Heteromys* distributed in southeastern México: *H. desmarestianus, H. gaumeri, H. goldmani*, and *H. nelsoni.*

Although our phylogenetic analysis of COI sequences did not include *H. anomalus* and *H. australis*, *H. nelsoni* is basal in relation to the other species, which is concordant with previous analysis of sequences of cytochrome *b* and nuclear genes [35]. The specimens of the newly discovered population in Chiapas from La Cascada to Cerro Mozotal (2,850 m) were grouped within the species *H. nelsoni* according to diagnostic morphological characters of the species and the barcodes obtained in this study. Within *H. gaumeri*, our data did not show strong geographic structure. Samples from Yucatán and Campeche had haplotypes that were shared; however, the only haplotype from Quintana Roo was in a basal branch.

The influence of environmental factors associated with vegetation types is an important factor that probably acted upon the diversification and speciation process of species with wider distribution and varied habitat, such as *H. desmarestianus* and *C. arenarius*. In contrast, species with restricted distribution, such as *H. nelsoni* and *C. siccus*, have low genetic variation. Genetic drift within these populations may have resulted in reduced genetic variability, which indicates that the species are vulnerable to extinction. In this regard, recognition and management of conservation units below the species level are crucial to avoid loss of genetic diversity in these species.

It is also necessary to continue with studies focused on estimating the levels of genetic variability in mammals from throughout their distribution with the additional aim of investigating the phylogenetic and taxonomic status in tropical habitats. This will help in proposing conservation policies for species associated with these habitats in southeastern México. In addition, this region needs to be surveyed with more attention to detail concerning the microhabitat of each species. These analyses demonstrate the ability of DNA barcoding to discriminate cryptic species and the amount of variation that an endemic and restricted species can have within its distributional area.

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Table 3. Average Genetic Distances (Minimum-maximumn) between *Heteromys desmarestianus goldmani, H. d. desmarestianus, H. gaumeri,* and *H. nelsoni,* Under the Evolutionary Model Most Appropriate for the Dataset, General Time Reversible (GTR, below Diagonal) and Kimura 2 Parameter (K2P, above Diagonal)

	1	2	3	4
1) *H. d. goldmani*	1.46 (0.15-3.19)	8.19 (6.44-9.43)	15.84 (10.32-18.45)	18.24 (15.45-19.67)
2) *H. d. desmarestianus*	8.30 (6.57-9.55)	1.09 (1.09-1.09)	13.14 (12.20-14.10)	16.43 (15.08-17.63)
3) *H. gaumeri*	16.34 (10.51-19.27)	13.32 (12.39-14.28)	0.66 (0.46-1.07)	18.64 (17.26-20.14)
4) *H. nelsoni*	19.08 (16.58-20.34)	17.43 (16.33-18.37)	19.16 (17.74-20.69)	0.41 (0.41-0.41)

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APPENDIX 1

Specimens examined of *Chaetodipus* including localities (type locality indicated with an asterisk) with latitude and longitude, haplotype number (catalog and GenBank accession numbers in parentheses) in the mammal collection of the Centro de Investigaciones Biológicas del Noroeste (CIB). *Chaetodipus arenarius*: *C. a. albescens* (San Felipe, Baja California*). San Felipe (31.022778 N, 114.841611 W), haplotype 1 (2903, HQ690033), haplotype 2 (2904, HQ690034). *C. a. helleri* (San Quintin, Baja California*). San Quintin (30.348333 N, -115.818333 W). Haplotype 3 (6803, 6804, 6807, HQ690035), haplotype 4 (6805, HQ690036). *C. a. sabulosus* (S side Scammon´s Lagoon, Baja California*). Guerrero Negro (27.895556 N, 113.971667 W). Haplotype 4 (9337, HQ693909), haplotype 7 (1684, HQ690040), Haplotype 8 (9336, HQ690041). *C. a. paralios* (Barril lat 28° 20´ N on Gulf of California, Baja California*). El Barril (28.296389 N, 112.871556 W). Haplotype 5 (11628, 11629, 11632, 11633, HQ690037), haplotype 6 (11630, 11631, HQ690038). *C. a. arenarius* (San Jorge, near Comondú, Baja California [Sur]*). San Jorge (25.726111 N, 112.074167 W). Haplotype 9 (6743, HQ690042), haplotype 10 (6742, 6744, HQ690043), haplotype 11 (6745, HQ690044), haplotype 12 (6746, HQ690045), haplotype 13 (6748, HQ690046). *C. a. albulus* (Magdalena Island, Baja California [Sur]*). Isla Magdalena (24.346111 N, 112.154167 W). Haplotype 16 (5071, HQ690049), haplotype 17 (5943, 5088, 5934, 5935, 5936, 5937, 5938, 5941, HQ690050). *C. a. ramirezpulidoi* (El Mogote, Baja California Sur*). Mogote (24.166667 N, 110.350000 W) haplotype 18 (158, 162, HQ690051), haplotype 19 (171, 163, 174, HQ690052), haplotype 20 (172, HQ690053), haplotype 21 (173, 175, HQ690054), haplotype 22 (168, HQ693908).

Chaetodipus siccus (Ceralbo [Cerralvo] Island, Baja California [Sur]*). Isla Cerralvo (24.161111 N, 109.863889 W). Haplotype 23 (5600, 5611, 5613, HQ690055), haplotype 24 (5601, 5603, HQ690056), haplotype 25 (5602, HQ690057), haplotype 26 (5604, HQ690058), haplotype 27 (5614, HQ690059), haplotype 28 (5616, 230, 231, 233, 234, 235, 5594, 15280, HQ690060), haplotype 29 (5617, HQ690061), haplotype 30 (15279, HQ690062).

Chaetodipus dalquesti ammophilus (Santa Margarita Island, Baja California Sur). Isla Margarita (24.406778 N, 111.823111 W). Haplotype 14 (5950, 5951, 5952, 5954, 5959, 5961, HQ690047), haplotype 15 (5953, 5962, HQ690048). *C. d. sublucidus* (La Paz, Baja California [Sur]*). La Paz (24.137583 N, 110.467361 W). Haplotype 31 (252, HQ690063), haplotype 32 (254, HQ690064), haplotype 33 (255, HQ690065), haplotype 34 (1925, HQ690066), haplotype 35 (1926, HQ690067), haplotype 36 (1927, HQ690068), haplotype 37 (1928, HQ690069), haplotype 38 (1931, HQ690070). *C. d. dalquesti* (4 mi SE Migriño (32 km SSE Todos Santos), Baja California Sur, Mexico* lat. 23° 10´ N, long. 110° 07´ W. Migriño (23.740000 N, 110.200556 W). Haplotype 39 (13039, HQ693907), haplotype 40 (6978, HQ690071), haplotype 41 (6979, HQ690072), haplotype 42 (6980, HQ690073), haplotype 43 (6981, HQ690074), haplotype 44 (13172, HQ690075). Outgoup: *Chaetodipus baileyi* (HQ690076), *Chaetodipus rudinoris* (HQ690077) *Chaetodipus spinatus* (HQ690078).

APPENDIX 2

Specimens examined of *Heteromys* and *Liomys* including localities with altitude (m), latitude and longitude, and haplotype number (voucher and GenBank accession number in parentheses). All specimens are housed in the mammals collection of El Colegio de la Frontera Sur in San Cristóbal de Las Casas (ECO-SC-M), except specimens indicated with CIB which are housed in the Centro de Investigaciones Biológicas del Noroeste.

Heteromys d. desmarestianus. Chiapas: 100 m SE Ejido Loma Bonita, 232 m (16.19400 N, 91.30999 W), haplotype 7 (1306, GU681750; 1360, GU681774). Cerro Chipote: 1.77 Km al S Ejido Loma Bonita, 600 m (16.18379 N, 91.31109 W), haplotype 6 (1521, HQ693910), haplotype 7 (1532, HQ693911). RIBMA, enfrente del Ejido Playón de la Gloria, 180 m (16.18239 N, 90.92569 W), haplotype 7 (1873, GU681749; 2075, GU681752; 2076, GU681747).

Heteromys d. goldmani. Chiapas: Ejido Ojo de Agua: 5.6 Km al SE de Bellavista, 2150 m (15.59809 N, 92.29419 W), haplotype 1 (1612, HQ693912; 1970, GU681753; 1971, GU681751; 1973, GU681757), haplotype 3 (1613, GU681771; 1616, GU681759), haplotype 5 (1968, GU681754). Finca Irlanda: 26 km por carretera a Nueva Alemania, al NW de Tapachula, 1095 (14.92879 N, -92.28399 W), haplotype 1 (1831, GU681734; 1832, GU681755), haplotype 2 (1833, HQ693913), haplotype 4 (1830, GU681756).

Heteromys gaumeri. Quintana Roo: El Eden, 25 Km al NNE de Leona Vicario, 10 m (21.21699 N, 87.18299 W), haplotype 9 (2056, GU681746). Reserva El Eden. 23 Km al N de Leona Vicario, 10 m (21.21450 N, -87.18419 W), haplotype 9 (2061, GU681743). Yucatán: Km 12 carretera Ticul-Muna, 20 m (20.44750 N, -89.62889 W), haplotype 8 (CIB 5451, GU681748), haplotype 11 (CIB 5449, GU681745). Rancho Hobonil, 2.5 km N, 1 Km W de Tzucacab, 37 m (20.01600 N, 89.01999 W), haplotype 10 (2245, HQ693914). Campeche: Ejido Río Caribe 49 Km por carretera de Candelaria, 81 m (18.19799 N, 90.54199 W), haplotype 8 (3170, HQ693915).

Heteromys nelsoni. Chiapas: Cerro Mozotal: 30 km al N de Motozintla por carretera Buenos Aires- El Porvenir, 2820 m (15.42549 N, 92.34069 W), haplotype 12 (1906, GU681763; 1917, GU681760; 1922, GU681761; 1943, GU681758; 1945, HQ693916), haplotype 13 (1918, GU681762). 1.2 Km del desvio de terraceria a la Comunidad

La Cascada, 2850 m (15.42599 N, 92.33399 W), haplotype 12 (2403, HQ693917).

Liomys pictus. Oaxaca: Rancho Don Pedro: 3.18 km NW de Montecillo Santa Cruz, 6 m (16.38800 N, 94.60900 W), 2639, HQ693918. Chiapas: Rancho San Isidro, 420 m (16.53899 N, 92.80599 W), 2047, HQ693919.

Liomys salvini. Chiapas: Comunidad El Cerrón, 8.33 Km NE de Pijijiapan, 240 m (15.74300 N, 93.15100 W), 1992, HQ693920. 3.95 Km NW del Faro de Puerto Arista, 20 m (15.95100 N, 93.82499 W), 967, HQ693921.

CONFLICT OF INTEREST

None declared.

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