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Molecular systematics and phylogeography of the endemic Osgood's deermouse *Osgoodomys banderanus* (Rodentia: Cricetidae) in the lowlands of western Mexico

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ABSTRACT

Osgoodomys banderanus is a recognized and endemic rodent species of western Mexico, an area known for its high biodiversity and number of endemisms. Phylogeographical relationships within this taxon were analyzed based on mitochondrial (*ND3*, *tRNA-Arginine, ND4L* and partial *ND4*) and nuclear (*GHR*) nucleotide sequences. We obtained a total of 112 samples from 22 localities, covering the complete distribution of the species. Phylogenetic analyses using Maximum Likelihood and Bayesian inference confirmed that *Osgoodomys* is a monophyletic group. In addition, phylogenetic and phylogeographic analyses detected three major clades, which do not coincide with the recognized subspecies of *O. banderanus*. The genetic lineages detected are the western clade (Nayarit, Jalisco and northern Colima), the central clade (Colima, Michoacán, and northern Guerrero) and the eastern clade (central and southern Guerrero). Genetic distances among clades (5–9%) and nucleotide substitutions (30–88) among haplogroups were high, especially in the southern group. Mountain ranges such as the Transmexican Volcanic Belt and the Sierra Madre del Sur, as well as the Balsas River act as geographical barriers for *Osgoodomys*. Our results suggest the presence of three independent species, which need to be characterized morphologically to confirm our hypothesis.

1. Introduction

The lowland region of western Mexico is considered a place of diversification for many different taxa (Poindexter et al., 2013; Vázquez et al., 2009) due to its long and complex geological and climatic history. These factors have promoted the diversification of the biota in the area, and some biogeographic, phylogenetic and phylogeographic studies have identified geographic structure in different species (e.g. Arbeláez-Cortés et al., 2014).

The Sierra Madre del Sur is found within this region, and the Mexican Pacific Ocean, Sierra Madre Occidental, and Transmexican Volcanic Belt form its borders. The local evolutionary processes of this region are interesting because the predominant ecosystem in this region is the tropical dry forest, which is continuous over an extensive area without apparent barriers to genetic flow among lowland populations (Arbeláez-Cortés et al., 2014). The Mexican tropical dry forest represents an example of an inverse latitudinal diversity gradient because it is more diverse than its South American counterpart, as demonstrated by the high degree of endemism and pronounced species turnover displayed by very low among-site floristic similarity (De-Nova et al., 2012). Additionally, the tropical dry forest represents one of the four most extensive types of vegetation of Mexico (Becerra and Venable, 2008).

The evolutionary processes that have taken place in the lowlands of western Mexico are quite contrasting. On one hand, there is evidence that genetic breaks are possible without obvious geographic barriers (Hernández-Canchola and León-Paniagua, 2017). In these cases, it has been suggested that local diversification processes are consequences of severe past events that promoted the genetic divergence of multiple taxa, which were then maintained over time by ecological or behavioral factors (Avise, 2000; Jaramillo-Correa et al., 2008; Hernández-Canchola and León-Paniagua, 2017). On the other hand, other research suggests that the exceptionally rugged topography has produced geographical barriers including mountain ranges, canyons or altitudinal gradients, which promote allopatric speciation through vicariance (Becerra and Venable, 2008). These two explanations of the origin of the biota are not necessarily mutually exclusive (Hernández-Canchola and León-Paniagua, 2017). One way to investigate evolutionary processes is through phylogeographic studies, which focus on the recent history of species by analyzing the geographical and temporal variation

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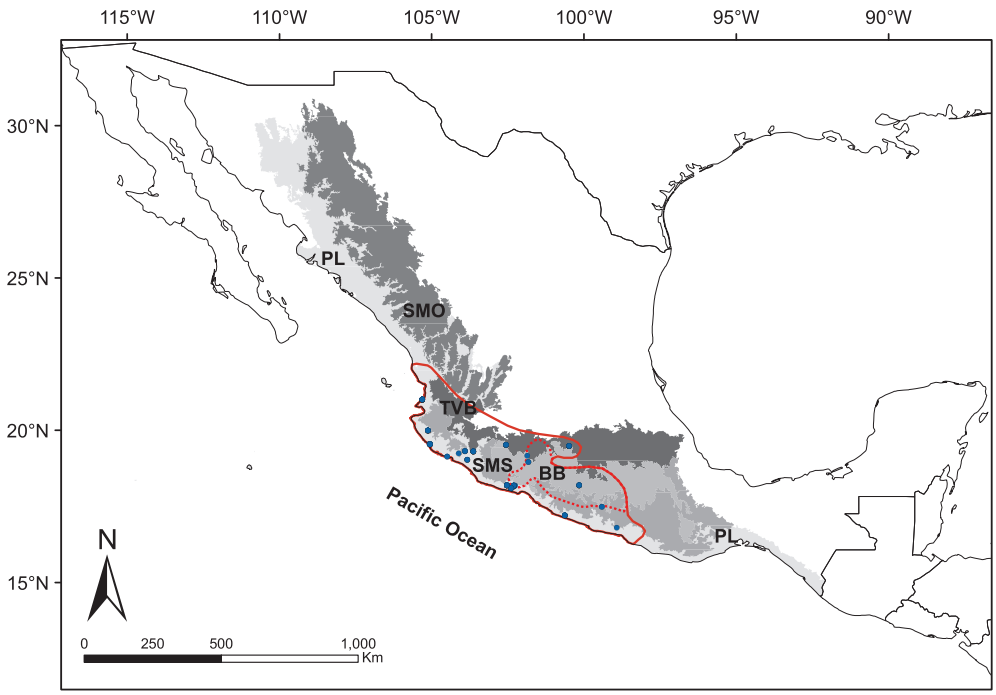


Fig. 1. Geographical distribution of samples of *Osgoodomys banderanus*. The blue points represent samples used in this work. The solid red line represents the geographic range of *O. b. banderanus*, and the dotted red line the range of *O. b. vicinior*. PL is Pacific lowlands, SMS is Sierra Madre del Sur, BB is Balsas Basin, TVB is Transmexican Volcanic Belt and SMO is Sierra Madre Occidental; biogeographic regions according to Morrone et al. (2017).

in genetic information within a single species or among closely related species (Avise, 2000).

The diversification events that shaped western Mexico are particularly relevant in Mexican mammals. The tropical dry forest of the Mexican Pacific Slope is home to most of the genera and species endemic of Mexico. Mammals endemic to this area include the *Megasorex* (shrew) and *Musonycteris* (bat) genera, many species of bats, and the only Mexican endemic carnivore, *Spilogale pygmaea* (skunk) (Ceballos et al., 2014). This region has also been relevant in the diversification processes of the order Rodentia that has the highest number of mammal species at both the national and global levels. There are genera endemic to western Mexico from two of the cricetine tribes (Rodentia: Cricetidae); in the tribe Neotomini these are *Hodomys* and *Xenomys*, and in the tribe Peromyscini, the genus *Osgoodomys* (Bradley et al., 2004; Ceballos et al., 2014).

One of the endemic and monotypic genera of rodent in western Mexico is *Osgoodomys banderanus*. This mouse is found from 0 to 1500 m above sea level (Álvarez, 1968), and its range extends from southern Nayarit to southern Guerrero, including the Balsas Basin (Hall, 1981; Fig. 1). The species was originally described as a member of genus *Peromyscus* (Allen, 1897), but Hooper and Musser (1964) later proposed the subgenus *Osgoodomys*, and Carleton (1980) finally pointed out some morphological characters which warranted its treatment as an independent genus. Currently, two morphological subspecies are recognized -*Osgoodomys banderanus banderanus* and *Osgoodomys banderanus vicinior*- which are differentiated by coat color, total length, and distribution (Álvarez, 1968). On the other hand, a research showed that there are four cytotypes within the species which do not correspond with the distribution of the subspecies, but rather to different habitats where the populations inhabit (Núñez-Garduño et al., 1999).

Due to the inconsistencies between morphological and cytogenetic studies, and because there is evidence of genetic breaks in western Mexico, we were interested in the genetic variation and evolutionary history of the monotypic and endemic rodent *O. banderanus*. We used genetic information from mitochondrial and nuclear loci from throughout the geographic range of this species to investigate the diversification processes and the taxonomic status of its biological units. Our objectives were to: (a) determine if there is genetic structure within the species; (b) determine whether that genetic structure is congruent with morphological or cytological data, or with geographic features; and (c) analyze the historical and demographic processes involved in the evolutionary history of this species. In this way, we may understand the mechanisms and processes that originated the unique biota that is found in western Mexico, an area of great biological relevance but few investigations into the diversification processes of mammals (Amman and Bradley, 2004; Castañeda-Rico et al., 2014; Ortega et al., 2009).

2. Materials and methods

2.1. Sample collection

We obtained tissue samples (9) and skin samples (34) from the mammal collection at the Museo de Zoología, Facultad de Ciencias - Universidad Nacional Autónoma de México, Mexico City, Mexico (MZFC-M), and one skin sample from the Universidad Autónoma Metropolitana, Unidad Iztapalapa, Mexico City, Mexico (UAM-I). Furthermore, we collected 57 specimens of *O. banderanus* in the Mexican states of Colima, Guerrero, Jalisco, Michoacán and Nayarit. We also took an ear sample from 10 specimens trapped at the Chamela Biological Station, Jalisco, Mexico. All material was deposited in the MZFC-M (Supporting Information, Table S1). All techniques used in this study were according to the guidelines published by the American Society of Mammalogists (Kelt and Hafner, 2010; Sikes et al., 2011) and followed Mexico's wildlife legislation (SEMARNAT SGPA/DGVS/11606/08257/06724).

2.2. DNA extraction, amplification and sequencing

We isolated total DNA from tissue and skin samples by a standard high-salt and choroform: isoamyl alcohol method. We also used the protocol of the Qiagen Dneasy Tissue Kit® (Qiagen Inc., Valencia, CA, USA) for some skin samples. Through polymerase chain reaction (PCR) we amplified the mitochondrial genes *ND3*, *tRNA-Arginine*, *ND4L* and partial *ND4* (hereafter mtDNA) using the protocols reported by León-Paniagua et al. (2007). We used the primers Gly and Nap2 (Engel et al., 1998), and 11 other primers to amplify most of the museum samples that consisted of fragmented or degraded DNA. Six of these were specific primers designed with the program OLIGOANALYZER 3.1 (Integrated

DNA Technologies, Inc., 2013; Supporting Information, Table S2). On the other hand, we amplified the nuclear gene *GHR* (Growth Hormone Receptor; hereafter nDNA) in a subset of the samples using the primers GHR1f and GHRend1f (Jansa et al., 2009; Supporting Information, Table S2), and the PCR conditions reported by Fernández et al. (2012). We included negative controls in each PCR run to check for contamination. We visualized the PCR products through electrophoresis using agarose gels (1%) stained with ethidium bromide. PCR products were purified and prepared to be sequenced in an ABI3730xl DNA analyzer (Applied Biosystems, Carlsbad California). We used some sequences available from GenBank including from *O. banderanus* and from related species for use as outgroups (Supporting information, Table S1). Sequences were edited in BIOEDIT 7.0.9.0 (Hall, 1999), and multiple alignment was performed using CLUSTAL W (Thompson et al., 1994).

2.3. Phylogenetic analysis

To select the best fit model of evolution, we used Akaike's information criterion in jModelTest 2.1.1 (Guindon and Gascuel, 2003; Darriba et al., 2012). The model selected for mt*DNA was GTR* + I + *Γ* with the following parameters A = 0.3702, C = 0.2337, G = 0.0*779* and T = 0.3181; nst = 6; a = 1.1440, I = 0.3820. HKY + *Γ was* the selected model for the nuclear gene, with the following parameters A = 0.2846, C = 0.2808, G = 0.2165 and T = 0.2182; kappa = 4.2978 (ti/tv = 2.3437); nst = 2; a = 0.6572.

To corroborate the monophyly of *O. banderanus*, we concatenated the mtDNA and nDNA sequences in 39 samples. We chose outgroups considering the putative close relatives to *Osgoodomys*: *Peromyscus*, *Habromys*, *Onychomys*, *Neotomodon*, *Podomys*, *Reithrodontomys* and *Isthmomys*; and two more distantly related genera, *Baiomys* and *Ochrotomys* (Supporting Information, Table S1). Intraspecific relationships were analyzed using 112 mtDNA sequences, and five sequences of *Peromyscus* species (*P. eremicus*, *P. gossypinus*, *P. sejugis*, *P. polionotus* and *P. melanotis*) as outgroup, because in previous phylogenetic analysis *Peromyscus* was considered as the sister taxon to *O. banderanus* (Reeder and Bradley, 2004).

We constructed Maximum Likelihood (ML) estimations in RAXML 8 (Stamatakis, 2014). Bootstrap support values were based on 5000 rounds of bootstrapping using the rapid bootstrap algorithm in RAXML, followed by a search of the best scoring ML tree. We used the model GTR + I + Γ for all data sets (concatenated and mtDNA sequences), since RAXML cannot implement other substitution models, but for concatenated data we made a partition to estimate the parameters separately for mtDNA and nDNA. Analyses based on Bayesian Inference (IB) were conducted in MRBAYES 3.2 (Hulsenbeck and Ronquist, 2001). On the combined data set we implemented separate models for each gene. The Metropolis Marcov chain Monte Carlo (MCMC) consisted of two independent runs of 10×10^6 generations in which trees were sampled every 10^3 generations. Convergence was assessed once the average standard deviation split of frequencies approached zero in the program TRACER 1.6 (Rambaut and Drummond, 2013). The first 20% of the trees were discarded as burn-in, and a majority rule consensus tree with values representing posterior probabilities was constructed with the remaining trees.

2.4. Haplotype network

The allele phase of each *GHR* nuclear gene was resolved using the coalescent-based Bayesian method of the Phase algorithm (Stephens et al., 2001; Stephens and Donnelly, 2003) in DNASP 5 (Librado and Rozas, 2009). We used the resulting highest probability haplotypes for further analysis.

To explore spatial distribution of genetic variation, we constructed haplotype networks using the Median-Joining algorithm (Bandelt et al., 1999) in the program NETWORK 6.4.1.1. The mtDNA haplotype network was built with 111 sequences; we excluded the sequence KF885885.1Chamela of 1309 bp. The nDNA network of the *GHR* gene was constructed with the 78-allele sequences.

2.5. Phylogeographic structure

We calculated two measures of population differentiation in PERMUT CPSSR 2.0: G_{ST} and N_{ST} (Pons and Petit, 1996; Burban et al., 1999). If N_{ST} is significantly higher than G_{ST} this could indicate that the species presents some degree of phylogeographic structure.

We explored genetic structure using mtDNA in the program GENELAND (Guillot et al., 2005a), which applies a spatial statistical model and MCMC technique to estimate the number of populations (K) and to locate genetic discontinuities among populations (Guillot et al., 2005b). Ten MCMC iterations were performed, K was allowed to vary from 1 to 10, and 10^6 MCMC were run using a thinning interval of 100 generations with a maximum Poisson-Voronoi rate fixed at 300. The posterior probability of subpopulation membership was computed for each pixel of the spatial domain (200 × 100 pixels) using a burn-in of 20%.

Populations were grouped according to the major clades and haplogroups identified in the phylogenetic trees and in the haplotype network. We found three main groups: western, central and eastern. Analysis of molecular variance (AMOVA; Excoffier et al., 1992) was performed on mtDNA groups using the program ARLEQUIN 3.5.1.3 (Schneider et al., 2000).

We did a Mantel Tests to evaluate whether there is a significant correlation between mean genetic distances (Kimura two parameter model, 2KP) and geographic distances (Km). The analysis was performed with mtDNA and nDNA using XLSTAT 2008 (XLSTAT, 2015), which uses a Pearson correlation with 10,000 permutations to assign a significance level. We set α value to 0.05.

2.6. Genetic diversity and demographic analyses

We used DNASP 5 (Librado and Rozas, 2009) to calculate haplotype diversity (*h*), nucleotide diversity (π) and number of segregating sites for the three groups and for each data set (mtDNA and nDNA). Finally, we calculated the haplotidic frequencies to estimate the genetic diversity and divergence between groups.

We performed Tajima's *D* (Tajima, 1989) and Fu's *Fs* (Fu, 1997) tests to evaluate whether sequences conformed to a neutral model of evolution. A significant negative value is consider a signal of recent population expansion. Statistical significance was determined using the coalescent simulator in DNASP 5 (Librado and Rozas, 2009).

2.7. Divergence time

Estimation of divergence time for major clades (time to the most recent common ancestor TMRCA) was conducted with the mtDNA data in BEAST 1.8.0 (Drummond et al., 2012). We used only unique haplotypes (85 sequences). A Yule speciation tree was used as the prior; Bayesian phylogenetic analyses were performed under the assumption of a relaxed molecular clock and a log normal distribution. We used three calibration dates. One was based on the fossil *Copemys russelli* from 14.8 Ma (Woodburne et al., 1990) as the minimum age of the ancestor for some neotomines. The second was the maximum (11 Ma) and minimum (7 Ma) estimations of the origin of the Neotominae group by Steppan et al. (2004). The third calibration date was the Peromyscine group radiation 4.5 Ma, with a 95% confidence interval of ± 1.1 Ma, by Engel et al. (1998). Two independent MCMC analysis were run for 20×10^6 generations; parameters were sampled each 10^3 generations. We checked convergence statistics for effective sample sizes in TRACER 1.6 (Rambaut and Drummond, 2013), and a consensus tree was generated using TREE ANNOTATOR 1.7.4 (available in the BEAST package) with 40×10^3 trees after discarding 10% of the trees as burn-in.

3. Results

3.1. Sequence variation

We obtained 112 mtDNA sequences (1331 base pairs, bp) and 39 nDNA sequences (843 bp) of *O. banderanus*. We did not find stop codons in the alignment of sequences translated into aminoacids. There were two insertions/deletions of one nucleotide in the positions 423 and 1330 in mtDNA alignment. Nucleotide frequencies for mtDNA were as follows: 36% A, 22.5% C, 8.9% G and 32.5% T; and in nDNA: 27.4% A, 28.9% C, 22.7% G and 21% T. All sequences were deposited in GenBank (Accession numbers: MH495819-MH495969; Supporting Information, Table S1).

3.2. Phylogenetic analysis

Phylogenetic analyses based on BI and ML (Supporting Information, Fig. S1) with the combined data set (38 samples, 2183 bp) were largely congruent. We confirmed the monophyly of *Osgoodomys* by the highest support values. Our results indicated three major clades within *Osgoodomys*: eastern, central and western clades. *Peromyscus eremicus* was the sister species of *O. banderanus*, with a genetic distance (2KP) between species of 20% in mtDNA and 2.8% in nDNA.

Analysis of mtDNA (112 samples, 1331 bp) under the BI and ML methods produced trees that showed congruence with the combined data topologies (Fig. 2). Our results uncovered the three major clades with high support values (BP ≥ 80, pp ≥ 0.99), except for a bootstrap value in ML < 70% for the node that divided the western and central clades.

The eastern clade included individuals from central and southern Guerrero. This clade represents the first cladogenetic event among *Osgoodomys* populations, and it is located at the southern of the *Osgoodomys*' distribution. This clade is evidently divergent from the others, with a mean genetic distance in mtDNA of 8.8 and 9.1%, respectively (Supporting Information, Table S3). The genetic distance in mtDNA within the eastern clade ranged from 0.08 to 1.1%. The central clade included individuals from the states of Colima, Michoacán and northern Guerrero. Genetic distance within this clade ranged from 0.07 to 2.8%, and the mean genetic distance between central and western/eastern clades was 5%. Finally, the western clade included individuals from states of Nayarit, Jalisco and northern Colima. The genetic distance within this clade ranged from 0.08 to 1.5%.

Phylogenetic analyses were not consistent with the proposed distribution of the two subspecies *O. b. banderanus* and *O. b. vicinior*. While the central clade included mainly individuals of the *O. b. banderanus* subspecies, it also contained individuals of the *O. b. vicinior* subespecies from the localities of Ichamio, Michoacán, and Campo Morado, Guerrero. The eastern clade included individuals of the *O. b. banderanus* subspecies from the localities Coacoyulichan and Palma del Cayaco, Guerrero, but it also included two individuals of the *O. b. vicinior* subspecies from Chilpancingo de los Bravo, Guerrero.

3.3. Haplotype networks

The mitochondrial median joining network consisted of 73 haplotypes, which were divided in three main haplogroups (Fig. 3) identical to the three major clades found in the phylogenetic analyses. The first haplogroup was located in the state of Guerrero and is differentiated from the other two haplogroups by 88 mutations. The second group extended from Colima, Michoacán, to northern Guerrero, and is separated by 30 mutations from the third group, which is formed by samples from Nayarit, Jalisco and northern Colima. The nuclear network was constructed with 36 alleles (Fig. 3). Differences among nuclear alleles were of one or two mutations; only in two cases were three mutations found between alleles. Alleles 1, 3, 5, 6, and 8–11 were exclusive to the western group, while alleles 13–34 were exclusive to the central group. Nevertheless, alleles 2, 4, 7, and 12 were shared among localities from the western and central groups. Finally, alleles 35 and 36 were only present in the eastern group.

3.4. Phylogeographic structure

The test for phylogeographic structure of haplotype variation across the distribution of the species showed that N_{ST} (0.207) was significantly higher ($p < 0.05$) than G_{ST} (0.126), indicating that closely related haplotypes were more likely to co-occur in the same region. GENELAND software detected five genetic clusters in the sample. We identified the same three main groups described in the phylogenetic and phylogeographic analyses, but the western and central groups presented substructure (Supporting Information, Fig. S2). In the western group, samples from Nayarit formed a separate cluster from the samples from Jalisco and northern Colima. The central group was divided into two clusters, one from southeastern Michoacán and another with samples from Colima, central Michoacán and northern Guerrero. Finally, the eastern group remained conformed by samples from central and southern Guerrero.

The AMOVA analysis indicated that 72.5% of the variation is attributable to differences among the three groups, whereas 17% of the variation is due to the differences among localities within each group, and 10.5% of the variation is explained by differences among individuals within the groups. All Φ values were significant ($p < 0.05$; Table 1).

The Mantel test revealed that the genetic distance matrix is significantly correlated with the geographic distance matrix of mtDNA ($r = 0.55$, $p < 0.0001$) and nDNA ($r = 0.51$, $p < 0.0001$), respectively (Supporting Information, Fig. S3), suggesting isolation by distance among the three groups or populations.

3.5. Genetic diversity and demographic analyses

According to the results of the phylogenetic and phylogeographic analyses, there are three well-supported groups: western, central and eastern. Genetic diversity indices for the mtDNA and nDNA for each group showed low values of nucleotide diversity (0.005–0.015), in contrast with high values of haplotype diversity (0.667–0.986; Table 2). The number of synonymous and non-synonymous mutations was similar in the western and eastern groups, whereas the central group had more non-synonym mutations.

Tajimás *D* was negative and non-significant for the three groups. Fu's *Fs*, which is based on haplotype distribution, was negative in all cases but it was significant only for the central group, revealing that for this group there is an excess of rare haplotypes relative to the expectation under neutrality. The mismatch distribution (Fig. 4) showed a multimodal distribution for both the eastern and western clades, indicating demographic stability in those populations, while the central clade had a unimodal distribution, characteristic of populations that have undergone a recent expansion. Nevertheless, all groups had non-significant H_{rg} and *SSD* values, suggesting that the hypothesis of population expansion cannot be rejected for any of the groups.

3.6. Divergence times

The tree topologies found in the molecular dating analysis were identical to the BI and ML trees. The result of divergence time estimation revealed that *Osgoodomys* is a monophyletic group that diverged from the rest of the species *c.* 4.73 Ma (95% of higher posterior density, HDP: 3.25–6.23). The eastern clade within *Osgoodomys* split from the western and central clades approximately 3.84 Ma (95% HDP: 2.19–5.49), while the split between the western and central clades was dated *c.* 3.21 Ma (95% HDP: 1.66–4.46) (Fig. 5).

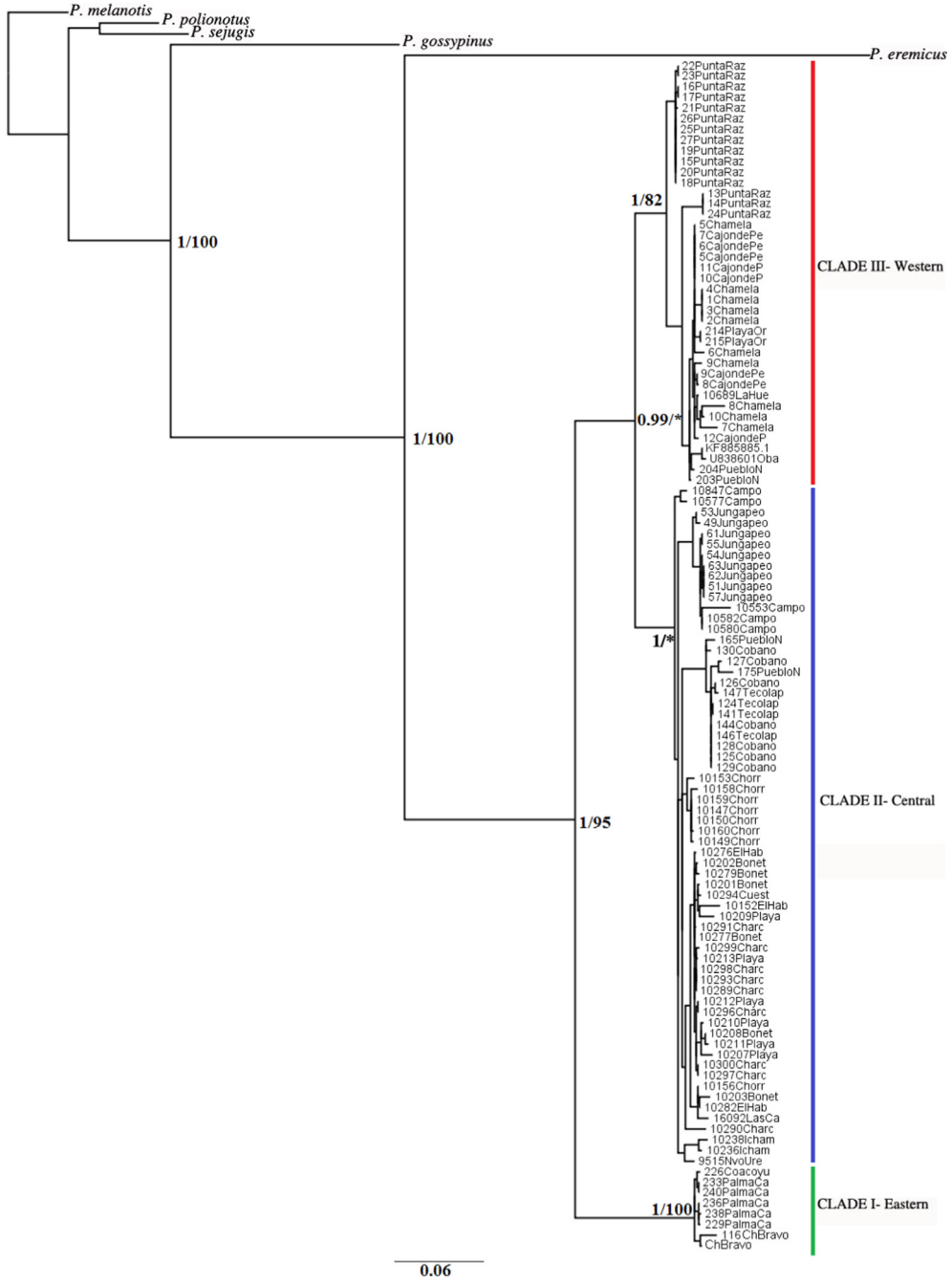


Fig. 2. Phylogenetic hypothesis of *Osgoodomys* based on *ND3*, *tRNA-Arginine*, *ND4L* and partial *ND4* mtDNA genes, using Bayesian inference and Maximum Likelihood analyses. The tree topology resulted in three major clades. Posterior probability values (> 0.7) and bootstrap values (> 70%) are separated by a slash at each node.

4. Discussion

4.1. Monophyly of Osgoodomys

Our concatenated results showed that *Osgoodomys* is a monophyletic group. The genetic distance using mitochondrial loci between *Osgoodomys* and *Peromyscus* (20%), is similar to the value reported by Platt II et al. (2015) using *cytochrome-b* gene (15%).

Osgoodomys is recognized as a distinct genus from *Peromyscus* based on morphological features such as the loss of accessory reproductive glands, pronounced supraorbital crests, reduction of the carotid circulatory pattern and the lack of sphenopalatine vacuities (Carleton, 1980; Musser and Carleton, 2005). Additionally, Hooper (1958) documented the unusual nature of the phallus of *O. banderanus.* The phylogenetic relationships among genera are similar to those found in previous studies that included more samples of *Peromyscus* species. *Osgoodomys* and other genera such as *Habromys*, *Megadontomys*, *Neotomodon* and *Podomys* are mixed with other *Peromyscus* species, confirming once more that *Peromyscus* forms a paraphyletic assemblage (Engel et al., 1998; Bradley et al., 2004, 2007; Miller and Engstrom, 2008; Platt II et al., 2015).

4.2. Intraspecific relationships

In our analyses, the mtDNA and the nDNA exhibited different levels of resolution. These differences between mitochondrial and nuclear loci

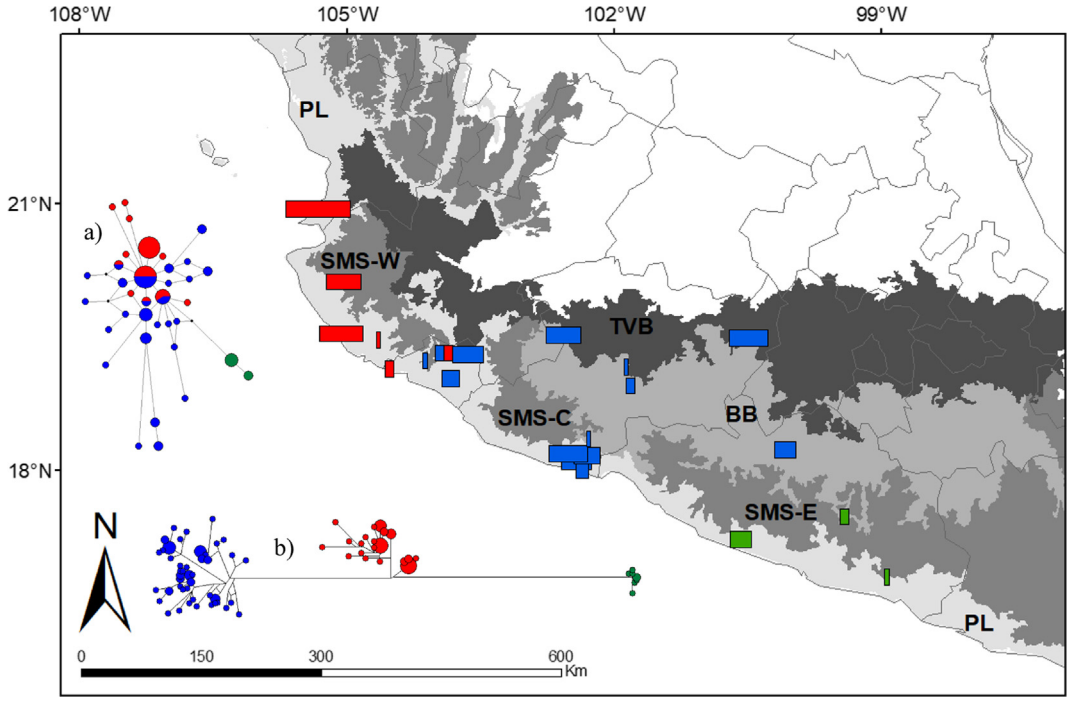


Fig. 3. Phylogeographic structure detected in *Osgoodomys*. The red color shows the western haplogroup, blue the central haplogroup, and green the eastern haplogroup. The map shows the distribution of each genetic group, and the bar size is proportional to number of samples per locality. At the bottom left, the networks for each molecular marker: (a) nuclear gene *GHR* and (b) the mitochondrial loci *ND3*, *tRNA-Arginine*, *ND4L* and partial *ND4*. The circle size is proportional to the frequency of haplotypes or alleles, and the line length to the number of mutations. PL: Pacific lowlands, BB: Balsas Basin, TVB: Transmexican Volcanic Belt. The province SMS: Sierra Madre del Sur is divided in the western, central and eastern subprovinces denoted by letters W, C and E, according to Morrone (2017). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Analysis of Molecular Variance (AMOVA) based on *ND3, tRNA-Arginine, ND4L* and partial *ND4* sequences of three genetic groups (western, central and eastern) of *Osgoodomys*.

Source of variation	d.f.	Percentage of variation	Fixation index
Among groups	3	72.5	Φ_{CT} = 0.7411
Among populations within groups	18	17	Φ_{SC} = 0.1534
Within populations	38	10.5	Φ_{ST} = 0.8740
Total	110		

All Φ values are statistically significant ($p < 0.05$).

can be attributable to a smaller effective population size and a higher mutation rate of the mtDNA (Piganeau and Eyre-Walker, 2009), and in general, nDNA has slower mutation rates which may lead to findings of incomplete lineage sorting. It is necessary to include more nDNA sequences from eastern group to analyze the genetic structure among the three groups. However, for each group, both markers revealed high haplotype diversity (~0.9) and a relatively low nucleotide diversity, so there is a high probability that two randomly chosen haplotypes are different, and a low probability that two randomly chosen homologous nucleotides are different (Avise, 2000). The nDNA network did not define a genetic structure and there were shared alleles between localities from the western and the central groups, however the mtDNA haplotype network, like the phylogenetic analysis, revealed three haplogroups that did not share any haplotypes. This mitochondrial result suggests the absence of admixture among individuals from the different haplogroups. In addition, the five clusters generated by GENELAND emphasize the internal structure of the western and central groups, which have a broader range distribution compared with eastern group.

The genetic distances for mtDNA within each clade were similar to the intraspecific values documented for other small rodent species, such

Table 2
Values of nucleotide diversity of a) mitochondrial loci and b) nuclear gene in *Osgoodomys*. Number of individuals per group (*N*), segregating sites (*S*), haplotype number (*H*), haplotype diversity with standard deviation (*dh/DE*), nucleotide diversity with standard deviation (*π/DE*), synonymous mutations (*mS*), non-synonymous mutations (*mNS*), haplotype richness adjusted to 7 (*rh*), genetic divergence within populations (*DHs*), total genetic divergence (*DHt*), and relative genetic differentation (*DGst*).

Haplogroup	*N*	*S*	*H*	*dh/DE*	*π/DE*	*mS*	*mNS*	*rh(7)*	*DHs*	*DHt*	*DGst*
(a) mtDNA											
Eastern	7	19	6	0.952/0.096	0.0052/0.0014	6	11	5.00	0.957	1	0.43
Central	64	145	46	0.986/0.0060	0.0152/0.0007	61	83	5.72	0.965	1	0.35
Western	40	78	21	0.938/0.0004	0.0119/0.0008	36	40	4.85	0.952	1	0.48
Total	111	271	73	0.987/0.0040	0.0359/0.0023	137	120	–	0.957	1	0.042
(b) nDNA											
Eastern	2	2	2	0.667 (0.204)	0.0016 (0.0005)	1	1	0.941	0.925	0.958	0.034
Central	22	22	26	0.965 (0.014)	0.0044 (0.0003)	9	13	0.953	0.928	0.951	0.024
Western	15	32	12	0.807 (0.055)	0.002 (0.0003)	7	7	0.866	0.907	0.982	0.077
Total	39	32	40	0.942 (0.015)	0.004 (0.0003)	15	19	–	0.920	0.963	0.0454

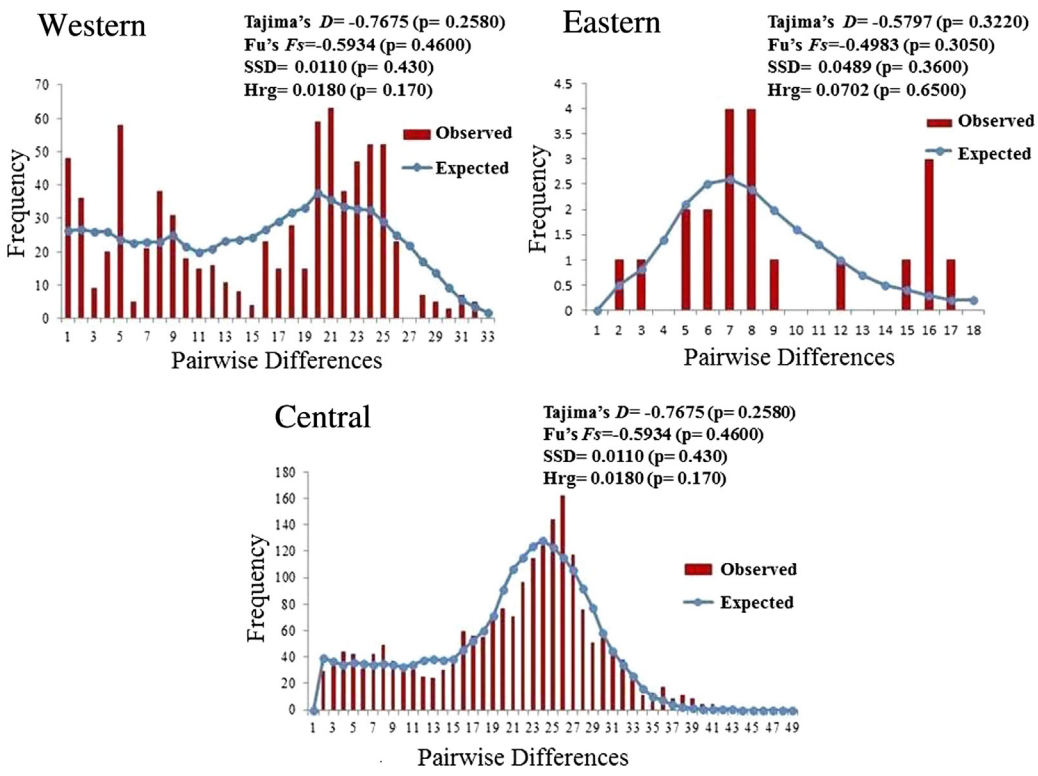
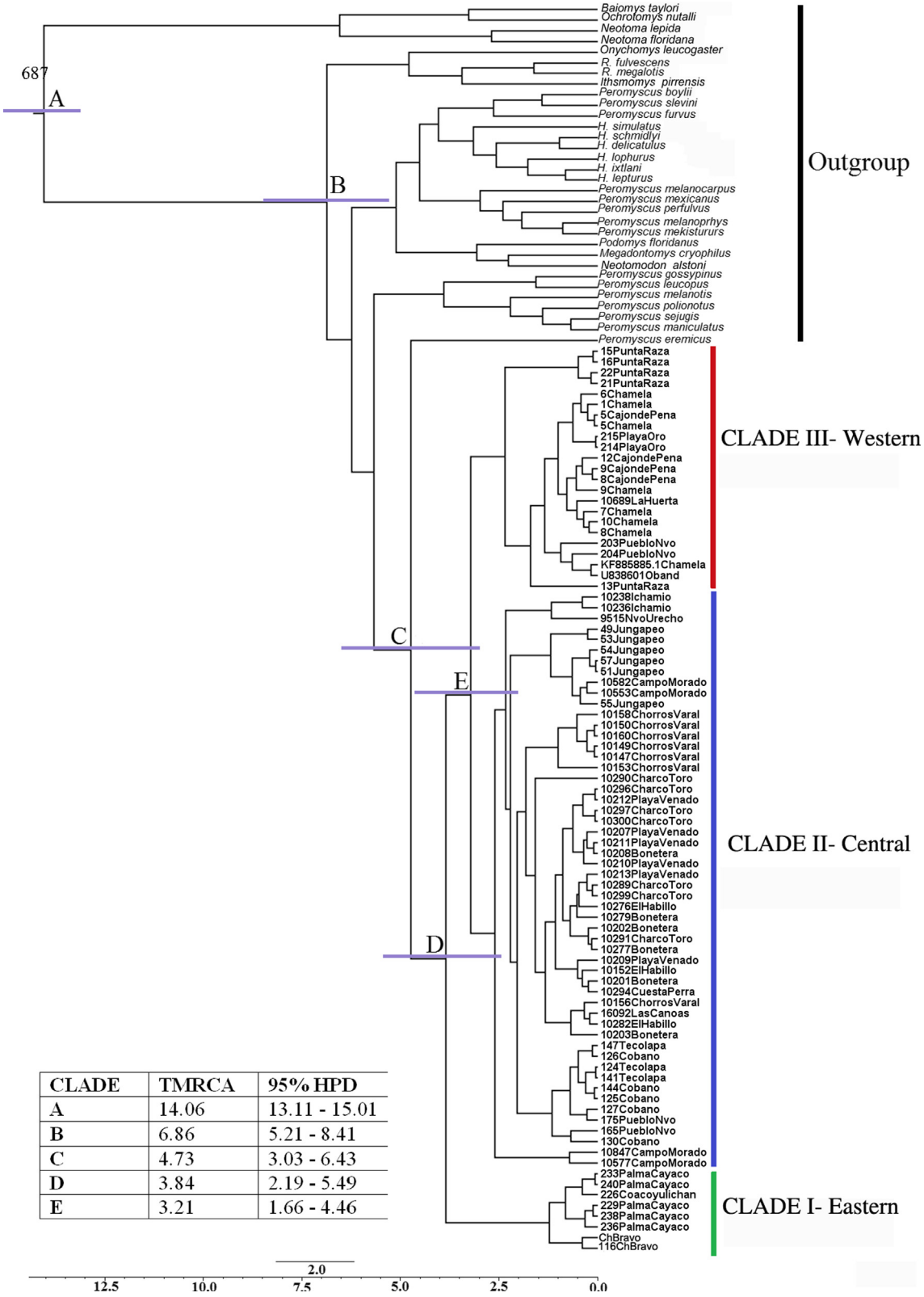


Fig. 4. Observed mismatch distributions and their fit to expected model of sudden demographic expansion for Osgood's deermouse based on mtDNA sequences of the western, central and eastern groups. The bars indicate the observed distribution, and the dotted line indicates the expected distribution. SSD: Sum of Square Deviations, H_{rg}: Harpending's raggedness index. Neutrality tests Tajima's *D* and Fu's *Fs* are shown.

as *Peromyscus sejugis* (0.7%; Walker et al., 2006), *P. maniculatus* (0.68%; Walker et al., 2006) and *P. furvus* (1.5%; Ávila-Valle et al., 2012). Nevertheless, if we consider a genetic distance ≥ 3% to recognize different species with *ND3*, *tRNA-Arginine*, *ND4L*, partial *ND4* genes (Chirhart et al., 2001; Ávila-Valle et al., 2012), the genetic divergence among clades is high, particularly in the eastern clade. Indeed, the divergence values among the three major clades (2KP genetic distance 5–9%) are comparable to the genetic divergence reported by Castañeda-Rico et al. (2014) between two recognized species of the *Peromyscus melanophrys* group (9.3% between *P. melanoprhys* and *P. perfulvus*).

Our results are not consistent with the recognized subspecies of *O. banderanus* (Hall, 1981). The subspecies *O. b. banderanus* is described as having a wide distribution from southeastern Nayarit to western Guerrero excluding the Balsas Basin, whereas the subspecies *O. b. vicinior* is restricted to the Balsas Basin. Osgood (1909) in his description of *O. b. vicinior* and *O. b. banderanus*, specified that some morphological characters vary according to the location of the samples and to confirm its validity would require analyzing numerous series of specimens. However, there has not been a recent and exhaustive review of morphological differences along the geographical distribution of the two subspecies. Our results are based on the largest number of DNA sequences of *O. banderanus* until now, and suggest that the classification of the two subspecies should be reviewed. Furthermore, in our phylogenetic hypothesis we found that individuals of two morphological subspecies are mixed in various clades, and individuals of the subspecies *O. b. banderanus* are present in more than one clade. Nevertheless, considering the genetic distances calculated with *ND3*, *tRNA-Arginine*, *ND4L*, partial *ND4* genes (5–9%), the three clades in phylogenetic and phylogeographic analyzes, in addition to nucleotide substitutions (30–88) we suggest that *Osgoodomys* is composed by three independent species. The posterior probability values obtained in Bayesian inferences support the presence of three species, notwithstanding, we found a low bootstrap value between central and western groups in ML construction. This phenomenon has been previously detected in genetic of population analyzes, where was suggested that Bayesian methods fares better than ML approaches (Beerli, 2006), but we recommend to add more loci to clarify and verify that there are three species (instead of only two: central/western and eastern) within *Osgoodomys*. Our conclusion is supported by the genetic species concept (Bradley and Baker, 2001; Baker and Bradley, 2006), which has been useful to detect cryptic lineages that eventually have been recognized as different species in mammals, as in rodents (Hanson et al., 2010; Ávila-Valle et al., 2012; Almendra et al., 2014), opossums (Arcangeli et al., 2018), shrews (Ohdachi et al., 2006), or bats (Velazco and Patterson, 2013). Taking into consideration the original subspecies descriptions, we suggest that the western clade be recognized as *O. banderanus* (type locality in Valle de Banderas, Nayarit), the central clade as *O. vicinior* (type locality in La Salada, Michoacán), and the eastern clade as *Osgoodomys* new species 1. Nevertheless, these three or two hypothetical species need to be characterized morphologically to confirm our results.

The geographic limits among clades of *Osgoodomys* coincide with the Balsas River, the Transmexican Volcanic Belt and the Sierra Madre del Sur, as in other lowland mammal species (Amman and Bradley, 2004; Ortega et al., 2009; Hernández-Canchola and León-Paniagua, 2017). Mountain ranges such as the Transmexican Volcanic Belt and Sierra Madre del Sur are relevant geographic features for the diversification processes of lowland species such as *O. banderanus* that inhabit altitudes below 1500 m above sea level (Álvarez, 1968). The higher parts of the mountain systems represent insurmountable limits, especially to species that only inhabit tropical dry forest. Nevertheless, the lowlands of the Balsas Basis also influenced the evolutionary history of this rodent. Amman and Bradley (2004) evaluated the genetic variation of the pygmy mice *Baiomys* and proposed that the Balsas River and its tributaries may prohibit gene flow west of the Sierra Madre del Sur range and in the lower elevation zones between Transmexican Volcanic Belt and the eastern slopes of the Sierra Madre del Sur. In addition, this phylogeographical structure is similar to the one reported in the frugivorous bat *Sturnira parvidens* (Hernández-Canchola and León-Paniagua, 2017). The effect of geographic barriers could be reinforced by the low dispersion rate of this endemic rodent. Both genes indicated isolation by distance among the groups, and we relate this result with the dispersion ability of *O. banderanus*. In a study in Colima, Mexico, the average distance between successive captures of males and females of *O. banderanus* was 19 m (Poindexter et al., 2013). If we compare this



CLADE	TMRCA	95% HPD
A	14.06	13.11 - 15.01
B	6.86	5.21 - 8.41
C	4.73	3.03 - 6.43
D	3.84	2.19 - 5.49
E	3.21	1.66 - 4.46

Fig. 5. Divergence time estimation of the three main clades of *Osgoodomys*. Letters above purple bars correspond to the time to most recent common ancestor values (TMRCA) shown in the table. The horizontal bars show 95% confidence interval in Mya (95% HDP).

distance with other mammals, like bats or carnivores, *O. banderanus* has low vagility, likely greatly limiting gene flow between populations.

Finally, the phylogeographic structure detected within *Osgoodomys* is not congruent with the four cytotypes reported by Núñez-Garduño et al. (1999). In that study, the authors inferred the distribution karyotypes from Guerrero and Jalisco because no samples were analyzed in those Mexican states. The absence of cytotype samples from Guerrero is particularly unfortunate because the eastern group displayed the highest divergence respect to the rest of the samples, this area represents the southern limit of the species, and because both the

central and eastern clades are found there.

4.3. Biogeography

The complex topographic characteristics of Mexico and the climatic fluctuations that began in the Pliocene and continued in the Pleistocene have served as an incubator of peromyscine species (Dawson, 2005). The origin of genus *Peromyscus* began approximately *c.* 6 Ma (95% HPD: 3.37–9.08), followed by a rapid diversification where several major *Peromyscus* lineages evolved, including the genus *Osgoodomys* (Platt II et al., 2015). According to our data, the split of *Osgoodomys* and *Peromyscus* took place in the Early Pliocene, *c.* 4.73 Ma (95% HDP: 3.25–6.23), before the proposed split between *Peromyscus* and *Habromys* (León-Paniagua et al., 2007). Hooper (1958) proposed that *O. banderanus* is a relict species which may represent an early stage in the evolution of *Peromyscus*, an idea supported by our data. Besides, the origin of *Osgoodomys* during the Pliocene is congruent with the theory that lowland tropical species generally originated previous to the Pleistocene (Pennington et al., 2004; Zarza et al., 2008).

The phylogeographic structure detected in *Osgoodomys* coincides with lowlands surrounding the subprovinces of the Sierra Madre del Sur reported by Morrone (2017), who considered many endemism of different taxa to classify these subprovinces. The smallest areas inhabited by haplogroups of *Osgoodomys* are located in the western Sierra Madre del Sur subprovince (western haplogroup) and the eastern Sierra Madre del Sur subprovince (eastern haplogroup). Nevertheless, the specimens of *Osgoodomys* from the central clade inhabit the central Sierra Madre del Sur subprovince and the Balsas Basin, and this area represents the largest surface occupied by any of the haplogroups. We hypothesize that historical events related with geographical barriers promoted this phylogeographical structure, by both vicariance and dispersion events.

The three clades within *Osgoodomys* split during the Pliocene, like other cricetid species of the genus *Habromys* and the *P. melanophrys* group (León-Paniagua et al., 2007; Castañeda-Rico et al., 2014). The eastern group represents the first cladogenetic event within *Osgoodomys*, which occurred near the transition between stages of the Pliocene (*c.* 3.84 Ma, 95% HDP: 2.19–5.49). Samples from this clade revealed the highest number of nucleotide substitutions (88) of all of the samples, which could be explained by vicariance since the Balsas River is a lowland geographic limit between the central and eastern clades. The emergence of the arid Balsas Basin ocurred in the Late Pliocene or Early Pleistocene as a result of the rising of the Transmexican Volcanic Belt (De Cserna et al., 1974; Becerra, 2005; Bryson Jr. et al., 2011). In addition, this region was likely flooded periodically (Gómez-Tuena and Carrasco-Núñez, 2000; Hardy et al., 2013) and may be an effective barrier to *Osgoodomys* populations, as it is for other lowland and montane species (Amman and Bradley, 2004; Devitt, 2006; Zarza et al., 2008; Ortega et al., 2009; Hardy et al., 2013; Reyes-Velasco et al., 2013; Hernández-Canchola and León-Paniagua, 2017).

The split between populations from western and central clades occurred *c.* 3.21 Ma (95% HDP: 1.66–4.46), during the Late Pliocene. The divergence of these groups may be associated with the presence of the Transmexican Volcanic Belt. Although the uplift of this mountain chain was during the Miocene, it has been suggested that dispersal and differentiation on a matrix previously shaped by large-scale landscape events may be a major driver of speciation (Smith et al., 2014). As such, we hypothesize that the western clade dispersed to its northern distribution, and the occurrence of Transmexican Volcanic Belt promoted the differentiation of this population. This pattern of divergence to the north and south of the Transmexican Volcanic Belt has been reported in other vertebrate species, including reptiles, amphibians, rodents and fish (Mateos et al., 2002; Zaldívar-Riveron et al., 2004; Zarza et al., 2008; Hardy et al., 2013). This genetic break is similar to the one reported in the mouse *Peromyscus perfulvus* (Castañeda-Rico et al., 2014), a species with a geographic range and an ecological affinity that is highly similar to *Osgoodomys*. The subspecies of *P. perfulvus* split into two clades: one along the Pacific coast of Jalisco and other in the Balsas Basin in Michoacán, similar to the western and central populations of *Osgoodomys*. This evidence may suggest similar biogeographic histories (Musser and Carleton, 2005) and supports the relevance of mountain ranges in western Mexico as geographic barriers for rodents and other mammals. Finally, the central haplogroup is the only one that showed evidence of population expansion, possibly as a consequence of the differentiation of this lineage and the subsequent invasion by dispersion throughout the lowlands surrounding the central Sierra Madre del Sur and Balsas Basin.

The tropical dry forest ecosystem is present along most of the geographic range of *Osgoodomys* and its establishment in Mexico has been calculated at approximately 20–30 Ma (Becerra, 2005). Is likely that the tropical dry forest has had a very important influence in the dispersal and divergence of the *Osgoodomys* populations. This ecosystem is geographically and ecologically isolated from moister forest regions, but is in contact with other habitats due to the interdigitation of the mountain ranges (Ortega et al., 2009). The geological and historical events related to the Transmexican Volcanic Belt and the Sierra Madre del Sur have led to repeated changes in the distribution and limits of tropical dry forest, and as a consequence, its associated biota. The complex history, ecological particularities, and geographic limits of this ecosystem in western Mexico have promoted high biodiversity and the occurrence of many Mexican endemisms. The rodents *Osgoodomys*, *Xenomys nelsoni*, *Hodomys alleni, Peromyscus perfulvus* and other mammals like the pygmy spotted skunk *Spilogale pygmaea,* the mouse opossums *Tlacuatzin balsasensis* and *T. sinaloae*, or the bat *Musonycteris harrisoni* are endemic to the tropical dry forest in western Mexico (Becerra and Venable, 1999; Zaldívar-Riverón et al., 2004; Devitt, 2006; Ortega et al., 2009; Arcangeli et al., 2018).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.06.034.

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