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Phylogeography of the bark beetle *Dendroctonus mexicanus* Hopkins (Coleoptera: Curculionidae: Scolytinae)

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ABSTRACT

Dendroctonus mexicanus is polyphagous within the *Pinus* genus and has a wide geographical distribution in Mexico and Guatemala, We examined the pattern of genetic variation across the range of this species to explore its demographic history and its phylogeographic pattern. Analysis of the mtDNA sequences of 173 individuals from 25 Mexican populations allowed to us identify 53 geographically structured haplotypes. High haplotype and low nucleotide diversities and Tajima's *D* indicate that D. *mexicanus* experienced rapid population expansion during its dispersal across mountain systems within its current range. The nested clade phylogeographic analysis indicates that the phylogeographic pattern of *D. mexicanus* is explained by continuous dispersion among lineages from the Sierra Madre Occidental, the Sierra Madre Oriental and the Trans-Mexican Volcanic Belt. However, we also observed isolation events among haplotypes from the Cofre de Perote/Trans-Mexican Volcanic Belt/Sierra Madre Oriental and the Trans-Mexican Volcanic Belt/Sierra Madre del Sur, which is consistent with the present conformation of mountain systems in Mexico and the emergence of geographical barriers during the Pleistocene.

1. Introduction

Population genetic studies are fundamental to our understanding of how evolutionary factors have influenced the genetic structure of phytophagous insects (Roderick, 1996). Within *Dendroctonus* species, population genetic studies have commonly been carried out in a context that is independent of history and geographical landscape (Stock and Amman, 1980, 1985; Sturgeon and Mitton, 1986; Langer and Spencer, 1991; Kelley et al., 2000). In those studies where geographical perspective has been introduced, the landscape has been considered to be uniform, isotropic, and not linked to the history of the population (Anderson et al., 1979; Namkoong et al., 1979; Stock et al., 1984; Roberds et al., 1987; Kelley et al., 1999, 2000; Six et al., 1999; Zuniga et al., 2006; Schrey et al., 2008).

Phylogeographic analysis is an alternative that permits the linking of the patterns of genetic variation of populations to biogeographical histories (Avise, 2000). The combination of paleontological, biogeographical, and genetic data of taxa from Europe and North America has allowed for description of diverse phylogeographic patterns and deduction of historical events that may have yielded these patterns (Cruzan and Templeton, 2000; Hewitt. 2004; Schmitt, 2007). For example, it has been determined that the phylogeographies of various European coleopteran and scolytine species are the result of contraction and expansion events experienced during and after Pleistocene glaciations (Juan et al., 1995; Stauffer et al., 1999; Kohlmayr et al., 2002; Ritzerow et al., 2004; Faccoli et al., 2005; Horn et al., 20(6). North America differs significantly from Europe in geological history and geographical features (Brouillet and Whetstone, 1993). Therefore, many of the factors used to explain European phylogeographic patterns cannot be applied to this part of the world. In fact, phylogeographic studies of North American scolytines, including some *Dendroctonus* species (Cognato et al., 2003; Maroja et al., 2007; Mock et al., 2007) suggest that phylogeographies of these bark beetles can be complex and diverse.

The events shaping the geomorphology and structural geology of North America did not occur at the same time across the continent. Mexico in particular is characterized by a complex geological history (Perrusquia-Villafranca. 1998). The main physiographic features of Mexico were formed by the Laramide orogeny in the late Cretaceous ~65 Myr BP, but its present geomorphological configuration developed gradually and was not completed until the early Holocene. Therefore, the main mountain systems of Mexico, the Sierra Madre Occidental, the Sierra Madre Oriental, the Sierra Madre del Sur, and the Trans-Mexican Volcanic Belt are relatively recent compared to other mountain systems in North America (Maldonado-Koerclell, 1964; Brouillet and Whets tone, 1993).

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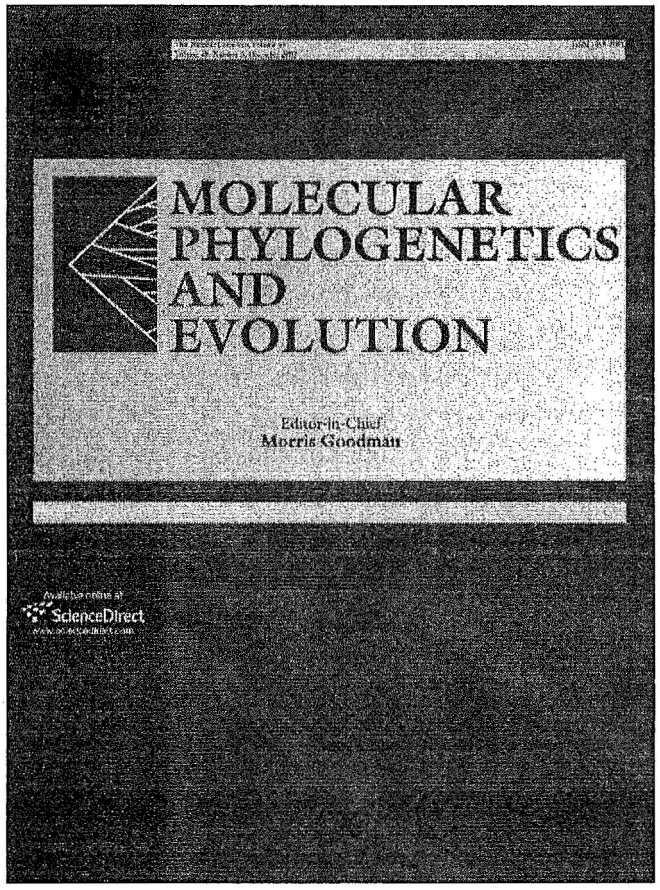

Coniferous forests have a long and extensive history in Mexico (Graham, 1993, 1999). The penetration of *Pinus* into Mexico occurred by two routes from the Rocky Mountains (Farjon and Styles, 1997) as a consequence of climate shifts associated with the uplifting of the western North American mountains. Floristic relationships and fossil records suggest that the first migration occurred during the Oligocene into the Sierra Madre Occidental (Miller, 1977; Graham, 1999), and the second in the early Pliocene (5 Myr BP) along the Sierra Madre Oriental (Martin and Harrell, 1957; Graham, 1999). However, pollen records from the mid-Miocene and Oligocene for the Sierra Madre Oriental and Chiapas suggest pines were present even earlier in Mexico and Central America (Millar, 1998).

There are no detailed data about climatic shifts in Mexico during the glacial periods; however, there is consistent evidence of these shifts from the last glacial maximum (~18,000 yr BP). The impact of glaciations on diverse taxa was not similar across North America. Apparently the effect on coniferous forests in Mexico was less severe than in Canada and the USA because its impact was more altitudinal than latitudinal, i.e., the effect was limited to the highest mountains (>3800 m) (Lachniet and Vazquez-Selern, 2005). Ice masses covering the highest mountains extended downward affecting the upper tree line, which dropped 600-800 m below its present position (Brown, 1985; Graham, 1993; McDonald, 1998). The paleoecological evidence from 12,500 to 9000 yr BP indicates that the climate was cold and moist, and coniferous forest reached their maximum development with a broader distribution than is present in Mexico today. After 9000 yr BP, the climate became warmer, high volcanic activity was common, and many regional changes in structural geology caused the connection and reconnection of hydrologic basins. All of these events contributed to forest fragmentation, and consequently could have promoted the isolation of bark beetle populations,

The purpose of the present study was to determine the phylogeographic structure of *Dendroctonus mexicanus* Hopkins. First, we examined the genetic diversity of mtDNA to explore whether or not the molecular signature depicts a continuous demographic expansion of populations occupying different regions and mountain systems. Second, we investigated whether or not the phylogeographic structure of D. *mexicanus* can be explained by the geomorphologic configuration of Mexico. Finally, we estimated divergence time among populations to determine whether these coincide with the current configuration of mountain systems in Mexico.

2. Materials and methods

2.1. Species

The geographic range of D. *mexicanus* in Mexico extends from the northern states of Chihuahua in the Sierra Madre Occidental (SMOC) and Nuevo Leon in the Sierra Madre Oriental (SMOR), to the southern states of Oaxaca in the Sierra Madre del Sur (SMS) and Chiapas in the Sierra Madre de Chiapas (Salinas-Moreno et al., 2004). This species also can be found in Guatemala, where, as in the southern states of Mexico, populations apparently are not abundant. D. *mexicanus* is considered a generalist species, i.e., polyphagous, because it colonizes 21 of the 47 pine species reported in Mexico predominantly from the Leiophyllae, Oocarpae, and Pseudostrobi subsections (Salinas-Moreno et al., 2004). Pinus *leiophylla, P. montezumae,* P. *oocarpa, P. pseudostrobus,* and P. *teocote* are its primary hosts. This bark beetle has two to four generations per year depending on temperature and elevation (Cibrian-Tovar et al., 1995; Salinas-Moreno et al., 2004), and is frequently found throughout its distribution in the same stands with D. *adjunctus* LeConte, D. *approximatus* Dietz, and D. brevicomis LeConte, D. *vitae* Wood, and D. *frontalis* (Salinas-Moreno et al., 2004).

2.2. Samples

Mountain systems in Mexico and Guatemala were visited on at least two occasions to collect samples covering most of the geographic range of D. *mexicanus*. To avoid bias in our inferences about phylogeographic structure (Petit et al., 2005), each mountain system was characterized by a sufficient number of populations, and each of them by at least five individuals. The SMOC, SMOR, and TMVB were represented by a minimum of four populations, and by more than 20 specimens in total. Due to the limited numbers of existing populations in the southern of Mexico and Guatemala, these areas were unfortunately not as well represented.

A total of 173 adult insects were collected from 25 geographically distinct populations of D. *mexicanus* from Mexico (Table 1, Fig. 3a). Insects were removed from recently infested trees to avoid analysis of genetically related individuals, except for populations from la Mesa, Predio Agua Blanca, and Milpillas in the TMVB. At these sites, we collected bark and logs from three different trees for each population, which were stored in separate boxes until adult emergence. Beetles from different boxes were analyzed. Because of the frequent coexistence of D. *mexicanus* with its sibling species, confirmation of specimen identification was based on morphological characters (Wood, 1982), the male seminal rod (Wood, 1982) and the chromosome number both of females and males (Lanier, 1981), all of which are unique and distinct characters of these species (Zufiiga et al., 2002).

In addition, we performed a phylogenetic reconstruction to assess that sequences used in this study belong exclusively to the species D. *mexicanus*. We included the sequences from sibling species deposited in GenBank by Kelley and Farrell (1998), and some of D. *frontalis* specimens obtained by us for this study. The sister genus *Tomicus* (in this case *T. piniperda),* which belongs to the tribe Tomicini was chosen as the outgroup (Wood, 1982). The reconstruction was performed at CIMMYT CRII (Crop Research Informatics lab.) using a Sun Microsystems Workstation with two AMD Opteron™ 246 Processors and 2 GB RAM. A Maximum Likelihood (ML) analysis was done in PAUP*(v.4.0b10; Swofford, 1998) using a heuristic search strategy with tree-bisection-reconnection, ACCTRAN, and 10 random-taxon-addition replicates, starting from a random tree. The optimal model for sequence evolution for our data set was assessed using the Akaike information criterion in MODELTEST v.3.7 (Posada and Crandall, 1998). Confidence at each node was assessed using a bootstrap analysis based on 5000 pseudoreplicates with 10 random-taxon-addition replicates per pseudoreplicate. The hypothesis of monophyly of D. *mexicanus* sequences was evaluated by the Shimodaira-Hasegawa test in PAUP·.

2.3. Mitochondrial DNA: extraction, amplification, and sequencing

A 246-bp fragment of the cytochrome oxidase I (COI) gene was amplified by polymerase chain reaction (PCR) from total genomic DNA from each bark beetle. This fragment is situated between positions 2505 and 2751 in the *Drosophila yakuba* mitochondrial genome, and was used because exploratory alignment analysis using all sequences of *Dendroctonus* deposited in the GenBank, and a panel of 15 sequences (700 bp) of D. *mexicanus* previously obtained by us, showed that the second half of the COI gene (and in particular the fragment used here) has an appropriate level of nucleotide divergence to assess differences among D. *mexicanus* populations and sibling species. To amplify this fragment, we designed the primers dmex685 (5'-CTTGCTACATACCATGGATCCC-3') and dmex1048 (5'-CTGGGTAATCAGAATAACGGCG-3') using complete COI sequences from Dendrccronus species deposited in the GenBank (Kelley and Farrell, 1998). PCR was performed in a 25 uL reaction volume using 5 ng of DNA, 1 x PCR Buffer, 2.5 mM

Table 1
Regions, mountain systems, locations, geo-references, number of specimens analyzed, and number of haplotypes identified in *D. mexicanus* populations

Regions, mountain system and locations	Lat/long	N	Haplotypes[a]	Host
Northern region				
Sierra Madre Occidental (SMOC)				
1. Tres Ojitos, Chihuahua	29°16′41″N/108°00′48″W	1	24	*Pinus leiophylla*
2. El Yeposo, Bocoina, Chihuahua	27°50′20″N/107°49′00″W	4	24, 29, 39, 40	*Pinus leiophyla*
3. Ejido Guachochic, Chihuahua	26°50′00″N/107°03′52″W	5	02(4), 16	*Pinus leiophylla*
4. Sierra Fria, Aguascalientes	22°10′00″N/102°36′00″W	10	02(4), 08, 10(3), 13, 17	*Pinus leiophylla*
Sierra Madre Oriental (SMOR)				
5. Potrero de Arteaga, Coahuila	25°23′48″N/100°46′25″W	10	43(6), 44, 45, 46, 47	*Pinus teocote*
6. Galeana, Nuevo Leon	24°50′24″N/100°04′08″W	7	03, 19, 23, 24(4)	*Pinus teocote*
7. San Cristóbal, St. Luis Potosí	22°36′00″N/100°27′00″W	1	24	*Pinus pseudostrobus*
8. Las Peñas Borradas, S.Luis Potosí	22°01′40″N/100°36′14″W	4	20, 22, 24, 25	*Pinus montezumae*
9. Los Encinos, Queretaro	21°17′06″N/99°10′49″W	10	02(5), 15(3), 24(2)	*Pinus teocote*
10. Los Herrera, Queretaro.	20°53′56″N/99°34′34″W	10	02(4), 11, 15, 24(4)	*Pinus pseudostrobus*
11. Carpinteros, Hidalgo	20°34′01″N/98°33′00″W	7	02(3), 24(4)	*Pinus teocote*
Central region				
Trans-Mexican Volcanic Belt (TMVB)				
12. La Mesa, Michoacán	19°45′43″N/100°03′47″W	5	02, 04, 24(3)	*Pinus leiophylla*
13. Tlatelolco, State of Mexico	19°44′00″N/98°22′00″W	10	01(2), 02(5), 15, 24(2)	*Pinus leiophylla*
14. Predio Agua Blanca, Michoacán	19°41′22″N/100°44′10″W	9	02(2), 09, 24(6)	*Pinus leiophylla*
15. Cofre de Perote, Veracruz	19°32′18″N/97°11′28″W	10	48(5), 49, 50, 51, 52, 53	*Pinus leiophylla*
16. Opopeo, Michoacán	19°29′20″N/101°35′28″W	9	02(4), 24, 28, 41(2), 42	*Pinus leiophylla*
17. La Charanda, Michoacán	19°26′02″N/102°03′36″W	6	02, 04, 05, 24(3)	*Pinus leiophylla*
18. Milpillas, Michoacán	19°25′12″N/102°09′00″W	9	02(5), 06, 07, 24, 41	*Pinus leiophylla*
19. El Pedregal, Michoacán	19°24′29″N/101°18′00″W	2	02(2)	*Pinus pseudostrobus*
20. Ajusco, DF	19°13′49″N/99°13′08″W	8	02(5), 14, 15, 24	*Pinus teocote*
21. Zentlalpan, State of Mexico	19°09′00″N/98°48′00″W	9	24(2), 27, 31(4), 32, 33	*Pinus leiophylla*
22. Cerro Gordo, State of Mexico	19°08′35″N/100°08′24″W	2	02, 26	*Pinus leiophylla*
23. Amecameca, State of Mexico	19°08′31″N/98°42′36″W	10	02(5), 06(2), 21, 24, 30	*Pinus leiophylla*
24. CICYTEC-IPN, State of Mexico	19°04′00″N/98°58′00″W	8	02, 12, 14, 15(2), 16, 18, 24	*Pinus montezumae*
Southern region				
Sierra Madre del Sur (SMS)				
25. Santa Catalina, Oaxaca	17°05′29″N/96°17′29″W	7	34(2), 35, 36, 37, 38(2)	*Pinus montezumae*

[a] Haplotype with number individuals in parenthesis.

$MgCI_2$, 150 uM of each dNTP, 0.1 uMof each primer, and 1.5 units of *Taq* polymerase (Perkin-Elmer). Amplification conditions were: an initial hot-start of 5 min at *95°C,* followed by 35 cycles of 30 5 at 95 *oC,* 1 min at 51 *°C,* 45 5 at 72 °C, and a final extension for 10 min at 72 C. PCR products were purified using the Qiagen PCR purification kit (QIAGEN) and directly sequenced. To assess whether pseudogenes could have been amplified due to fragment size, complementary DNA analyses, i.e., restriction fragment length polymorphism (RFLP) and single-strand conformation polymorphism (SSCP) of the fragments, were carried out (data not shown). All probes showed the absence of additional bands in the PCR products, which would have been indicative of pseudogenes. Sequencing was performed on both strands with PCR primers using the big-dye terminator sequencing kit (Applied Biosystems) and carried out with an ABl 377 sequencer (Applied Biosystems). All obtained sequences were compared, edited manually, and aligned with Clustal X (Thompson et al., 1997). Haplotypes were identified using Collapse v.1.0 (http://darwin.uvigo.es).

2.4. Poplliation history analysis

Based on comparisons between mtDNA haplotype and nucleotide diversities (see Grant and Bowen, 1998), we explored the demographic history of D. *mexican* us populations. The haplotype diversity *(h,* the probability that two randomly chosen haplotypes are different), and nucleotide diversity *(n,* mean weighted sequence divergence between haplotypes) were estimated using Arlequin v.3.1 (Excoffier et al., 2005). Additionally, assuming neutrality, we estimated Tajima's D statistic, which can also be used to infer population demographic history. Tajima's D statistic is expected to be negative when genetic structure has been influenced by rapid range expansion (Tajima, 1989). To compare observed frequencies of pairwise differences with those expected under a model of demographic expansion, mismatch distributions were generated using DnaSP v.4.10 (Rozas et al., 2003). A multimodal distribution is expected when there are no changes affecting population size, but unimodal distributions are predictable when sudden demographic expansions have occurred (Rogers and Harpending, 1992). The *R2* statistic and its associated probability ($P < 0.05$) (Rarnos-Onsins and Rozas, 2002) after 10,000 replicates were used to assess the significance of the distribution fit to that of an expanding populations. All these estimations were calculated per geographical regions and mountain systems. Regions were established according to geographic latitude. Thus, populations from the Sierra Madre Occidental, Sierra Madre Oriental, Sierra Fria, and Sierra Gorda integrated the northern region, TMVB populations the central region, and only the population of Santa Catalina-Oaxaca from the SMS the southern region (Table 1).

2.5. Phylogeographk structure analysis

To determine whether the observed phylogeographic structure in D. *mexicanus* is coincident with the geomorphologic conformation of Mexico, we examined the phylogeographical pattern of this species by analysis of molecular variance (AMOVA) and nested clade phylogeographic analysis (NCPA) (Excoffier et al., 1992; Templeton et al., 1995). Both methods are complementary, and provide a more thorough assessment of process affecting the distribution of genetics variation. While NCPA method has been widely used to infer complex population histories, recently its legitimacy has been questioned (see controversy in Molecular Ecology, 2008). We have retained the NCPA analysis for three reasons: (a) this method together with statistical phytogeography can provide a more detailed and reliable picture of the population history, (b) the NCPA analysis

has been evaluated using real evolutionary sceneries. although does not allow to evaluate the relative support for different alternative scenarios. and (c) the NCPAanalysis was used in this study as one analytical approaches more to understand evolutionary history of D. *mexicanus.*

The amount of genetic structuring was estimated using Arlequin v.3.0 (Excoffier et al., 2005) following hierarchical level: (a) among northern. central. and southern regions. (b) between populations within regions. (c) within populations. The 0-statistics significance was computing using a nonparametric permutation test with 10.000 permutations. On the other hand. the haplotype network and nesting levels were constructed according to the procedures of Templeton et al. (1992) using the software TCS v.1.13 (Clement et al., 2000). Network ambiguities were resolved by criteria based on geography and coalescent theory (Crandall and Templeton. 1993). Phylogeographic inferences were derived using the most recently developed inference key (Templeton. 2004) after statistically evaluating the relationship between the TCS-generated network and geography using GEODIS v.2.0 software (Posada et al., 2000). We selected only those haplotype relationships that were statistically significant ($P < 0.05$) to discriminate historical events.

Because we assigned prior discrete groups by regions and mountain systems. we evaluated the statistical support for population expansion of demographically independent groups using coalescent analysis. We used MDIV software that implements the coalescent model described in Nielsen and Wakeley (2001) and estimated the parameters: $\theta (= 2N_e u)$. M (migration rate = number of migrants between populations per generations). and T (the divergence time between populations measured in units of female effective population time. (oNe). This program uses a Bayesian approach to simultaneously estimates population divergence times and migration rates between two populations that are assumed to have diverged from a common ancestral population. Based on our previous knowledge of Fst estimates for D. *mexicanus* in the TMVB using isozymes (Zuniga et al., 2006). and the geological age of the final conformation of the mountain systems of Mexico (~2 Myr BP), we ran MDIV multiple times for each data set using uniform prior distributions for M and T values between 0 and 20. Final results were obtained using a finite sites model (Hasegawa et al., 1985) with the transitions/transvertion ratio estimated directly from each data set; Markov chain Monte Carlo (MCMC) simulations for 5.000.000 steps. where the first 500.000 were discarded as burn-in time. The best MCMC convergences were obtained when Mmax was set to 20 and Tmax to 10.

Finally. the coalescent scaled parameter T was converted to Tpop (the amount of time that has elapsed since the populations arose from an ancestral group) between mountain systems and regions to explore the probability that the signatures of range expansion were congruent with the geomorphologic conformation of all mountain systems from Mexico. To calibrate population divergence time in generations before present (T_{pop}). we used the equality: $Tpop = [(T \cdot 0)/2L]1/u$ (Brito. 2005), where L is the sequence length (246 bp) and u the mutation rate per site per generation. The mutation rate per base per generation assumed a standard of 1.15% per million years per lineage for arthropod mitochondrial DNA (Brower. 1994).

3. Results

From 173 mtDNA COI sequences, 53 different haplotypes were identified (Accession Nos. from DQ022214 to DQ022267). Sequenced fragments had 169 monomorphic sites and 77 variable sites. The Akaike information criterion favored Tarnura-Nei + G model ($\alpha = 0.624$ for the v-distrlbutlon, and base frequencies: A = 0.29. C = 0.17. G = 0.13. T = 0.41). Maximum likelihood analysis performed with 53 haplotypes resulted in a single tree of -Ln 1808.20 (Fig. 1). The SH test support the monophyly of D. *mexicanus* haplotypes ($P > 0.99$); however. the relationships among haplotypes was unresolved. and there was not correspondence to geographical location. except those from Potrero de Arteaga (SMOC). the Cofre de Perote (TMVB) and Santa Catalina (SMS). *D. mexiconus* sequences are consistent with a constant rate of evolution along the tree branches (LRT= 40.80. P = 0.05).

3.1. Population history

The largest number of haplotypes was those from the Cofre de Perote (TMVB) with six and CICyTEC-IPN(TMVB) with seven (Table 1). Haplotypes 02 and 24 were found most frequently; the former was present in 16 of the 25 analyzed populations with a mean frequency of 0.491. and the latter was present in 18 populations with a mean frequency of 0.389. The remaining haplotypes were found in no more than three populations. Haplotypes found in the Cofre de Perote (TMVB).Santa Catalina (SMS). and Potrero de Arteaga (SMOR) populations were exclusive from those sites (Table 1). Haplotype and nucleotide diversities varied across populations (data not shown), but were not associated with the geographic range of populations. The mean haplotype and nucleotide diversities were h= 0.75 ± 0.14. and π= 0.007 ± 0.004. respectively. Among regions π and h values were similar. but the highest values of haplotype and nucleotide diversities were found in the central (TMVB). and southern (SMS) regions. Significantly negative Tajima's D values were obtained for the northern and central regions (Table 2). Mismatch analysis for each region and mountain system fits the unimodal distributions as expected for a model of recent population expansion. The R2 statistical only was not significant for the southern region (SMS) ($R2 = 0.175$, $P > 0.10$) (Table 2).

3.2. Phylogeographic analysis

0-Statistics. sum of squares. percentage of variation. and probability (P) associated with 0 values for the different hierarchical levels are summarized in the Table 3. AMOVA showed differentiation among the populations of the northern. central and southern regions of Mexico (0ct=0.02, P= 0.0001). and among populations within regions (0sc= 0.130, P= 0.0001). The variation within populations was not statistically different from zero (0st= 0.152, P>0.05).

The haplotype network was set to a level of six steps (Fig. 2). Two star-like patterns are found in the network linked by I-step clades represented by haplotypes 02 and 24 from the TMVB. More than one parsimonious link was observed among some of the haplotypes resulting in reticulations within the network. For example. the connection between haplotypes 02 and 24 was considered more likely than any other association; haplotypes 42 and 41 were linked to haplotype 02. considering the larger number of individuals analyzed from the same locality; haplotype 21 was linked to haplotypes 20 and 22 forming a chain. which. in turn. was linked to haplotype 24 assuming that the most probable connection is that among individuals of the same population and whose frequency is mainly expressed in the ancient haplotype. The rest of the ambiguities formed by haplotypes 10-17, 16-19. and 29-30 were solved using the same criteria.

Only six associations at levels of steps 2, 3, 4. and the total cladogram were statistically significant (Table 4); however, no inference on population history could be made on clade 4-3 because values for clade or nested clade distances were not significant. For clades 2-6 and 3-5 from northern and central of Mexico. we identified long distance colonization with restricted genetic flow. The values for clades 2-11 and 4-2 from the TMVB and the SMS suggest allopatric fragmentation events. Finally. for the total clad-

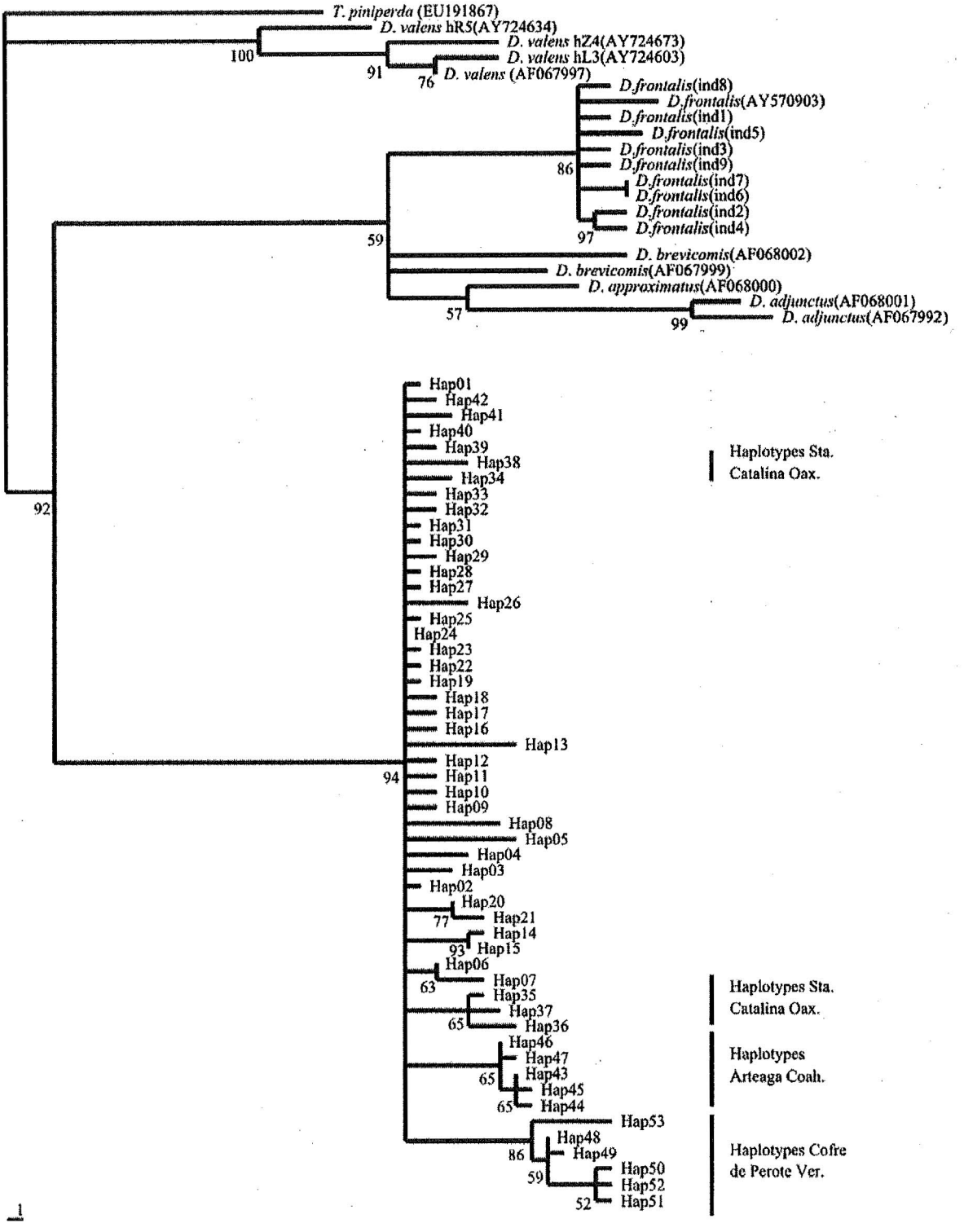

Fig. 1. Maximum-likelihood tree for D. *mexican* us COI haplotypes obtained with PAUP v4.0bl0 under Hasegawa-Kishino-Yano (HKY85) model, unequal base frequencies, an invariable sites parameter, and gamma-distributed rate heterogeneity parameter. Numbers below the branches represent bootstrap values based on 5000 pseudoreplicates.

ogram, the inference key indicates an allopatric fragmentation event among populations of the northern. central. and southern regions (Table 4).

Estimates of 0, M, *T*, and Tpop among regions and mountain systems are shown in Table 5. The scaled divergence times (T) varied widely. and the migration rates in general were less than 1, except between

Table 2
Genetic diversity and Tajima's *D* test of *Dendractonus mexicanus* by region, and mountain systems with significant geographical associations

Localities	Number of specimens	π (SD)	h (SD)	D	R2
Region					
Northern	69	0.0125 ± 0.0073	0.844 ± 0.030	−1.824**	0.052**
Central	97	0.0154 ± 0.0087	0.828 ± 0.030	−1.971**	0.038**
Southern	7	0.0155 ± 0.0101	0.905 ± 0.103	−0.358***	0.175***
Mountain systems					
TMVB	97	0.0154 ± 0.0087	0.828 ± 0.030	−1.971**	0.038**
SMOC	20	0.0098 ± 0.006	0.832 ± 0.075	−1.967**	0.089**
SMOR	49	0.0130 ± 0.0076	0.824 ± 0.036	−0.653***	0.084**
SMS	7	0.0155 ± 0.0101	0.905 ± 0.103	−0.358***	0.175***
All populations	173	0.0154 ± 0.0086	0.849 ± 0.020	−2.275*	0.026*

n, nucleotide diversity; *h*, haplotype diversity; D, Tajima's D test, *R2*, Ramos-Onsins and Rozas's statistics.
* P<0.01,
** P<0.05,
*** P>**0.10.**

Table 3
Results of analysis of molecular variance (AMOVA) of COI mtDNA sequence data from populations of D. *mexicanus*, variance components, and percentage variation explained at each hierarchical level

Source of variation	φ	Sum of squares	Percentage of variation (%)	*P*-value
Among regions (ϕ_{CT}) Northern, Central, and Southern	0.026	2.417	2.60	0.0001
Among populations within regions (ϕ_{SC}) Northern (1–11), Central (11–24), Southern (25)	0.130	16.678	12.66	0.0001
Within populations (ϕ_{ST}) (all population)	0.152	56.223	84.74	0.099
Total		75.318		

Population numbers are shown in parentheses.

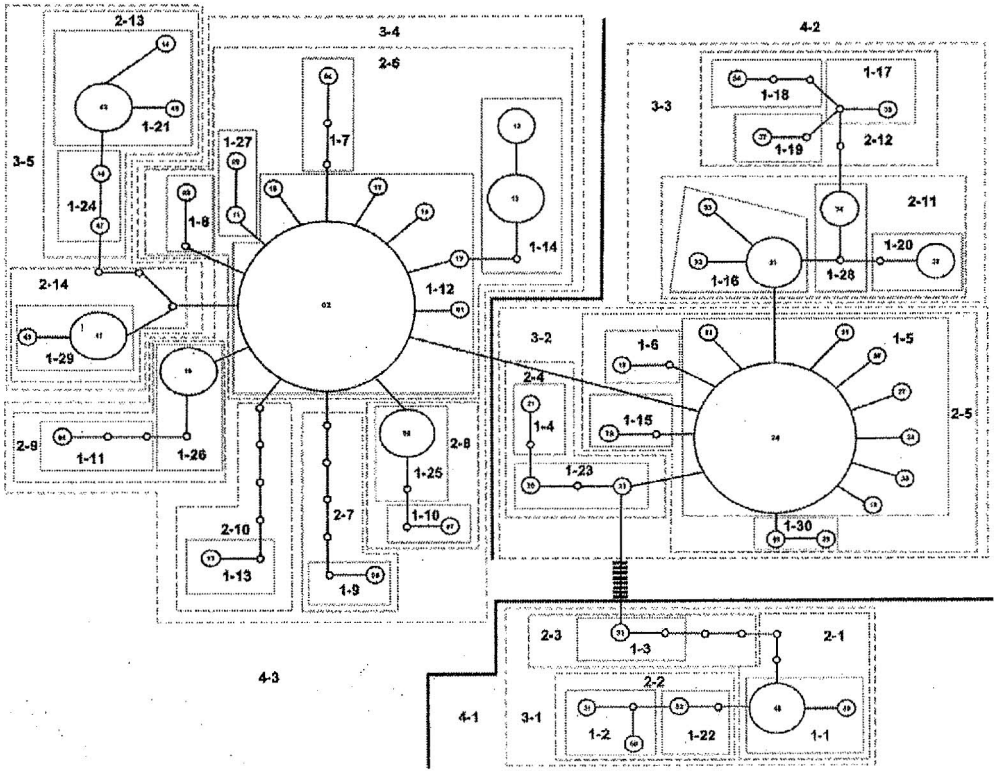

Fig. 2. Haplotypes network of the 25 populations studied under 95% cladogram estimation in TCS. Each line indicate a mutational change, numbers inside circles identify different haplotypes (see Table I). Empty circles symbolize lost ancient haplotypes. and squares represent hierarchy of nesting, Haplotypes 20 and 12 are considered **ancestral.**

the northern/central regions (3.48), and among those mountain systems including within them (SMOC/SMOR = 2.32, SMOC/TMVB = 2.88, and SMOR/TMVB = 5.25) mountain systems. These M values show the biogeographical connection between these regions,

Table 4
Phylogeographic inference from nested clade analysis of *Dendroctonus mexicanus*

Clade	Steps followed in inference key	Historical/demographic events
2-6	1,-2,-3,-5,-6,-7,-8- Yes	Restricted gene flow/dispersion with some long distance dispersion
2-11	1,-2,-3,-5,-15- No	Past fragmentation or allopatric fragmentation
3-5	1,-2,-3,-5,-15- No	Past fragmentation and/or long distance colonization
4-2	1,-2,-3,-4,-9- No	Allopatric fragmentation
4-3		Inconclusive outcome
Total cladogram	1,-2,-3,-4,-9- No	Allopatric fragmentation

Only clades with significant association between haplotype, clades and geographical distribution are shown.

Table 5
Results of the migration/isolation model obtained with the MDIV software

	0	*M*	*T*	T_{MRCA}	$_{e}$Ne	T_{pop} (Myr)
Regions						
Northern/Central	12.57 ± 0.76	3.48 ± 0.51	0,112 ± 0.20	1.3722	546539	0.124 ± 0.013
Northern/Southern	9.43 ± 0.56	0.08 ± 0.51	0,752 ± 0.20	1,1949	410039	0.627 ± 0.010
Central/Southern	13.70 ± 0.67	0.12 ± 0.51	0.528 ± 0.20	1.2979	595769	0.639 ± 0.012
Mountain systems						
Perote/SMOC	6.34 ± 0.55	0.04 ± 0.51	1.856 ± 0.20	2.6439	275643	1.040 ± 0.010
Perote/SMOR	5.49 ± 0.41	0.04 ± 0.51	1.744 ± 0.20	2.8111	238617	0.846 ± 0.007
Perote/SMS	5.59 ± 0.52	0.04 ± 0.51	1.920 ± 0.20	3.0912	243143	0.949 ± 0.009
Perote/TMVB	9.43 ± 0.56	0.04 ± 0.51	1.312 ± 0.20	1.8669	410252	1.094 ± 0.010
SMOC/SMOR	13.48 ± 0.67	2.32 ± 0.51	0.128 ± 0.20	22.7071	586134	0.152 ± 0.012
SMOC/SMS	6.38 ± 0.49	0.08 ± 0.51	1.008 ± 0.20	1.4358	277491	0.569 ± 0.009
SMOC/TMVB	8.56 ± 0.58	2.88 ± 0.51	0.272 ± 0.20	1.2449	372004	0.206 ± 0.010
SMOR/SMS	5.47 ± 0.43	0.04 ± 0.51	1.152 ± 0.20	1.7181	237717	0.557 ± 0.008
SMOR/TMVB	7.72 ± 0.54	5.25 ± 0.51	0.144 ± 0.20	1.2841	335656	0.098 ± 0.010
SMS/TMVB	10.11 ± 0.55	0.08 ± 0.51	0.768 ± 0.20	1.0664	439660	0.687 ± 0.010

$_{TMRCA}$ and $_{Tpop}$ are measured in units of 2N = 2Neu and u is the mutation rate per sequence per generation. *0*, theta; M, number of migrants; T, the scaled divergence time; T_{pop}, population divergence time in generations before present; T_{MRCA}, the most recent common ancestor; 0Ne, effective population sizes.

4. Discussion

4.1. Population history

The relatively low *n* and high h for the entire data set, regional subsets, and mountain systems indicate a model of range expansion for D. *mexicanus* across its geographic distribution (category 1 from Grant and Bowen, 1998). This model is supported by negative values of Tajima's D statistics and mismatch distributions observed for all regions and mountain systems. A similar relationship between and h has been found in some European scolytines, and *Dendroctonus* and *Ips* species from the USA using different COI mtDNA sizes (Stauffer et al., 1999; Kohlmayr et al., 2002; Cognato et al., 2003; Ritzerow et al., 2004; Faccoli et al., 2005; Horn et al., 2006; Maroja et al., 2007; Mock et al., 2007). These studies found that the range expansion model observed in these species is linked to postglacial expansion of populations from Pleistocene refuges and host fragmentation; however, it seems unlikely that our results can be explained by similar processes.

Paleoclimatic evidence and pollen records indicate that Pleistocene glaciations had a minor effect on coniferous forests in Mexico, allowing dispersal of these forests (including pine, fir, and spruce) through reduced altitudinal borders (Brown, 1985; Graham, 1993). In addition, there is no evidence-that support the existence of Pleistocene refuges for coniferous forests or bark beetles in Mexico. Therefore, considering the wide distribution of pine forests in Mexico during the Pleistocene and the limited effect of glaciations, it is possible that D. *mexican* us populations also had a wide geographic range. In fact, the areographical analysis of *Dendroctonus* species in Mexico indicate that they employed mountain systems as corridors separated by barriers that exert a low selective filter effect (Salinas-Moreno et al., 2004). The similar nucleotide diversity values observed in D. *mexican* us across its geographic range is indicative of a strong connectivity among its populations.

By contrast, intraspecific relationships of alleles, the distribution of richness (abundance) and evenness (how the variation is distributed) of the haplotypes, and AMOVA results indicate, that the phylogeographic structure of D. *mexicanus* could be strongly linked to the final geomorphological conformation of Mexico. The physical isolation of mountain systems by basin formation, and intensive volcanic activity disrupted the range expansion of D. *mexicanus* and the interconnectivity of its population, Based on the prediction of coalescence theory that older alleles (those with greater population frequencies) are more likely to have wider. geographic distributions than younger alleles, the haplotypes 02 and 24 (ho2 and *h24)* might be considered ancestral by their wide geographic range, and because they are present in 16 (64%) and 18 (72%) populations, respectively. In addition, they probably had their origin in the central region of Mexico (TMVB), because both integrated more interior clades of the total cladogram, they are present in almost all populations from this region or mountain system, and many unique haplotypes located in the TMVB a re linked to them by one single mutation step (Fig. 3c). The TMVB begins at the Santiago River basin extending from the Pacific Ocean to the central portion of the state of Veracruz in the Gulf of Mexico. Its formation was progressive, starting in the west during the Miocene (~23 Myr BP),and ending in the east during the Pliocene-Pleistocene (~2.5 Myr BP) (see Fig. 3b).

The presence of the ancestral haplotypes *h02* and *h24* in populations from the SMOC and SMOR strongly suggests a common phylogeographic history between D. *mexican* us populations located in these mountain systems and those from the TMBV (Fig. 3d). Migration rates are consistent with this common history (with exception of the Cofre de Perote), because the highest migration rates observed tended to occur between the northern-central regions, or well among mountain systems included into these regions. In spite of the fact that SMOC and SMOR are older than the TMVB (the SMOC had its origin in the late Cretaceous, and developed during

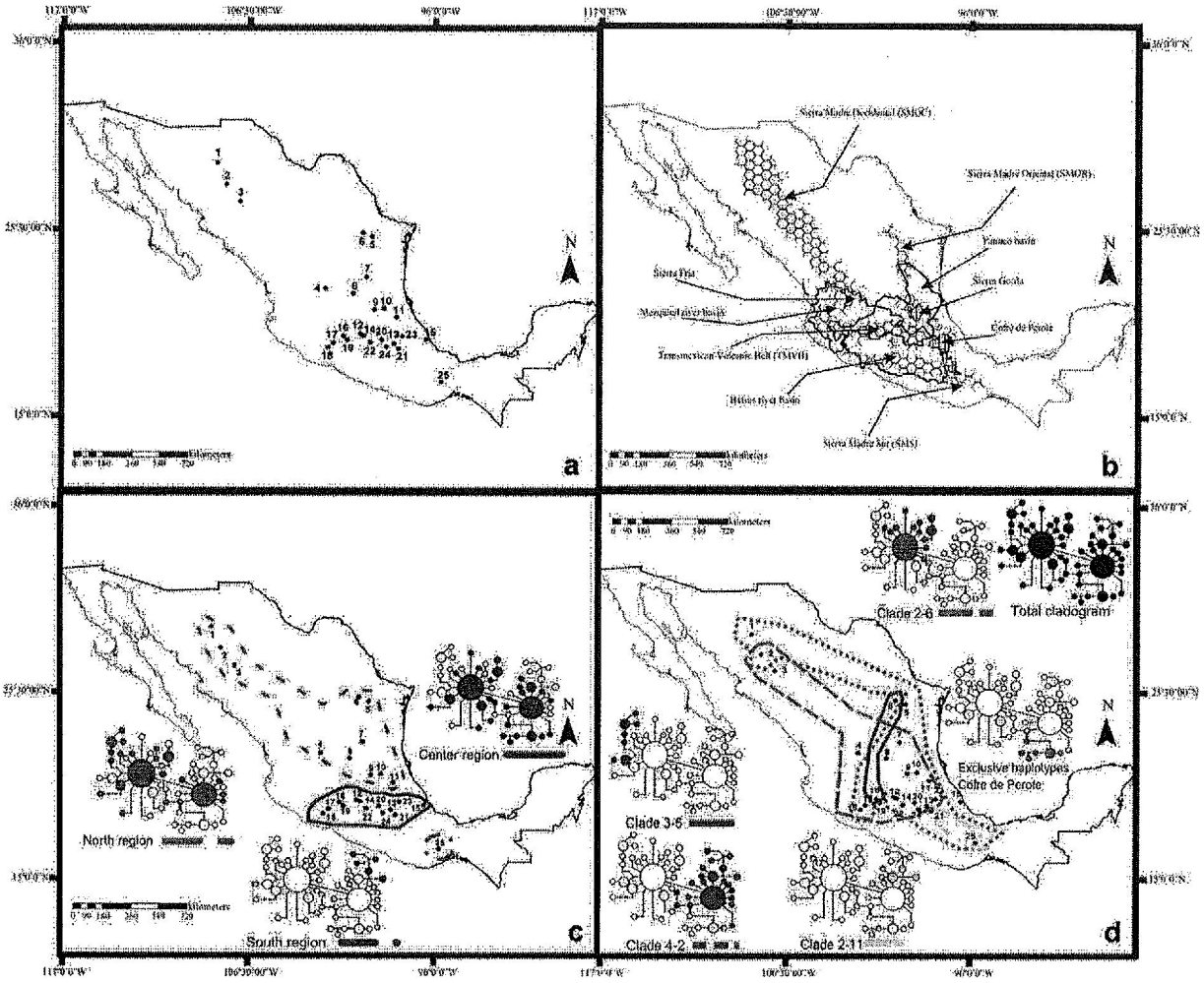

Fig. 3. Haplotype geographic relationships of *Dendroctonus mexicanus* in Mexico: (a) populations studied, (b) Mountain systems and Mexican Basins, *(c)* clades nested by geographic region, (d) *clades* with significant geographical associations.

the Oligocene and Miocene, the SMOR was formed during the Eocene), biogeographical patterns of Coleoptera have shown that the SMOC and western TMVB are closely related, and that the eastern TMVB shares many biogeographical elements with the southern end of the SMOR and SMS (Marquez and Morrone, 2004; Morrone, 2005; Corona et al., 2007). Thus, the long distance colonization process inferred indicate that there was continuous migration of bark beetles among the TMVB, and the SMOC (clade 2-6), and SMOR (clade 3-5). Applying this information to the total cladogram (see Crandall and Templeton, 1993), it suggests that probably founders carrying ancestral haplotypes *(h02, h24)* moved from the TMVB toward the SMOC, including the Sierra Fria, and SMOR.

Based on the Tajima's D value, mismatch distribution, and diversity index, one potential bias could be present in the estimating of divergences times using MDIV which assumes constant population sizes. To explore this potential bias, we performed a Bayesian analysis for constant size and exponential population growth models using BEAST *v.1.4.8* (Drummond and Rambaut, 2007). Our results did not allow identification of any difference between constant size or exponential population growth models (Bayes factor < 20) (data not shown). Therefore, there is not reason to believe that the MDIV assumption of a constant population size has been violated.

The connections between the SMOC-TMVB and the SMOR-TMVB were definitively interrupted when the Rio Mezquital and RIO Santiago basins and Panuco River basin, respectively, were formed. This geographic interruption resulted in isolation among bark beetle populations of these mountain systems, dispersal northward and genetic differentiation along the SMOC and SMOR. The presence in the SMOC and SMOR of unique and older haplotypes support this hypothesis, and τ_{pop} estimated among populations of the SMOC/TMVB (0.206 [± 0.010] Myr BP), and SMOR/TMVB (0.098 [± 0.010] Myr BP) agree with the formation of these basins,

Our results also show that phylogeographic history of D. *mexicanus* haplotypes in the Cofre de Perote is different with respect to the haplotypes of the TMVB, SMOR, and SMS (Fig. 3d). The presence of exclusive haplotypes in the Cofre de Perote, and the low migration estimated with regard to the TMVB, SMOR, and SMS (Table 5), indicates a geographical and reproductive isolation of this population, Based on geological history of TMVB, there is a plausible explanation for this isolation. The geological age of the Cofre de Perote region is recent (1.57-0.2 Myr BP), its isolation from the west end and center of the TMVB occurred during the late Pleistocene with formation of the Oriental Basin (see Fig. 3b). The Oriental Basin was then reconnected with Mexico and Apan basins through

important water fluxes (Miller and Smith. 1986); during the early Holocene this region was characterized by intensive volcanic activity. These factors could encourage particular environmental conditions where population sizes of D. mexicanus decreased. and effects of genetic drift were sufficient to promote its divergence, Thus. the Tpop (1.094 [± 0.010] Myr BP) estimated among the Cofre de Perote and the TMVB (clade 3-1) populations agree with the geological formation of the Oriental Basin. If this observation is accurate. then we would predict that taxa whose geographic range is exclusive to the TMVB would show similar genetic patterns. In fact. isolation by distance in west-east direction was observed in D. mexican us populations from the TMVB using allozymes (Zuniga et al., 2006); unfortunately. Zuniga et al. (2006) not include samples of the Cofre de Perote-Ver. (TMVB). On the other hand. the Cofre de Perote isolation of the SMOR and SMS is likely a consequence of the altitudinal gradient established after conformation of the east end of the TMVB. The gradient descends from 4500 m asl at the top of the Cofre de Perote Mountain down to zero meter at the sea. The vegetation changes across of this altitudinal gradient (G6mez-Pompa, 1973; Narave, 1985). In the high range (~2800-1700m asl) there are pine and oak-pine forest descending into clouds forest (~1600-1200m asl). These communities disappear. with oaks forest emerging at medium altitude (~1100-600m asl), which are then replaced by tropical deciduous forest at lowlands (~1000-500 m asl). Biogeographical studies also have shown that an altitudinal gradient for entomofauna similar to latitudinal gradient proposed for fauna (Lobo and Halffter, 2000), that is, in high altitude there is presence of Boreo-Montane species from Holartic origin, but in medium and low altitudes the species present are predominantly of Neotropical origin.

The divergence (inferred) among haplotypes from the Southern (SMS) and Central region (TMVB) (clades 2-11. 2-12, 4-2) show a more extreme isolation than that observed between the northern (SMOC. SMOR) and central (TMVB) regions (Fig. 3d). In general, migration estimated among SMS and the two other regions were low, which confirm its scarce biogeographical connectivity in the past. The inferred allopatric fragmentation event shows geographical isolation early between SMS and TMVB. in spite of the fact that both share biogeographical elements. The SMS is a geologically complex mountain system, with pre-Cambrian units, volcanoes from the Mesozoic, and formations from the middle Tertiary similar to the SMOC. Its isolation from the central region (TMVB) occurred during the late Pliocene or early Pleistocene when the Balsas depression appeared (DeCserna et al, 1974). The isolation of the SMS might be associated with the formation of the Balsas depression and Tehuacan-Cuicatlan valley. This depression is an important barrier, which could have fragmented pine forests, and consequently promoted geographical isolation of D. mexicanus populations (see Fig. 3b). The T_{pop} estimates indicate that divergence of these clades also occurred in the Pleistocene ~0.687 [± 0,010) Myr BP, which agrees with the formation of this depression. Because we were unable to obtain D. mexicanus samples from others localities in the SMS, where its populations are less abundant, some caution in interpreting our inferences about this region is prudent. It is widely recognized that the description of phylogeographic patterns are more biased by the number of population sampled than by the number of individuals sampled within populations (Petit et al., 2005).

ln summary, while phylogeographic inferences based on one single marker should obviously be interpreted with care, our result show that the phylogeographic structure of D. mexicanus describes an expansion range model among populations of different geographical regions and mountain systems. In addition, divergence times strongly suggest that the phylogeographic structure was determined first by Mexican mountain systems conformation, and secondly, by dispersal abilities of the insect within each mountain systems,

These results explain why D. mexicanus populations do not exhibit a well defined genetic structure within mountain systems. Geographical isolation of populations within the TMVB was not found to increase the spatial genetic differentiation among populations (Zuniga et al, 2006). Similar results were obtained by Schrey et al. (2008) for D. frontalis from five relatively close forests in Mississippi USA during an outbreak. On the other hand, high genetic variation has been observed within bark beetles population in many studies using different molecular markers: multi-loci enzymes (Sturgeon and Mitton, 1986; Langer and Spencer, 1991; Six et al., 1999; Zuniga et al., 2006). RAPD-PCR (Carter et al., 1996), mtDNA (Stauffer et al., 1999; Cognato et al., 2003; Ritzerow et al., 2004: Faccoli et al., 2005; Horn et al., 2006: Maroja et al., 2007; Mock et al., 2007). and microsatellites (Maroja et al., 2007; Schrey et al., 2008). This variation can be explained by changes in population longevity. population size. dispersal potential. and the use of different hosts in sympatric conditions. Genetic homogenization among dernesjpopulations depends on distance and the insects dispersal abilities. limited dispersal can produced disequilibrium between selection-gene flow or genetic drift-gene flow. which can give rise to local genetic differentiation among populations within the same forest area, high genetic variation within geographic populations by Wahlund effect (Lowe et al., 2004), or the presence of single haplotypes within them. Finally, while the specimens collection in this study was not designed to test the host plants effect on variation distribution of this bark beetle, an exhaustive collect and well designed, including syrnpatric and allopatric populations of D. mexicanus on different host plants across their distribution ranges. could help to understand the relative effect of hosts in structuring populations in Mexico, Testing these hypotheses could help us understand local areas genetic patterns. and contribute to improving bark beetles management strategies.

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