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BIOLOGÍA EVOLUTIVA

Filogeografía comparada de *Oryzomys couesi* y *Ototylomys phyllotis*; implicaciones históricas y geográficas en la conformación de México y Centro América

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

TANIA ANAID GUTIÉRREZ GARCÍA

TUTORA PRINCIPAL DE TESIS:

DRA. ELLA GLORIA VÁZQUEZ DOMÍNGUEZ
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UNAM
POSGRADO
Ciencias Biológicas

COORDINACIÓN

Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 03 de diciembre de 2012, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **GUTIÉRREZ GARCÍA TANIA ANAID** con número de cuenta **506021367** con la tesis titulada: **"FILOGEOGRAFÍA COMPARADA DE *Oryzomys couesi* Y *Ototylomys phyllotis*; IMPLICACIONES HISTÓRICAS Y GEOGRÁFICAS EN LA CONFORMACIÓN DE MÉXICO Y CENTRO AMÉRICA"**, realizada bajo la dirección de la DRA. ELLA GLORIA VÁZQUEZ DOMÍNGUEZ**:**

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Vocal:	DR. JOAQUÍN ARROYO CABRALES
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Suplente	DRA. LIVIA SOCORRO LEÓN PANIAGUA

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F. a 12 de febrero de 2013.

DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA
COORDINADORA DEL PROGRAMA

c.c.p. Expediente de la interesada.

AGRADECIMIENTOS INSTITUCIONALES

Al Posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México y al Instituto de Ecología, por aceptarme en el Programa de Doctorado en Ciencias Biológicas y ser cede para la realización de mis estudios y de esta tesis.

Al Consejo Nacional de Ciencia y Tecnología, por el otorgamiento de la beca para estudios de posgrado bajo el registro 175434.

A los proyectos PAPIIT IN215205-2, IX238004, IN21907 y CONACyT 101861 por el financiamiento para la realización del trabajo de campo y de laboratorio, además de la divulgación de los resultados de esta tesis.

A la Dra. Ella Vázquez Domínguez, quien fue directora de esta tesis Doctoral y tutora principal durante mis estudios de posgrado.

Al Dr. Enrique Martínez Meyer y al Dr. Daniel Ignacio Piñero Dalmau, integrantes de mi comité tutoral del Doctorado en Ciencias Biológicas, por su paciencia y atención al vigilar y evaluar semestralmente tanto este trabajo como a la alumna que lo realizó.

Al jurado de examen, sinodales de esta tesis: el Dr. Juan José Morrone Lupi, el Dr. Joaquín Arroyo Cabrales, el Dr. Daniel Ignacio Piñero Dalmau, el Dr. Ricardo López Wilchis y la Dra. Livia Socorro León Paniagua, por sus valiosas observaciones, comentarios y correcciones a este trabajo y a mi formación académica.

A los técnicos del Instituto de Ecología de la UNAM, el Biól. J. Gerardo Rodríguez Tapia (Unidad de Geomática), el M. en I. Alejandro René González Ponce (Unidad de Cómputo), Ing. Erik Daniel Valle Vidal (Unidad de Cómputo) y a la Dra. Alejandra Vázquez Lobo (Laboratorio de Genética y Evolución).

Al equipo del Laboratorio de Genética y Evolución del Instituto de Ecología, a los estudiantes de Ella Vázquez y a mis compañeros del Posgrado en Ciencias Biológicas.

Al equipo de McMaster Ancient DNA Centre, Ph.D. Hendrik Poinar, Melanie Kuch, Jacob Enk, Christine King y Kirsten Bos, por su apoyo en mi trabajo con muestras antiguas.

A las personal de las colecciones biológicas que proporcionaron gran parte de las muestras de ambas especies utilizadas en este estudio y que están mencionados en la tesis.

AGRADECIMIENTOS PERSONALES

Durante la elaboración de esta tesis y de mis estudios de posgrado conté con el apoyo y cariño incondicional de las personas más importantes para mí, mi familia. Les estoy agradecida por esforzarse conmigo y quiero además que sepan que los extrañé cada día. Mamá, papá, Juan, Viviana, los amo.

A la familia Macías Preza, MG, JM, Pepe, Óscar y Vale, por su apoyo, confianza y cariño durante los últimos años del posgrado, por recibirme en su casa como vago, agregada cultural, vecina y amiga. Gracias. "Ya llegó Tania!"

A Ella, por creer en mí, por aceptar ser mi tutora y amiga. Por dejarme crecer personal y académicamente. Por darme la libertad y guiar mi doctorado a la vez. Porque un alumno, quizás nunca superará al maestro.

A ti, Pepe, por ser mi compañero y amigo, por todo lo que aprendimos.

A mis amigas y amigos de México y del mundo, porque con cariño y paciencia soportaron mi ausencia en la época en la que la tesis o el estudio lo requirieron. Porque están ahí cuando los necesito. Todos ellos y a su particular manera colaboraron en este trabajo. No sólo creyeron en mí, sino que no me dejaron caer. Los quiero.

A mis abuelos, tíos y primos, por su apoyo. A Yeni, quien ha sido parte también de esta travesía de familia.

A toda "la banda" que me hace falta mencionar y además a revisores, profesores, personas que en cierta manera influyeron en el "cada día" de mi doctorado.

Índice

Resumen

La filogeografía comparada es el estudio de las similitudes en la distribución geográfica de la variación genética de un complejo de especies codistribuidas. Se realiza a partir del análisis filogeográfico de cada especie de un conjunto de especies y de la comparación de los resultados entre ellas. La historia climática, la historia geológica, las particularidades topográficas, la susceptibilidad diferencial de las especies a eventos climáticos (e.g. glaciales), las particularidades ecológicas y la historia de vida de las especies, entre otros, generan tanto patrones filogeográficos congruentes como incongruentes entre especies con distribuciones compartidas. Una región con historia geológica, climática y biológica compleja e ideal para estudios de filogeografía comparada es la que incluye México (M) y América Central (CA). La fauna actual de esta región incluye una mezcla de especies tanto del norte como del sur del continente asociadas con el Gran Intercambio Biótico de América y entre las cuales están los roedores *Oryzomys couesi*, especie semiacuática, y *Ototylomys phyllotis* que es semiarborícola. En este trabajo se utilizaron secuencias de un fragmento de citocromo b de estas dos especies que se codistribuyen en parte de México y América Central, para: 1) determinar la diversidad y estructuración genética dentro y entre poblaciones de cada especie a lo largo de su distribución geográfica, 2) determinar los patrones de dispersión y determinar el posible lugar de origen de cada especie, 3) comparar los patrones filogeográficos en sus áreas de distribución compartidas y 4) proponer los posibles eventos y procesos de diversificación de acuerdo con los resultados genéticos obtenidos, las características ecológicas de los roedores y las historias geológica y climática de la región. Con esta finalidad, se analizaron cerca de 700 pares de bases de cada especie utilizando diferentes herramientas filogeográficas: distancia genética, diversidad genética, estructuración genética, redes de haplotipos, reconstrucciones filogenéticas, estimación de tiempos de divergencia, entre otros.

De acuerdo con los resultados de filogeografía intraespecífica, el origen de *Ototylomys phyllotis* se localiza cerca del centro de su distribución geográfica actual, entre Honduras y El Salvador. Su diversidad genética actual es alta a moderada ($h=0.990\pm0.003$; $\pi=0.0448\pm0.0019$) y los patrones filogeográficos obtenidos muestran que se divide en cuatro filogrupos con distancias genéticas de 3 a 7% entre ellos. De estos filogrupos, tres coinciden con la clasificación taxonómica basada en características morfológicas y con las principales barreras geológicas en M y AC. Por otro lado, el origen de *Oryzomys couesi* se ubica cerca al límite sur de su distribución, entre Costa Rica y Colombia. Su diversidad genética es alta a moderada ($h=0.994\pm0.002$; $\pi=0.02597\pm0.0015$) y la especie se divide en al menos seis filogrupos con distancias genéticas entre ellos de hasta 7%. Estos filogrupos están relacionados mayormente con el clima y la ecología y en menor medida con la geología de la región.

De acuerdo con la filogeografía comparada, ambas especies tienen una estructura genética influenciada principalmente por la distancia geográfica y coinciden en dos de sus filogrupos, uno en América Central y otro en la península de Yucatán. Sin embargo, tuvieron una susceptibilidad diferente a las barreras geológicas y eventos climáticos, por lo que sus patrones de origen y dispersión son distintos. Las características ecológicas e historia de vida especie-específicas de estos roedores generaron respuestas distintas a los mismos eventos vicariantes, lo que resultó en la ausencia de patrones de estructuración genética concordantes. Este trabajo muestra que la información obtenida a partir de la filogeografía comparada es fundamental para conocer la historia biogeográfica de M y AC.

Abstract

Comparative phylogeography is the study of the geographic distribution of the genetic variation of codistributed species. It is performed by the phylogeographic analysis for each species out of a set of species and the subsequent comparison of the resulting patterns between them. Both the congruence and incongruence of the phylogeographic patterns observed between species are associated with the climatic and geologic history, landscape features, differential susceptibility of species to climatic processes (e.g. glacial periods), ecologic features and life history, among others. Mexico (M) and Central America (CA) have an intricate geological and biogeographical history that renders this area as ideal to study patterns of historical divergence, genetic differentiation and structuring of endemic populations. Current fauna from the region includes a mixture of North and South American components, many associated with the Great American Biotic Interchange (GABI), such as the semiaquatic rodent *Oryzomys couesi* and the semiarboreal rodent *Ototylomys phyllotis*. In this study, sequences of a fragment of cytochrome b from those species were used with the following aims: 1) to determine the genetic diversity and genetic structure patterns within and between populations along the entire geographic distribution for each species, 2) to determine the historic distribution patterns and suggest the most plausible geographic area of origin for each species, 3) to compare their phylogeographic patterns comprising the area where the species are codistributed and 4) to propose the diversification events and evolutionary processes in accordance with the genetic results obtained and the ecological features of both species, and with the geologic and climatic history of the region. For this purpose, approximately 700 base pairs of cytochrome b from individuals of each species were analyzed using different phylogeographic tools: genetic distances, genetic diversity, genetic structure, haplotype networks, phylogenetic trees, time of divergence, inter alia.

The intraspecific phylogeography results show that *Ototylomys phyllotis* origin is located near the center of its current geographic distributional range, in the Honduras and El Salvador area. Its genetic diversity is high to moderate (h=0.990±0.003; π=0.0448±0.0019) and has a phylogeographic pattern showing four phylogroups with genetic distances of 3-7%. Three of these phylogroups coincide with the morphological groups and with the main geological regions in M and CA. On the other hand, the origin of *Oryzomys couesi* is located near the southern limit of its geographic distributional range, between Costa Rica and Colombia. Its genetic diversity is also high to moderate (h=0.994±0.002; π=0.02597±0.0015) and is divided in six phylogroups with genetic distances of up to 7%. These phylogroups are mostly related with the climate of region and ecology of the species and less with the geological barriers.

The results of the comparative phylogeography analysis show that both species have a genetic structure influenced mainly by the geographic distance. The two species coincide in two phylogroups located in America Central and in the Yucatan peninsula. However, they have different susceptibility to the geological barriers and climatic events; hence their origin and dispersal patterns are not similar. Their ecological features and species-specific life history characteristics generated mixed responses to the same vicariant events, which result in the absence of congruent patterns of genetic structure. This work shows that information obtained by comparative phylogeographic analyses is fundamental to the understanding of the biogeographic history of M and CA.

Introducción

Las historias geológica y climática de México (M) y Centro América (CA) han generado patrones de estructuración genética comunes en las especies que se distribuyen en esta región. Los cambios geológicos y climáticos pueden crear patrones repetidos de división de taxones en subpoblaciones discretas; al estudiar dichos patrones es posible evaluar la dinámica de dispersión, la diferenciación geográfica y la formación de especies nuevas (Bermingham y Martin, 1998), entre otras. El estudio de estos patrones es posible mediante la filogeografía comparada, que permite identificar los eventos que influyeron en la distribución de la variación genética de especies codistribuidas. Los estudios de filogeografía comparada se basan en la hipótesis nula de que cada especie tiene una historia particular y cualquier coincidencia en aquella de otra especie es resultado del azar (Bermingham y Martin, 1998), y por otro lado el supuesto de que una historia de evolución similar produce patrones filogeográficos comunes (Avise, 2000). La historia geológica de CA implica la conexión de dos masas terrestres (América del Norte y América de Sur) a través del origen diferencial de cadenas montañosas y la continuidad de tierras bajas, lo cual permitió el intercambio de la biota particular a cada región. La distribución posterior de la fauna se vio afectada también por la formación de complejos volcánicos al sur de México y la emersión de la península de Yucatán. La historia climática de las regiones mencionadas desde su conformación geológica hasta la actualidad incluye fluctuaciones en variables ambientales (e.g. precipitación y temperatura), que afectaron directamente la distribución e interacción de las comunidades de la zona. Existe controversia respecto a la respuesta particular y en conjunto de los organismos de esta región a los cambios ambientales que hubo durante el Plioceno y hasta la actualidad, además de la composición diferencial de las comunidades presentes (Hershkovitz, 1969; Halfter, 2002; Arita y Vázquez-Domínguez, 2003; Orellana *et al.*, 2003; Hasbún *et al.*, 2005; Wüster *et al.*, 2005). Durante este periodo también hubo taxones cuyo origen y dispersión parece que fue independiente de los eventos de formación de México y CA, los cuales se distribuyeron y ocuparon áreas distintas de acuerdo con su ecología y vagilidad, como en el caso de las especies del genero *Oryzomys* (Engel *et al.*, 1998).

Dos especies que incluyen América Central dentro de su distribución y que se diferenciaron durante la radiación previa al último gran glacial que inició hace 2.4 millones de años (Shackleton *et al.*, 1984), son los roedores *Oryzomys couesi* (Alston, 1876) y *Ototylomys phyllotis* (Merriam, 1901). El primero es un roedor semiacuático distribuido desde Colombia hasta Texas; el segundo, un roedor

semiarborícola que se distribuye desde Costa Rica hasta Chiapas. Aunque ambas especies han sido utilizadas en estudios sobre filogenia y divergencia de otros géneros de roedores y la información respecto a su ecología y genética es limitada, son candidatos ideales para un estudio de filogeografía comparada ya que, de acuerdo con la región de estudio, podría haber patrones filogeográficos concordantes en ambas especies asociados a los cambios históricos tanto en la geología como en el clima de M y CA, que permitirían evaluar qué tanto estos procesos influenciaron la distribución y permanencia de ambas especies en la región. Sin embargo, de acuerdo con las diferencias en el tipo de hábitat, forma de dispersión, historia demográfica y otras características de las especies, los patrones filogeográficos serían no concordantes a nivel local. Por lo tanto, los objetivos de este trabajo son: 1) determinar la diversidad y estructuración genética dentro y entre poblaciones de *Oryzomys couesi* y *Ototylomys phyllotis* a lo largo de su distribución geográfica, 2) determinar los patrones de dispersión y sugerir el lugar de origen de cada especie, 3) comparar los patrones filogeográficos de ambas especies en el área en la que se codistribuyen para identificar las concordancias entre ellos y, 4) proponer los posibles eventos y procesos de diversificación de acuerdo con los resultados genéticos obtenidos, las características ecológicas de estos roedores y las historias geológica y climática de la región.

Con la finalidad de cumplir con estos objetivos y de tener un contexto completo que incluya las bases de un estudio filogeográfico, las historias geológica y climática de la región y la descripción de los patrones filogeográficos de ambas especies, este trabajo se divide en cuatro capítulos.

El capítulo 1 describe cómo desarrollar un estudio de filogeografía comparada desde la elección de las especies o regiones hasta la interpretación de los resultados. Se destacan aspectos de la metodología que se deben de considerar y se incluye un resumen de las metodologías disponibles más actuales para el análisis de la información genética desde la perspectiva filogeográfica.

El capítulo 2 analiza cómo las barreras geológicas han generado patrones genéticos concordantes en diferentes especies que se distribuyen en América Central. Incluye un breviario de la historia geológica de la región, el análisis de los patrones de dispersión, migración y diversificación de las especies durante la conformación de este bloque terrestre y finaliza con la propuesta de las divisiones más evidentes en las conformaciones genética y geológica de la historia evolutiva de dichas especies.

El capítulo 3 lo conforma la filogeografía de *Ototylomys phyllotis*. Es el estudio de la historia de este roedor semiarborícola desde su origen en el centro de América Central hasta alcanzar su distribución actual. Incluye la descripción de su estructura genética y el efecto de los principales eventos geológicos y climáticos de la región en su estructura y diversificación.

Finalmente, el capítulo 4 incluye la filogeografía de *Oryzomys couesi* y la comparación de los patrones filogeográficos de ambas especies. También incluye un análisis de sus características ecológicas y la evaluación del efecto de éstas y de otras características como la morfología, la filogenia y la distancia geográfica, sobre la estructuración genética de las dos especies.

Capítulo 1

Artículo: **Comparative phylogeography: designing studies while surviving the process**

Publicado en: **Bioscience**

Comparative Phylogeography: Designing Studies while Surviving the Process

TANIA A. GUTIÉRREZ-GARCÍA AND ELLA VÁZQUEZ-DOMÍNGUEZ

Comparative phylogeography *(CP) can be defined as the study of the effects of evolutionary history and biogeography on the distribution of genetic variation of codistributed species. CP studies have intensified in recent years, which is a natural progression from an extensive history of intraspecific phylogeography research. On the basis of a thorough review of published studies that specifically deal with CP, our objective in the present review is to provide a comprehensive guide to the discipline that will help those wishing to develop a CP project. We describe the characteristics that shape a CP study and summarize the field's prime theoretical, methodological, and analytical requirements; frequent hypotheses tested; and current achievements and limitations, including a variety of illustrative examples throughout. We finally highlight some new approaches in CP and briefly discuss future directions for the field.*

Keywords: biogeography, evolution, geological history, phylogeny

Over 20 years ago, John Avise coined the term *phylogeography* (Avise et al. 1987), a discipline in which the geographical distribution of the genetic variation of natural populations is studied in a historical context. As such, intraspecific phylogeography helps to decipher spatial and temporal patterns of population structure (i.e., genetic differences within and among populations) and to explain the ecological and evolutionary processes responsible for those patterns (Avise 2000). An astounding number of intraspecific phylogeographic studies have been published since the field's birth (see Knowles 2009), information that allowed the identification of species codistributed in space and time that showed similar or contrasting phylogeographical patterns in gene genealogies (see box 1 for a glossary of terms). This awareness motivated the design of studies to test whether taxa with overlapping distributions share a common history and laid the foundation for what later came to be known as *comparative phylogeography* (CP). It was Bermingham and Avise (1984) who established the theoretical grounds for this new framework in the study of historical distributions of species (Avise 2000). In earlier CP studies, whether a similar evolutionary history would produce shared intraspecific phylogeographical patterns among codistributed species was explored. CP was therefore defined as the study of the effects of evolutionary history and biogeography on the distribution of genetic variation in codistributed species.

Bermingham and Moritz (1998) briefly described the concepts and application of CP and explained how CP analyses could contribute to broader studies of ecology and evolution. The latter was possible, they reasoned, because congruence among the evolutionary, demographic, and distributional histories of taxa could be described with the use of a CP approach and could, in turn, be explained by the ecological and geological landscape. They also indicated some of CP's early pitfalls (e.g., the use of a single gene system, mitochondrial DNA) while emphasizing some of the improvements needed: (a) to use unlinked molecular markers and to develop new methodologies for testing evolutionary congruence, (b) to continue the development of coalescence theory and to increase the statistical rigor of phylogeographic analyses, and (c) to increase the precision of the time estimation of cladogenesis (Bermingham and Moritz 1998).

Briefly described, the goal of most CP studies has been the search for general patterns in the effect of environmental (historical) changes on several species in a particular space and time. Despite consensus about this objective, several gaps and questions remain: Is there a common methodology for CP? What are the limitations when trying to decipher intraspecific and interspecific patterns? What are the requirements for selecting species, genetic markers, and sampling schemes? In the present review, we have prepared a comprehensive overview of CP to serve as a resource for the design and assessment of CP studies. Accordingly, we first describe the characteristics that shape a CP study, then summarize CP's prime theoretical, methodological, and

BioScience 61: 857–868. ISSN 0006-3568, electronic ISSN 1525-3244. doi:10.1525/bio.2011.61.11.5

Box 1. Glossary.

Allopatric. *Allopatric distribution* refers to species that occur in geographically separated areas; *allopatric fragmentation* occurs when a geographical (e.g., river, mountain) or anthropogenic (e.g., road) barrier separates an originally continuous distribution.

Allozymes. Multiple variants of the same enzyme produced by different alleles.

Cladogenesis. Evolutionary change and diversification resulting from the branching off of new taxa from common ancestral lineages.

Cryptic species. Genetically distinct evolutionary lineages (i.e., individuals along significantly divergent branches of a phylogenetic tree) that are morphologically indistinguishable.

Ecological plasticity. Degree to which an organism can tolerate deviation from its optimal environmental conditions. A term related to *phenotypic plasticity*, which is the potential for a single genotype to develop into multiple alternative phenotypes under different environmental conditions.

Evolutionary significant unit (ESU). A population (or populations) of a species that is genetically distinct from other populations; in phylogeography, an evolutionarily distinct phylogroup can be considered an ESU. ESUs have many applications in conservation.

Gene genealogies. The pattern of similarities between DNA sequences contains information about their evolutionary history. Therefore, the ancestry of a sample of homologous (of the same gene) DNA sequences from a population back to their most recent common ancestor is the gene genealogy of the sample.

Gene tree or species tree. The relationship between species is usually represented as a bifurcating tree with the branching points representing speciation events (*species tree*). The ancestry of genes taken from these species can also be represented as a tree, with the branching points representing ancestral genes (*gene tree*).

Genetic divergence. A process in which genetic changes accumulate over time in an ancestor by the action of different evolutionary processes that cause measurable differences between individuals, populations, or species. Measured by the extent to which two sequences differ from one another.

Haplotype. Refers to half of a genotype. A combination of alleles (for different genes) that are located closely together on the same chromosome and that tend to be inherited together; for example, mitochondrial DNA in most animals is maternally inherited. Therefore, unique haplotypes (DNA sequences) are transmitted from mothers to their offspring. Haplotypes can be detected with molecular techniques and analyzed via gene trees.

Host–parasite. Of or relating to a biological interaction or relationship in which an organism obtains its nutrients from one or very few host individuals, normally causing harm but not causing death, at least not immediately.

Lineage. Descent of an organism; one or a series of populations (*demes*) that share a common history of descent not shared by other populations. A genetic lineage is a series of mutations that connect an ancestral genetic type (i.e., allele, haplotype) to its descendants.

Molecular marker. Fragment of DNA used to generate data from one or more genomes (e.g., mitochondrial and chloroplast DNA). Depending on its location on the genome and mechanisms of inheritance, a molecular marker can be unlinked (inherited independently) or linked (inherited together with other genes).

Molecular clock. On the basis of the idea that DNA sequences evolve at roughly constant rates, the dissimilarity of two sequences can be used to calculate the amount of time that has passed since they diverged. Molecular clocks are incorporated into phylogenetic analyses and calibrated with fossil record data or with rates of molecular change.

Mutualism. Biological interaction between pairs of species that brings mutual benefit; the individuals in a population of each mutualist species grow, survive, or reproduce at a higher rate when in the presence of individuals of the other species.

Taxa (singular: *taxon*). A taxonomic unit, whether named or not (i.e., a population or group of populations of organisms) whose members are phylogenetically related. These units have common characteristics that differentiate the unit (e.g., a geographic population, a species, a genus, a family, an order) from other units.

Sister species. The most closely phylogenetically related species (or taxa) to a given species (or taxon).

Stochastic variance. The coalescent or stochastic variance is the variability in gene divergence time that arises in the evolutionary and demographic history of populations as a natural consequence of genetic drift. As a result of this stochastic genealogical component of divergence, information about the historical demography of the species of study and that of its ancestor need be considered when analyzing and modeling phylogeographic patterns.

Symbiont. Close and often long-term interactions between different species that include mutualistic, commensal, or parasitic relationships and that can be obligate (i.e., both symbionts entirely depend on each other for survival) or facultative (i.e., they can but do not have to live with the other organism).

Sympatric. Overlapping, as in *sympatric distribution*.

Vicariance. Biogeographic process of speciation in which the range of distribution of a species is severed by a geographical barrier, causing the posterior isolation of populations (disjunct populations).

analytical requirements; frequent hypotheses tested; and current achievements and limitations. We end by highlighting some new approaches in CP and by briefly discussing future directions for the field. Illustrative examples are included throughout for clarification and to facilitate understanding.

What is a CP study about?

A CP study consists of a phylogeographic analysis of two or more species or taxa and a comparison of their respective phylogeographic patterns (Avise 2008). As such, CP is not an independent field, but an approach integrating both ecology and evolution. In general, a CP study has two phases (Bermingham and Martin 1998, Victoriano et al. 2008): It begins with a descriptive survey that includes the collection of genetic data from the species (e.g., sequences, multilocus genotypes) and phylogenetic analyses of that data. The intraspecific data obtained at this phase provide insight into phylogenetic relationships of populations, genetic diversity and genetic distance between lineages, effective population size, gene flow, and the timing of diversification events. The second phase is the comparative component and includes diverse analyses to test for congruence between the evolutionary and distributional histories of each species; it also includes evaluation of the geographical, ecological, and biological hypotheses that could explain those histories. The aim at this phase is to evaluate whether the evolutionary histories of these species show a shared response to the same historical events. In figure 1, we have summarized the information commonly used for developing a CP study following this two-phase model.

Main factors to consider when designing a CP study

Below we outline the major factors that must be considered during the design phase of a CP study.

Choosing the species of study. By evaluating two or more species that share part of or all of their geographical range, one can detect not only dispersal and vicariance events (as in traditional biogeographical approaches) but also hybridization, secondary contact, suture zones, and population and demographic dynamics, to name a few patterns. Most phylogeographic studies are designed on the basis of some initial abiotic and biotic information (figure 1), which is essential in choosing the species of study as well as the geographical scale and method of analysis. Investigating the biology and ecology of taxa is an important first step, because many species-specific characteristics, such as environmental requirements, life history characteristics, dispersal abilities, and ecological associations, can have an effect on the species' response to biogeographical processes and ultimately on patterns of genetic diversity (Ditchfield 2000). Accordingly, species-specific features can be criteria for selecting species to include in a CP study (figure 1). The species' relationship (i.e., sister species or distantly related species) and the distribution of gene genealogies (i.e., concordant or discordant), if they are known a priori, can also be considered in the selection of species for a CP study. Some general phylogeographical outcomes can be expected on the basis of this information, as is shown in figure 2.

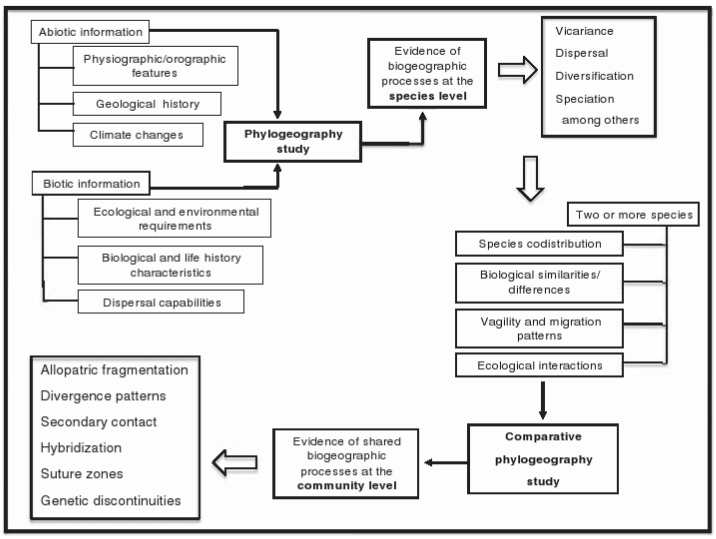

Figure 1. Summary of the information commonly used to perform a comparative phylogeography study, based on the different studies examined for the present review (see also supplemental table S1, available online at http://dx.doi.org/10.1525/bio.2011.61.11.5*).*

For instance, by studying sympatric sister species or codistributed taxa with comparable ecological preferences and dispersal abilities, one can evaluate whether their phylogeographic structures result from recently derived differences or from more ancient historical processes (figure 2). Species in archipelagos are a good example of the latter, particularly if they represent independent dispersal or colonization events—that is, if they arrived on the islands at different times or from different places. Such species are often exposed to similar environments among the islands and may consequently have similar evolutionary patterns. Kirchman and Franklin (2007) evaluated such a scenario: They studied three bird species

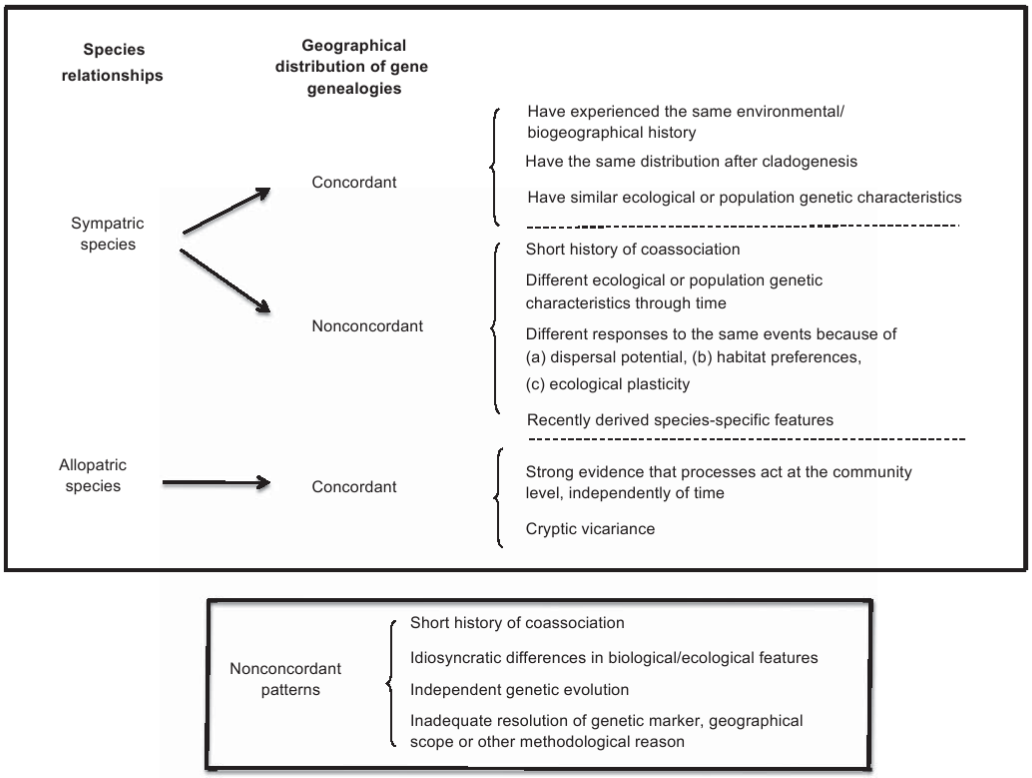

Figure 2. Summary of some simple general outcomes of a comparative phylogeographic study in accordance with the codistributed species relationships and the geographical distribution of their genealogies. More complex patterns occur, but the ones included here are the most frequently found in the literature.

from the Vanuatu islands that are morphologically similar within the islands but have different habitats outside Vanuatu with respect to their distribution, morphology, and degrees of geographic variation. Following a CP approach, Kirchman and Franklin (2007) were able to conclude that these codistributed species had different historical migration and colonization events but have similar levels of phenotypic variation as a result of their parallel evolution on the islands (see supplemental table S1, available online at *http://dx.doi.org/10.1525/bio.2011.61.11.5*).

Even phylogenetically distant species, with an affinity for a particular habitat, are good candidates for CP studies. An example is the study of Fedorov and colleagues (2008), who evaluated the phylogeographic history of the wood lemming (*Myopus schisticolor*), a species closely associated with the boreal forest of the taiga zone in Europe and Asia. Federov and colleagues (2008) compared the lemming data with those of other boreal forest species across Eurasia and found that they all had similar phylogeographical patterns associated with late Quaternary glacial and geological events. Their results showed that the primary events that shaped the historical dynamics of the taiga zone and the evolutionary history of the species were successive range expansions and contractions of the boreal forest (table S1).

Regarding ecological associations and interactions, mutualism and the host–parasite system are ideal systems to explore evolutionary history in CP studies (Razo-Mendivil 2010). Historical processes and geographical barriers that affect both the host and its symbiont can cause parallel differentiation and, consequently, congruent phylogeographical patterns. Nevertheless, because of species-specific characteristics (e.g., dispersal ability), a host and its symbiont can show different histories. For example, Parker and colleagues (2004) evaluated the CP of two species of legumes (*Amphicarpaea*) and their root nodule bacteria (*Bradyrhizobium*) and found no evidence of parallel cladogenesis. Their results suggest that different bacterial lineages colonized *Amphicarpaea* at different times, after evolving in association with other legume species (table S1).

Some taxa have been less studied under specific comparative phylogeographical frameworks and therefore contain good species to consider; the relatively fewer number of

studies in which fungi, plants, parasites, and marine species were used became evident during our review. Some studies done with fungi—although not explicitly expressed as CP—have shown strong phylogeographic patterns (Printzen 2008), whereas others have described or confirmed new species that originated from cryptic speciation events (Otálora et al. 2010). Nonetheless, still lacking for fungi is an analysis of the distribution of genetic variation and of gene genealogies in a CP context.

CP surveys of freshwater species have primarily involved fish, whereas surveys of other vertebrate and invertebrate species are lacking. Goldstien and colleagues (2006) emphasized the need to evaluate phylogeographic hypotheses among multiple marine taxa; they also highlighted the importance of studying particular regions, such as locations at which currents converge and cause compositional and temperature changes, which may represent biogeographical barriers. Likewise, Schaal and colleagues (1998) stressed the difficulties of working with plants in phylogeography; as a result, considerably fewer comparative studies have been done with plants than with animals.

Finally, choosing taxa for a CP study often depends on the availability of previous phylogeographical studies from the region of interest, as well as on the congruence of the patterns observed therein, independent of ecological history or phylogenetic relatedness (Taberlet et al. 1998). Likewise, although the chosen species must be codistributed, numerous other characteristics are also taken into account, including physiological similarities (Bermingham and Martin 1998), differences in body size and feeding adaptations (Pastorini et al. 2003), habitat preferences and vagility (Hugall et al. 2002), differences in reproductive cycles (Churikov and Gharret 2002), sexual dimorphism (Smith et al. 2000), and ecological preferences and levels of endemism (Huhndorf et al. 2007). Taxon-specific considerations may also be necessary; for example, ecophysiology and seed dispersal forms are important to consider when selecting plant species for CP analyses (Schönswetter et al. 2004).

Geographical scale and sampling strategies. The geographical scale is undoubtedly important when delimiting the region of study in CP. Selection of the study area should depend on the biogeographical question and the distribution of species; however, it often relies on existing geographical, geological, and historical information about the region. Likewise, the availability of samples of the species of interest—be they from museums and public or private collections or from the wild—is an important factor to consider.

The questions evaluated in CP studies are most frequently of a global scale, mainly because the entire geographical range of the species is commonly evaluated. However, some examples actually cover a variety of scales. An example of a global-scale study is that of Albach and colleagues (2006), who investigated eight closely related and sympatric species of the *Veronica alpina* plant complex from alpine habitats across Europe and North America. Following a CP approach, they described the phylogeographical patterns of species on each continent and located their potential Pleistocene refugial areas. All of the studied species showed concordant phylogeograpical patterns, which are directly associated with the similar environmental processes (i.e., Pleistocene climate changes) that affected the Northern Hemisphere (figure 2). In addition, the authors also did an intercontinental comparison and found higher genetic diversity levels and stronger genetic structure for the species from North America, due to less-severe population contractions (table S1).

As for the sampling requirements, both comparative and intraspecific phylogeography undoubtedly require thorough sampling in order to have adequate inference power. Extensive geographical sampling can provide considerable insight into the evolutionary processes of closely related taxa and at different levels of organization, from populations to species (Avise 2000). Different sampling strategies together with a variety of population data have shown that adequate sampling of the genetic variation is crucial for the statistical evaluation of phylogeographical hypotheses (Morando et al. 2003, Bonett et al. 2007). However, as is noted in table S1, sample numbers differ significantly among studies, and the number of samples per locality or population varies as much as the taxonomic level of sampling (Domínguez-Domínguez and Vázquez-Domínguez 2009), which can range from the subspecies level to that of the genus (Taberlet et al. 1998, Zink et al. 2001).

Determining the best sampling strategy is not an easy task. The a priori assessment of sampling adequacy can be particularly challenging, because genetic diversity is not always randomly distributed across a species' geographical range. Increasing the number of samples may not provide a better representation of the distribution of genetic variation, nor does it guarantee better resolution in the analyses performed. However, the higher the number of samples is, the better the framework is for a CP study (Victoriano et al. 2008). Likewise, strong differences in sample size between localities or populations and insufficient coverage of the species' range can both lead to limitations in hypothesis testing or even to erroneous interpretations. If sufficient sampling size and geographic coverage cannot be achieved (as they would be in the ideal scenario), it is acceptable to instead sample a higher number of populations, thereby maximizing the sampling of geographic distribution. Particular attention should be given to locations near putative barriers or filter barriers, which can potentially affect phylogeographical patterns. Previous studies or preliminary sampling might help, for example, to identify specific areas where patterns of genetic variability should be recovered (Buckley 2009). In addition, a careful sampling design (i.e., to try to sample all individuals in the population) must be planned when studying endangered species with limited ranges or reduced populations.

The importance of sample number was highlighted in a comparative study of the salamander *Desmognathus monticola* in the Appalachian Mountains (Bonett et al. 2007). The study design included intensive sampling of a genetically

diverse region and incorporated individuals that were an isolated population. On one hand, the phylogeographical results confirmed that a minimum number of haplotypes, which occur in less than 1.2% of the species' native distribution, was crucial in determining that the isolated population of *D. monticola* was not native to the region (i.e., it was introduced) and that it needed to be extirpated rather than conserved (table S1). On the other hand, examples exist in which few but properly distributed samples have been enough to evaluate demographic histories, the presence of refugia, and processes of colonization and expansion (Ditchfield 2000, Churikov and Gharrett 2002, Kirchmand and Franklin 2007).

Importance of the genetic markers used. The key elements defining a phylogeographic pattern are (a) how much genetic divergence is present among populations, individuals, or haplotypes (i.e., how genetically different are the entities measured?) and (b) how the haplotypes are distributed geographically (Avise 2000, Ditchfield 2000). In addition, the detection of such patterns depends on the genetic structure that can be revealed by the particular molecular marker used. Genetic information in phylogeography has been commonly obtained with the use of mitochondrial markers; for example, cytochrome b has proven useful in the description of intraspecific and comparative phylogeographic patterns of many taxa (see table S1 for other commonly used molecular markers). Nevertheless, the advantages of using more than one gene (mitochondrial, chloroplast, nuclear, or a combination of these) have become more and more evident (Templeton 2002). It is well known that stochastic variance can limit confidence in the interpretation of historical processes, particularly for CP studies, if they are based on only one gene. Accordingly, using a variety of genes is helpful for good phylogenetic resolution among taxa but also for identifying differences among populations or species that were not previously recognized taxonomically (Domínguez-Domínguez and Vázquez-Domínguez 2009), for obtaining a stronger evaluation of geographical barriers and dispersal routes, and for making stronger inferences about different evolutionary significant units. Likewise, if different genes show equivalent divergence among populations, individuals, or haplotypes, it can be considered evidence of a lack of gene flow, the separation of populations, or a history of isolation (Zink et al. 2001). Notably, an important principle in CP is that similar divergence values observed among genes in codistributed species strongly suggests common biogeographical processes (Taberlet et al. 1998, Riddle et al. 2000a, 2000b).

One should nevertheless consider that histories—for example, of local extinction and recolonization—might vary among species, despite a common geographical history. Consequently, genetic differentiation and genetic divergence values can be difficult to compare among genes (Hugall et al. 2002). In addition, it is important to remember that even though some genetic markers are adequate for identifying genetic differences between populations or taxonomic groups, not all of them provide information about the causes or factors associated with such differences. For instance, allozymes are not always selectively neutral, and allozyme evaluations capture only a small fraction of the variation contained in an organism's genome, because not all variation in the DNA will translate into variable protein products (Freeland 2005). Accordingly, they are not the best choice to determine a geographical pattern of genetic differentiation between populations. Finally, if levels of genetic variation among the species of interest are known at the beginning of a CP study, one can choose the molecular marker that has the highest genetic variability (see Mateos 2005). Another crucial factor to consider when choosing both the species and the genetic markers is undoubtedly the methods of analysis and statistical testing, a topic we review in the following section.

CP analyses: Phylogenetic, genetic, and phylogeographical methodologies

The techniques and methods followed in CP studies vary greatly (see table S1); a detailed survey of methods is out of the scope of the present review, but in this section, we highlight those most frequently used and their primary references.

Phylogenetic approaches. Comparison of the molecular phylogeny, taxonomy, and geographical distribution of several taxa occupying the same area, as is routinely done in CP studies, provides insight into the complexity of the evolution and history of codistributed species (Riddle et al. 2000b, Pastorini et al. 2003). Therefore, obtaining a high-quality phylogeny is a critical element in the search for the repetitive patterns of taxonomic subdivision evaluated in phylogeography. The phylogenetic approaches applied in CP studies are diverse, but in common among them is their use of gene trees instead of species trees. For example, in order to show vicariant events, researchers may use (a) a gene tree for each of the species studied, in which individual haplotypes are grouped by locality (Riddle et al. 2000b, Hugall et al. 2002); (b) a tree in which the branches represent each individual or haplotype, regardless of the species' localities (Mateos 2005); (c) a tree that includes all of the species in each locality (Pastorini et al. 2003); or (d) multiple trees, on which the geographic distributions of the species are overlapped (Riddle et al. 2000b). Phylogenetic methods have been employed for a diverse array of evolutionary and phylogeographical inquiries, such as estimating times of divergence (table S1; see Mateos 2005); discerning taxonomic differentiation at the subspecies, species, or congeneric level (Ditchfield 2000, Demastes et al. 2002); inferring the temporal scale of diversification; testing hypotheses regarding the chronological development of historical events (Leaché et al. 2007); and identifying cryptic species and their phylogeographical histories (Razo-Mendivil et al. 2010). Although diverse types of phylogenetic analyses are necessary in CP, it is important

Box 2. Statistical testing in phylogenetic analyses.

Phylogenetic evaluations must include a statistical component, particularly for support regarding similarities among patterns observed between different genes or among multiple species. Phylogenetic inferences have intrinsic supporting statistics (bootstrap values or posterior probabilities), but other approaches also exist, including comparisons among genetic distance percentages or bootstrap values (Pastorini et al. 2003) or between mean divergence values among localities (Hugall et al. 2002). Specific statistical tests of phylogeographic hypotheses using phylogenetic trees have been done by different means. For example, Sullivan and colleagues (2000) used three statistical methods in order to test competing hypotheses of concerted (i.e., concordant among species) versus independent responses to past climatic fluctuations in the highland rodent *Reithrodontomys sumichrasti* and the *Peromyscus aztecus–hylocetes* complex. The results of Kishino–Hasegawa/Templeton test and parametric bootstrap tests that showed that the observed phylogeographic incongruence between the two groups were significant, which supports the independent-response hypothesis. They also used a randomization test of reconciled tree maps, which indicated a significant history of covicariance between the two groups, which in turn supports the concerted-response hypothesis. Through combining these complementary methods of analysis, Sullivan and colleagues (2000) concluded that there has been some correlation in the responses of these two taxa to past climatic and geologic events but that the responses have not been entirely concordant (see supplemental table S1, available online at *http://dx.doi.org/10.1525/bio.2011.61.11.5*). Other methods employed for statistically testing phylogenetic results include the Brooks parsimony analysis (Taberlet et al. 1998), a comparison between user-defined trees and empirical ones with statistical tests (Ditchfield 2000).

to be aware of the limitations of these methods and of the need to incorporate new forms of statistical testing (box 2; Vázquez-Domínguez et al. 2009).

Genetic and demographic analyses. Although many CP studies include a qualitative or quantitative comparison of the phylogenies of the species studied, this is not a prerequisite. Indeed, many examples exist in which the species' response to barriers and filter barriers—key elements in phylogeography—have been evaluated using a combination of genetic and demographic analyses (Templeton et al. 1995, Zink et al. 2001, Churikov and Gharrett 2002, Michaux et al. 2005, Templeton 2009). Such analyses have included measures of genetic diversity, divergence, and structure; tests of molecular variance; the use of minimum-length unrooted trees; the application of nested clade phylogeographical analysis (NCPA); the estimation of mismatch distributions and of effective population sizes; and the use of statistical parsimony networks or other algorithms that depict the relationship between haplotypes. One comprehensive example of such an approach is the work of Goldstein and colleagues (2006), who evaluated the CP of three intertidal limpet species distributed along North Island and South in New Zealand. Their analytical approach incorporated estimates of genetic diversity, molecular variance, minimum spanning networks, and NCPA. They confirmed moderate to strong genetic discontinuities among the North and South Island populations due to allopatric fragmentation, a pattern broadly concordant across the three species (table S1, figure 1). Although such methods may be sufficient to detect phylogeographical patterns, there are also theoretical and mathematical methods that have been specifically developed for both intraspecific and comparative phylogeographic analyses and that allow for a more comprehensive description and evaluation of such patterns and their associated processes (Hickerson et al. 2006a, 2006b, Richards et al. 2007, Carstens et al. 2009, Moussalli et al. 2009).

Novel theoretical and mathematical frameworks. The recent increase in CP studies, as well as the use of more genes and a higher number of species, has motivated the development of new theoretical and mathematical frameworks (e.g., coalescence theory; see box 3), together with specific methodological approaches for phylogeographical analyses (box 4). Simultaneous divergence patterns—one of the central interests in CP—are still an analytical challenge because of the confounding effects of the differences among taxon pairs on parameters that affect genetic divergence (e.g., mutation rates, ancestral population sizes, ancestral subdivision, generation time, migration). To cope with this problem, different maximum-likelihood and approximate Bayesian methods have recently been developed that specifically take into account that some phylogeographic information includes multiple data sets that should be simultaneously analyzed (box 4).

As was mentioned previously, inferences in CP require statistical models for hypothesis testing that incorporate the variance present both in demographic models and in coalescent stochasticity. This is important because multiple historical events with temporal and spatial differences can result in similar phylogeographical patterns that appear to be the consequences of a single event (Riddle and Hafner 2006). The approximate Bayesian computation under a hierarchical coalescent model (hABC) developed by Hickerson and colleagues (2006b, 2007) specifically considers the problem of statistically testing biogeographic hypotheses and takes into account coalescent stochasticity, uncertainty in taxon-specific demographic parameters, and the genetic variance associated with coalescent and mutational processes. Leaché and colleagues (2007) applied the hABC method to test for simultaneous vicariance across 12 codistributed taxa that share a common phylogeographic pattern of genetic divergence across the center of the Baja California peninsula. The hABC results did not support a shared vicariant history, and Leaché and colleagues (2007) concluded that genetic

Box 3. Theoretical and mathematical frameworks: Coalescence theory in comparative phylogeography.

Initially, it was considered in phylogeographical analysis that branches on the gene tree could be interpreted as evidence for the occurrence of specific historical demographic events in a geographical context. However, gene trees are random outcomes of stochastic population-level processes, and such randomness has profound implications for the interpretation of the estimated gene tree (Nielsen and Beaumont 2009). The advent of coalescent theory has been fundamental in dealing with this problem, because the theory provides a mathematical framework that describes the distribution of gene trees in populations by relating the patterns of common ancestry within a sample to the size and structure of the overall population (Kuhner 2008, Knowles 2009, Nielsen and Beaumont 2009). The coalescent is a stochastic model that describes the ancestral or genealogical process for a sample of gene copies, which is well suited to data analysis, because it generates testable predictions about variation in a sample and because it yields efficient simulation algorithms. Because the same coalescent process holds for a wide variety of different reproductive schemes, it is considered to be a very robust model. Accordingly, coalescent-based population genetic models, together with diverse analytical and simulation methods, have been developed that account for changes in demography and for stochastic lineage sorting, which have helped to connect demographic models with gene trees.

Box 4. Methodological approaches for phylogeographical analyses.

Maximum-likelihood and Bayesian methods use the full information content of the data and can therefore, in principle, separate simultaneous and variable-divergence histories present in comparative phylogeography (CP) information. However, simulation-based methods, such as approximate likelihood and approximate Bayesian computation, are more suitable to simultaneously analyze multiple phylogeographical data sets, especially those involving complex models and idiosyncratic biogeographical histories (Hickerson et al. 2006b). Moreover, approximate Bayesian computation is a statistical technique that can be used to infer parameters and to choose between models in the complicated scenarios encountered in CP. For example, on the basis of gene-sequence and microsatellite data, approximate Bayesian computation has been used to choose between competing models of human demographic history and also to infer growth rates and times of divergence (Beaumont 2010).

Summary statistics are also applicable to CP analyses (Knowles 2009) and have the advantage that they are relatively unbiased and can summarize relevant information regarding a parameter of interest (e.g., effective population size). As an example, Hickerson and colleagues (2006a) used simulation-based approximation methods to review a set of summary statistics and to estimate their power to test for simultaneous vicariance across comparative phylogeographic data sets: They simulated the behavior of seven summary statistics, considered different divergence times using multiple taxon pairs, and evaluated simple hypotheses to test simultaneous vicariance given variable population sizes. They found that different summary statistics were superior (i.e., achieved the highest statistical power) when testing older divergence-time hypotheses or when testing more recent divergence-time hypotheses.

diversity in this region was structured by two different historical events (table S1, figure 2).

Temporal approaches. The different temporal components of the evolutionary history of species can be inferred by evaluating the phylogeographic signal carried by different levels of genetic diversity: Genotype and allelic frequencies can generally change over a few generations, whereas changes at the DNA-sequence level are relatively slow. Taking this into account, Garrick and colleagues (2008) proposed a framework in which direct and indirect approaches were combined to quantify the temporal elements in a variety of molecular data, which permits explicit assessment of congruence at those variable temporal depths. This approach can be used to evaluate the influence of ancient relative to that of more-recent factors and processes that have an impact on genetic structure. Remarkable examples include CP studies with symbiont species in which these temporal phylogenetic analyses have helped to clarify the changes in specialization in symbiosis between plants and bacteria (Parker et al. 2004).

Conservation approaches. Several years ago, Moritz and Faith (1998) suggested, in a conservation context, the use of a diversity measure (phylogenetic diversity) based on the branch lengths of phylogenetic trees to evaluate independent evolutionary units. To our knowledge, this approach has only been used in a CP context by Smith and colleagues (2000), who compared the phylogeographic structure of two bird species that have different patterns of endemism across six mountains in Africa. They estimated how much of the total phylogenetic diversity was shared among the regions and how much was unique to each one. On the basis of their results, they identified specific geographical regions that harbor evolutionarily distinct populations, which are highly significant in the conservation of these species (table S1).

Hypotheses in CP

A geographical component—particularly the climatic and geological history of the region of interest—is of course a common feature of all phylogeographic studies. Accordingly, many of the hypotheses that are intended to be tested in CP studies are related to these factors. Frequently tested climatic hypotheses involve global temperature changes; past climatic fluctuations; and glacial cycles, mainly during the Quaternary period. For example, one can assess the classical hypothesis that highland organisms shifted in concert,

elevationally or latitudinally, in response to glacial cycles (Sullivan et al. 2000, Schönswetter et al. 2004). Interestingly, concordant phylogeographic patterns are not necessarily the rule when sympatric species are evaluated. Michaux and colleagues (2005) tested such a hypothesis with their study of two European rodents (genus *Apodemus*), in which they used mitochondrial DNA sequences of samples from throughout the rodents' range. They found that the two species survived the Quaternary glaciations in different ways and from different refugia. This study shows the importance of taking into consideration biological characteristics like ecological plasticity when evaluating survival through climate change. It also confirms that even closely related species can have different phylogeographic histories (table S1, figure 2).

Another source of information to establish hypotheses for CP are geological reconstructions, which are often based on paleontological and stratigraphical data and which have helped to determine the chronology of orogenic processes such as volcanism and river or mountain formation. Such geological reconstructions imply a sequence of evolutionary events from which geographical hypotheses can be established and later tested by evaluating the phylogeography of species. An example of this is the CP study of Huhndorf and colleagues (2007) that revealed the effect of volcanic activity on the fragmentation of montane rainforests in east-central Africa. Using mitochondrial DNA sequence variation, Huhndorf and colleagues (2007) estimated phylogeographic patterns and divergence times for populations of three species of montane endemic rodents. These estimates were supported by molecular dating of the formation of biogeographical barriers shaped by the eruptive events. Their results demonstrated that this type of fragmentation played a major role in the diversification of these montane endemic rodents during the middle to late Pleistocene (table S1).

Historical and phylogeographical hypotheses may be built on the basis of fossil evidence; however, fossils are scarce for most taxa. In fact, we did not find any examples of studies that included fossil evidence for different but codistributed species. There are studies in which species sharing a recent common ancestor were investigated and in which a molecular-clock approach, which facilitates the estimation of divergence times and other events, was frequently incorporated. One example is the study of Hemmer and colleagues (2010), who performed a CP study of the jaguar (*Panthera onca*) on the basis of fossil remains of the lower dentition in combination with modern DNA assessments. Their results showed that the ancestral population was of African origin, from which the jaguar dispersed over Europe 1.95–1.77 million years ago (mya) and that its transcontinental dispersal to North America happened around 0.99–0.78 mya, with a later diversification in South America.

Vicariant and dispersal models are often evaluated in CP studies as biogeographical hypotheses. Although examples are numerous for both models, to illustrate the former, we highlight the work of Riddle and colleagues (2000b), who evaluated the phylogeographic population structures of 12 mammalian, avian, amphibian, and reptilian species. They constructed phylogenetic trees for each taxon, estimated their net divergences, and compared the different phylogeographic groups (called *phylogroups*) that they found. Their results support previously hypothesized vicariant events in the evolution of Baja California–peninsular biota, from the late Miocene to the middle Pleistocene (table S1). Example hypotheses involving dispersal models include instances of continent-to-island migration and recolonization, founder effects, and growth and decrement of population sizes. For instance, Kirchman and Franklin (2007), whose study was discussed previously, combined analyses of haplotype (DNA sequence) diversity and genetic structure with demographic and phylogenetic approaches. They found evidence to support the hypothesis of recent colonization and subsequent expansion in two of the three species of birds that they examined.

One may come across instances in which biogeographical hypotheses do not explain the distribution of genetic variation or the phylogeographic patterns observed. Many factors can lead to this lack of congruence, including the instability of species assemblages or communities through time (Taberlet et al. 1998). Codistributed species can also have different, independent responses because of idiosyncrasies in their biological and ecological characteristics, because they just recently came to share their geographical distribution, or because the gene (or genes) evaluated has (or have) different evolutionary rates in the species studied (figure 2; Sullivan et al. 2000). One should also consider the particular scenario in which the geological events are temporally nested, occurring at the same site but at significantly different times. For example, the repeated elevations of the Rocky Mountains and the most recent glacial cycling associated with those mountains have produced barriers and events of very different ages (Spaeth et al. 2009). This is an essential consideration in CP studies, because it can result in a lack of congruence or, more importantly, in false congruence among species. A potential solution was suggested by Riddle and Hafner (2006) that included temporal nesting within a taxon combined with fossil dating and Brooks parsimony analysis.

New approaches for CP analyses

Several new approaches have recently become available for the performance of CP analysis. We describe them here.

Hypothesis testing. Further advancing the latest methodological frameworks proposed for CP analyses, Hickerson and Meyer (2008) extended the hABC model (Hickerson et al. 2006b, 2007) to develop a new hierarchical approximate Bayesian computation model (HABC). This model uses coalescent population genetics to estimate ancestral demographic patterns (e.g., population size expansion or contraction) across codistributed taxa. Moreover, the HABC model can explicitly test simultaneous vicariant and dispersal events in multiple codistributed taxon pairs. It is also capable of distinguishing simultaneous isolation, even with a few individuals. As was discussed previously, this sampling

limitation is often encountered in phylogeographic surveys. Another advantage of the HABC model is that one can analyze different phylogeographic data sets at once in order to make cross-taxon-pair inferences about biogeographical processes while explicitly allowing for uncertainty in the demographic differences within each taxon pair. Hickerson and Meyer (2008) used the HABC model on two comparative phylogeographical data sets (mitochondrial DNA sequences from multiple codistributed taxa) to test two hypotheses of marine allopatric speciation. Their results showed how the model is able to detect whether either hypothesis is a dominant process across the codistributed taxon pairs.

Multivariate analyses (e.g., principal component analysis) are an efficient tool for the evaluation of genetic variability in different contexts, including phylogeography. They have shown great potential in the detection of spatial genetic patterns and in the exploration of recent phylogeographic events, mainly because of their ability to summarize multivariate genetic information into a few synthetic variables (Jombart et al. 2009). Ciofi and colleagues (2006) explored the challenging scenario of inferring colonization dynamics of populations or lineages that have derived from relatively recent dispersal events within islands and that have limited dispersal. They studied phylogeographical and dispersal events of the giant Galápagos tortoise (*Geochelone nigra*) using mitochondrial DNA and microsatellites and a variety of multivariate, genetic, and phylogeographic analyses. The analyses revealed a strong association between geographical distance and genetic distinctiveness, concordant with the rather recent colonization events on the island (table S1). Previous studies for this species were unable to distinguish colonization processes, probably because of inadequate analytical methods.

Carstens and colleagues (2009) recognized the need to have more means of testing the predictions of null hypotheses in CP. They proposed a method involving information-theoretic metrics in which information theory is used to quantify the probability of multiple hypotheses given the phylogeographical data in question. They used both empirical and simulated data from previous studies of the salamander (*Plethodon idahoensis*) to generate a ranking of 17 models, each of which represented a set of historical evolutionary processes. This approach allowed Carstens and colleagues (2009) to quantify the relative strength of support for each of the 17 models instead of simply rejecting various hypotheses.

Supertrees. Supertrees result from combining many smaller, overlapping phylogenetic trees into a single more comprehensive tree. On the basis of this premise, Victoriano and colleagues (2008) searched for a shared phylogeographical signal in lizards with partially overlapping ranges (three species of *Liolaemus*) using a supertrees method. Their results are consistent with the hypothesis that the species responded in parallel to shared historical processes, which in turn influenced their phylogeographical structure (table S1). This method requires well-resolved phylogenies, with reasonable sampling throughout the species' distribution. The obtained genealogies are analyzed with the supertrees approach to evaluate whether any combinations of species show significant spatial codivergence. An advantage of these recently developed methods is that they incorporate the assessment of significant statistical signals through randomization tests. Another advantage is that with these methods, one can perform statistical tests of a shared phylogeographical signal between taxa, even if the taxa have only partially overlapping geographical distributions instead of complete codistribution, as is usually required when estimating spatial divergence.

Niche and paleodistribution models. Species-distribution modeling and its historical counterpart, paleodistribution modeling, which both explicitly incorporate spatiogeographic data (abiotic variables such as temperature, precipitation, and topography), are increasingly used analytical approaches in phylogeography (Hickerson et al. 2010). Hugall and colleagues (2002) compared the phylogeography of several endemic vertebrates from the Australian tropical forest with spatial models of predicted species distribution under different paleoclimate scenarios. They identified late Pleistocene–Holocene historical refugia and broad patterns of extinction and recolonization shared among species (table S1). Furthermore, these new modeling techniques, in combination with coalescent simulations, also provide a means for generating realistic phylogeographic hypotheses, even for taxa that have a scarce fossil record (Richards et al. 2007).

Indeed, these approaches have enabled the evaluation of the impacts of past climate fluctuations at a very fine scale, together with speciation mode and migration during Pleistocene cold cycles. In a study aimed at comparing spatial patterns of population persistence and isolation across three species of rainforest skinks (*Saproscincus*) and at investigating their responses to late-Quaternary climate fluctuations, Moussalli and colleagues (2009) found high concordance between molecular data and paleodistribution models, which indicates conservatism of bioclimatic niches (i.e., different codistributed species have similar historical abiotic and environmental characteristics). The study showed that species with broader climatic niches maintained more genetic diversity and that this genetic diversity was more structured as a result of persistence through different historical climates. This result suggests that the observed differences in response to past climate change were associated more with differences in the species' climatic niches than to other ecological traits (table S1).

Conclusions

CP studies have been increasing in number at a much slower pace than those at the intraspecific level. We suggest that data-gathering limitations in conjunction with methodological and analytical difficulties are the primary reasons for this. Time is also a constraint, given that performing a CP study means having to obtain field and molecular data for more than one species and also means using additional and more complex analytical and statistical methodologies.

It is evident that testing and proving congruent phylogeographical patterns among taxa (as a result of a common evolutionary history) is no easy task, considering that even sister species can differ in their ecological characteristics and life-history features. Accordingly, much emphasis has to be placed on developing new analytical methods and on using different methodologies in concert in order to compare species and localities in a quantitative manner. Species-specific demographic and ecological characteristics can result in different outcomes of the same historical and biogeographical events and, consequently, in the loss of concordant phylogeographical patterns between species. Therefore, in the planning of a CP study, the information obtained through intraspecific phylogeographic evaluations is of fundamental value. It is also important to consider, among other things, the stochastic variation of loci; the degree of taxonomic resolution in the species of interest; and their ecological characteristics; together with fossil evidence and biogeographical, geological, and historical information relating to the geographical distributions being studied.

CP is no longer solely descriptive and qualitative, and one can now test more rigorously the comparative evolutionary histories of multiple species. In regions in which sufficient background information is available, a priori hypotheses can be statistically tested; moreover, for poorly known regions or taxa, new methods also allow the generation of alternative hypotheses. Many evolutionary and historical questions, such as models of speciation and diversification times, will continue to be answered with CP surveys; the scope of applications will also widen significantly to identify areas of endemism, patterns of hybridization, and evolutionary novelty with conservation value. The identification of selection patterns and that of ecologically driven speciation have been identified by Hickerson and colleagues (2010) as further opportunities for CP studies. Indeed, one of the most powerful properties of CP is that it allows evaluation of evolutionary patterns and processes involving entire communities and assemblages, which may broaden our theoretical and applied knowledge of evolution, ecology, and genetics at the community level. As was mentioned at the beginning of this review, over a decade ago, Bermingham and Moritz (1998) recognized some challenges that would improve the comparative approach in phylogeography. The present review testifies that those challenges have been amply met and, moreover, that many new challenges have been established or are to come. It also provides evidence that we have made considerable gains in our understanding of the influence of past events on current patterns of genetic diversity and on the geographical distribution of species.

Acknowledgments

We are deeply grateful to Christine King, John Avise, David Hafner, and Juergen Gadau for insightful comments that greatly helped us to improve this review. We also thank the members of our laboratory for enriching phylogeographic discussions that encouraged and motivated us to do the present review. Ulises Razo-Mendivil reviewed an earlier version of the manuscript. Partial funding was provided to EV by Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (Grants IN219707 and IN215205-2) and as a scholarship to TAG from Conacyt (175434).

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Table 1. Summary of comparative phylogeographic studies, including a brief description of the objectives or main questions analyzed, molecular markers used, taxa included (with sampling size if available), and genetic statistics and analyses performed. Main results are highlighted (see text for description of each example).

Objective / question	Molecular marker	Genus or species (# of samples)	Genetic statistics / analyses	Main results / conclusions	Reference
Vicariance hypothesis. Evaluation of the evolution of the Baja California Peninsular desert biota across 12 taxa.	COIII Cyt b	*Peromyscus eremicus* group (4 spp, 73 individuals); *Chaetodipus baileyi* group (2 spp, 51); *C. arenarius* (36); *Dipodomys merriami* group (60); *Ammospermophilus* (30); *Bufo punctatus* (44)	NJ, ML, MP, SD	Concordant intraspecific phylogeographical patterns support three vicariant events (mid Pleistocene, late Pliocene, and Pliocene, respectively). Results show a history of biogeographic and evolutionary independence in northern and southern regions of the Baja California Peninsular desert.	Riddle *et al.* 2000b
Comparison of phylogeographic structure of endemic birds across mountains in Africa. Evaluation of phylogenetic diversity index (PD) and levels of endemism.	Cyt b, CR	*Andropadus tephrolaemus* (32) *Nectarina oritis* (40)	HD, BCD, ML, MT, PD	Values of genetic diversity unique to each region, as well as some diversity shared among regions. Identification of specific regions that harbor evolutionary significant units (ESU's).	Smith *et al.* 2000
Testing concerted versus independent responses of codistributed rodents to past climatic fluctuations.	Cyt b	*Reithrodontomys sumichrasti* (43) Results compared to those found in *Peromyscus aztecus*/*P. hylocetes* complex	KHT, LRT, ML, MP, PBT, RRTM	Support for both hypotheses based on three statistical complementary methods. Phylogeographical incongruence together with history of covicariance as responses to past climatic and geologic events.	Sullivan *et al.* 2000
Predicting location of refugia based on paleoclimate modeling of a snail.	COII	*Gnarosophia bellendenkerensis* (121) Comparison with lizards and frogs, e.g. *Litoria*, *Gnypetoscincus*, *Carphodactylus*	DA, EPS, GD, MCMC, ML, NJ, PCM, SD	Snail phylogeography is consistent with predictions from the models in relation to the location and size of Pleistocene-Holocene climatic refugia, and also to extinction and recolonization patterns. Comparisons with vertebrate fauna revealed concordant as well as inconsistent (species-specific) phylogeographical patterns.	Hugall *et al.* 2002
Evaluation of the biogeographic history	Plant: chloroplast	Legume: *Amphicarpaea* (12)	KHY, ML, MP, NJ	Legume and its root nodule bacteria do not show parallel cladogenesis.	Parker *et al.* 2004

and phylogeography of a symbiotic relationship.	trnL region, nuclear ribosomal ITS, histone H3-D Bacteria: 16S rRNA, 23S, ITS	Bacteria: *Bradyrhizobium* (18)		Different *Bradyrhizobium* lineages evolved in association with other legumes and colonized *Amphicarpaea* species at distinct times.	
Testing of the hypothesis of a vicariant event responsible for concordant divergences in two freshwater fish species.	ND2	*Poecillia butleri* (13) *Poeciliopsis presidionis* (1), *P. latidens* (1), *P. fasciata* (2), *P. presidionis* (2), *P. tumeri* (1)	BA, GDV, LRT, MCk, ME, ML, MP, NJ	A Plio-Pleistocene vicariant event associated with geological activity caused divergence in three different lineages. Divergence times were estimated with the use of molecular clocks, supporting that divergences between species were a consequence of the same vicariant event.	Mateos 2005
Evaluation of the effect of Quaternary climate changes on sympatric rodent species.	Cyt b	*Apodemus flavicolis* (98) *A. sylvaticus* (102)	GD, GDV, ML, MP, MSN, NJ, TD	Species have different phylogeographical patterns and different European Quaternary refuges. Different patterns observed are associated with the species-specific ecological plasticity.	Michaux *et al.* 2005
Evaluation of evolutionary history in closely related sympatric species from alpine habitats in northern Europe and North America. Comparison of intercontinental phylogeography.	AFLP (genomic DNA), trnL intron, 3′ exon, trnL-trnF	*Veronica alpine* (107), *V. bellidioides* (68) *V. copelandii* (3) *V. cusickii* (11) *V. nutans* (3) *V. nipponica* (1) *V. stelleri* (4) *V. wormoskjoldii* (3)	AMOVA, f_{fp}, f_{pp}, GD, GDS, MP, NJ, PCoA	Concordant phylogeographical patterns as a result of similar environmental histories. Also, different phylogeographical patterns and Pleistocene refugial areas in each northern Europe and northern North America. Diversification patterns associated with higher genetic structure and diversity in species from North America.	Albach *et al.* 2006
Evaluation of the effect of vicariance on colonization and dispersal in islands, in recently derived lineages and species with limited dispersal ability.	CR micros	*Geochelone nigra* (631)	HD, MD, GD, GDV, AMOVA, NCA, BA, MiE, PCoA	Geographical distance and genetic distinctiveness strongly associated, concordant with recent colonization events. Evidence of chronological association between orogenic events (volcanic activity) and species evolution. Identification and proposal of different taxonomic units.	Ciofi *et al.* 2006
Evaluation of the effect of a marine barrier between the North and South islands in New Zealand in three	Cyt b	*Cellana ornata* (302), *C. radians* (321), *C. flava* (85)	AMOVA, GD, GDS, HD, MSN, NCA	Moderate to strong effects of the marine barrier. Concordant allopatric fragmentation patterns across species.	Goldstien *et al.* 2006

limpet species.					
Discerning the introduced or relict status of a population of an endemic amphibian species.	COX1	*Desmognathus monticola* (100)	HD, MSN, NCA	Results indicate that an extremely isolated population of the species was an introduction. Conservation applications designed based on the results that this not native population should be extirpated.	Bonett *et al.* 2007
Phylogeographic patterns of montane endemic rodents in fragmented areas of east-central Africa.	Cyt b D-loop	*Hylomyscus denniae* (21), *Hybomys lunaris* (13), *Lophuromys woosnami* (20)	HD, HKY, LRT, MCk, ML, MP, NJ	Geological reconstructions helped decipher chronology of events. Volcanic activity, phylogeographical patterns, and divergence times coincided and revealed that biogeographical barriers and fragmentation from eruptive events shaped the diversification of the species.	Huhndorf *et al.* 2007
Evaluation of historic population demography of three codistributed bird species in an archipelago.	CR I	*Gallirallus philippensis* (21) *Chalcophaps indica* (25) *Rhipidura spilodera* (17)	DA, EPS, GD, GDV, HD, HKY, MCMC, ML, MSN, T_{mrca}	Codistributed species, despite similar phenotypic variation within the islands, have historical and biogeographical differences. Results support the hypothesis of recent colonization and subsequent expansion in two species.	Kirchman and Franklin 2007
Testing for simultaneous vicariance across codistributed taxa along the middle of the Baja California Peninsula.	Cyt b, COIII, ATPase6, ND1, ND2, ND4	Mammals (12-136): *Ammospermophilus leucurus*, *Chaetodipus arenarius*, *C. baileyi* *Dipodomys merriami* *Peromyscus eremicus*, *Thomomys bottae* Reptiles (12-37): *Callisaurus draconoides*, *Uta stansburiana*, *Phrynosoma coronatum*, *Sceloporus zosteromus*, *Pituophis catenifer*, *Trimorphodon biscutatus*	HABC	The shared vicariance hypothesis is not supported. The structured genetic diversity observed in this region is the result of two different biogeographical events.	Leaché *et al.* 2007
Evaluation of Eurasian refugia, vicariant events, and recolonization of boreal species.	Cyt b	*Myopus schisticolor* (100) Comparison with a diverse array of boreal species across Eurasia	AMOVA, DA, EPS, GD, HD, HKY, MCk, MD, MJHN, ML, NJ, TD	Shared late Quaternary phylogeographical patterns across species. Successive range expansions and contractions shaped the historical dynamics of the species' shared environment.	Fedorov *et al.* 2008
Evaluation of concordant phylogeographical	Cyt b, 12S	*Liolaemus tenuis* (144), *L. pictus* (82), *L. lemniscatus* (52)	BA, GD, HD, MA, MAST, MDS, ML,	Concordant phylogeographical associations in all three species: groups consistent with bioclimatic zones.	Victoriano *et al.* 2008

patterns in partially overlapping lizard species, considering topography and endemicity			MRP, PRM, STA	Shared orogenic vicariant events and consequent divergence in two species. Identification of major refugial areas and dispersal routes.	
Comparison of spatial patterns of population persistence and isolation in three skink species, to evaluate late-Quaternary climate fluctuation in the Australian Wet Tropics.	ND4, tRNA	*Saproscincus basiliscus* (139), *S. tetradactyla* (35), *S. czechurai* (38)	GD, SD, AMOVA, DA, BA, PCM	Complex phylogeographical patterns reflect persistence of large, structured populations in the central zone. Support for a vicariant barrier separating northern and central regions. Species-specific characteristics shaped species distributions: mid-Holocene warm-wet climate was restrictive for high-elevation species, whereas cool-dry glacial maximum climate restricted more generalist and low-elevation species.	Moussalli *et al.* 2009

Abbreviations refer to: AMOVA=Analysis of Molecular Variance, BA=Bayesian analysis, BCD=Bray-Curtis distance, DA=Demographic analyses (e.g., growth, mutation rates, etc), ENM=Ecological niche modeling, EPS=Effective population size, f_{fp}=fixed private fragments, f_{pp}=polymorphic private fragments, GD=Genetic diversity (or nucleotide diversity), GDS=Genetic distance, GDV=Genetic divergence, HABC=Hierarchical Bayesian model, HD=Haplotype divergence or diversity, HKY=Hasegawa-Kishino-Yaro model, KHT=Kishino-Hasegawa-Templeton, KHY=Kishino-Hasegawa-Yano, LRT= Likelihood ratio test, MA=Multivariate analysis, MAST=Maximum agreement trees, MCk=Molecular clock, MCMC=Metropolis-Hastings Markov Chain Monte Carlo, MD=Mismatch distribution, MDS= Multidimensional scaling analysis, ME=Minimum evolution, MiE=Migration rate estimation, MJHN=Median-joining haplotype network, micros=microsatellites, ML=Maximum likelihood, MP=Maximum parsimony, MRP=Maximum representation with parsimony, MSN=Minimum spanning network, MT=Mantel test, NCA=Nested clade analysis NJ=Neighbour-joining, PBT=Parametric bootstrap test, PCM=Paleoclimate modeling, PCoA=Principal coordinate/Principal component analysis, PD=Phylogenetic diversity, PRM=Pairwise regressions models, RRTM= Randomization test of reconciled tree maps, SD=Sequence divergence (%), SPN=Statistical parsimony network, STA=Supertrees, TD=Time of divergence, T_{mrca}=Time to most recent common ancestor.

Capítulo 2

Artículo: **Consensus between genes and stones in the biogeographic and evolutionary history of Central America**

Publicado en: **Quaternary Research**

ARTICLE IN PRESS

YQRES-03405; No. of pages: 14; 4C:

Quaternary Research xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Quaternary Research

journal homepage: www.elsevier.com/locate/yqres

Consensus between genes and stones in the biogeographic and evolutionary history of Central America

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ARTICLE INFO

Article history:
Received 26 April 2012
Available online xxxx

Keywords:
Biogeography
Evolutionary history
GABI (Great American Biotic Interchange)
Middle America
Population genetics
Phylogeography

ABSTRACT

Results from genetic and geologic studies can be combined to elucidate some general patterns of the biogeographic and evolutionary history of Central America (CA) and of its biota. Based on an ample review of geologic, biogeographic and genetic studies, our aim was to examine how common genetic patterns can be linked with geologic processes. Considering information about geologic and tectonic evolution of CA, we subdivided the region into four tectonic blocks: Maya, Chortis, Chorotega and Chocó. Species exchange between North/South America and CA encompasses three events: a first migration during the Late Cretaceous–Early Paleocene, a second through a terrestrial corridor preceding the formation of the Isthmus of Panama (IP), and the third involving a major dispersion through the IP. Such events caused similar genetic differentiation patterns and left a signature on the diversification of extant taxa, which we propose as three evolutionary groups: 1) Mayan, characterized by marked genetic structure and divergence, multiple refugia and formation of cryptic species; 2) Mid-CA, defined by high differentiation at the population level and between highland and lowlands, associated with intense volcanic activity; 3) Panamian, distinguished by migration from north to south and vice versa via de IP, with markedly high species divergence and speciation.

Introduction

Central America is considered an outstanding region for the study of biological processes like speciation, extinction and diversification of flora and fauna, mainly because of its intricate geologic and biogeographic history, diversity of habitats and dynamic climatic and tectonic history (Cavers et al., 2003; Iturralde-Vinent, 2006; Daza et al., 2010; Gutiérrez-García and Vázquez-Domínguez, 2012). The increase of available genetic and phylogeographic information of a diverse array of taxa from this region, particularly from the Pliocene and Pleistocene, has helped decipher and date genetic and evolutionary processes associated with geological events. These studies have shown how the region's geologic and climatic history over the past several million years caused similar patterns of genetic differentiation for complexes of resident and/or migrant fauna (Daza et al., 2010). However, despite the association of the genetic results with the geology of the region, very few include a thorough description of the geological processes. Both genetic and geologic information are key for the understanding of historical patterns and processes that have shaped the diversification of species (lineages) at different spatial and temporal scales.

Based on the review of different studies and on our own data, it became evident that the common genetic and phylogeographic patterns of different taxa from this region correspond to large spatial and temporal scales, and consequently the evolutionary patterns identified are associated with geologic factors and processes corresponding to such scale (e.g., geomorphology, topographic barriers, volcanic chains, volcanic activity, large-scale climatic changes, intermittent connections, corridors). Factors that act at finer scales are related with the biota's life-history characteristics and it is only possible to associate them with evolutionary processes on a case-by-case basis and not as a general assessment. Hence, we believe that a thorough review, in which common genetic and phylogeographic patterns are highlighted and the interacting role of geologic factors is underlined, can help elucidate general patterns of the region's evolutionary history. Moreover, Quaternary researchers and readers from both biological and geological disciplines will benefit from gaining knowledge of how geologic information can be linked to other fields of study and also by the awareness of the current gaps in the study of the relationship between Central America geology, genetics and evolution.

The aim of this review is to examine how common genetic and phylogeographic patterns in Central America can be more thoroughly linked with geologic processes, for a more comprehensive understanding of the history of this region and the evolution and diversification of its biota. Most genetic information has been described for the Holocene onwards, and more specifically for the Quaternary. We

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http://dx.doi.org/10.1016/j.yqres.2012.12.007

Please cite this article as: Gutiérrez-García, T.A., Vázquez-Domínguez, E., Consensus between genes and stones in the biogeographic and evolutionary history of Central America, Quaternary Research (2013), http://dx.doi.org/10.1016/j.yqres.2012.12.007

start with a brief summary of the origin and development of Central America as a bridge connecting two landmasses (North and South America), based on different geologic and biogeographic studies, and describe the main geological events that ultimately affected the biota of the region. We next integrate information from a variety of systematic, phylogeographic and population genetic studies from different taxa, encompassing from the emersion of the region as a group of islands during the Miocene until the present, with emphasis on the Quaternary, to depict how some genetic and geological processes are linked in the evolutionary history of species. We do the latter by emphasizing the role of the corresponding large-scale geologic factors and briefly describe why others are less highlighted. Importantly, we show that there is an emerging pattern of differentiation, in which three main evolutionary groups can be linked to specific geological barriers such as mountain and volcanic chains, isthmuses and fault systems.

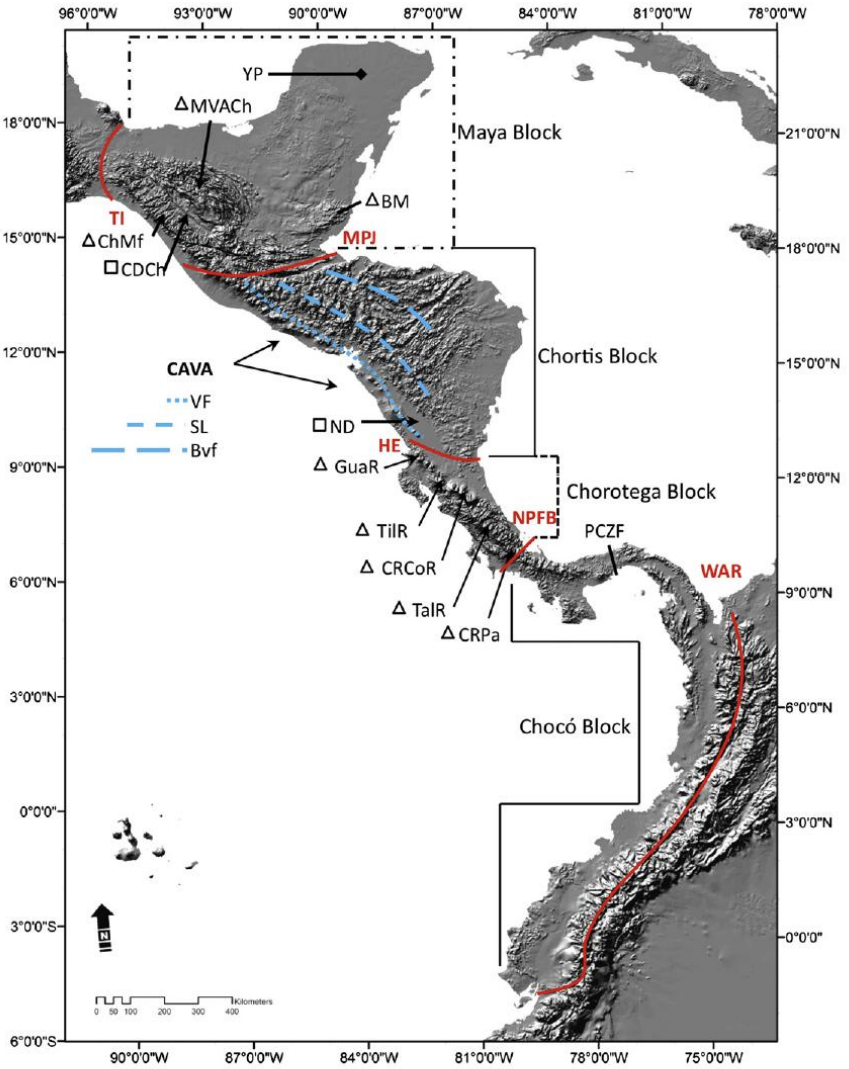

Figure 1. Summary of Central America's geology as used throughout the text, where main geological blocks, delimited by geological barriers, are shown. Abbreviations correspond to: BM = Belize mountains, CAVA = Central America volcanic arc, CDCh = Central depression of Chiapas, ChMf = Chiapas massif, CRCoR = Central range of Costa Rica, CRPa = Central range of Panama, GuaR = Guanacaste range, MVACh = Modern volcanic arc of Chiapas, ND = Nicaragua depression, PCZF = Panama channel zone fault, TalR = Talamanca range, TilR = Tilarán range, YP = Yucatan platform. Dashed (blue) lines indicate the three lines along the CAVA: Bvf = Behind the volcanic front, SL = Secondary line, VF = Volcanic front. Solid (red) lines depict the geological limits between each block, from north to south: IT = Isthmus of Tehuantepec, MPJ = Motagua–Polochic–Jocotán fault system, HE = Hess escarpment, NPFB = North Panama fracture belt, WAR = Western Andes range. Triangles indicate mountain ranges and squares depression areas and valleys. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Central America origin and development

We define Central America (CA) as a land area located between the Isthmus of Tehuantepec, Mexico, including the Yucatan peninsula, and the Andes range, Colombia (Fig. 1). In the genetic literature parts of this region have been indistinctly called Mesoamerica (Sullivan et al., 2000), Mesoamerica Isthmus (Cavers et al., 2003), Central America Nucleus (Crawford and Smith, 2005), Central America Isthmus (Wang et al., 2008) and Middle America (Parkinson et al., 2000; Razo-Mendivil et al., 2010). Hence, in order to explain our use of 'Central America', it is important to highlight that Mesoamerica refers to a social entity (Kirchhoff, 1943), an expression derived from anthropological studies that describe the distribution of a pre-Hispanic culture (Flannery, 1966; Blanton and Feinman, 1984). Thus, it can only be used to describe a biological area of distribution if it does not exceed this "social" limit. On the other hand, Middle America can be understood as a region between the United States and South America, sometimes including Caribbean islands and the West Indies (Winker, 2011).

Geological and genetic studies in this region have focused on events taking place from the gradual emergence of CA as an intermittent archipelago until it formed a continuous landmass. During this time the region was shaped by tectonic activity, including changes in sea level, island emersion, volcanism, massive earthquakes, faults along the continental edge and tsunamis, as a result of movements and interaction of the Cocos, North American, Caribbean and Nazca tectonic plates (White and Harlow, 1993; Rogers et al., 2002; Coates et al., 2004; Dávalos, 2004). The movement of the tectonic plates, together with processes like glacial and interglacial periods during the Miocene, caused sea-level changes that exposed groups of islands where at present stand CA and the Antilles. These islands have been called GAARlandia, Proto-Antilles or Proto-Greater Antilles (Iturralde-Vinent and MacPhee, 1999; Crawford and Smith, 2005). It has been suggested that this island chain emerged during the Late Cretaceous (80–70 Ma), a period that coincides with a decrease in sea level of ca. 60 m below the present level (Haq et al., 1987). Later, the emergent Greater Antilles were connected to northern South America during the Late Eocene–Early Oligocene transition (35–33 Ma). After a period of land exposure from the Miocene to the Early Pliocene (24–5 Ma), most of the islands emerged, whereas during the Late Miocene (9 Ma), 'stepping stone' connections between North and South America first became available (Dávalos, 2004; Heinicke et al., 2007; Montes et al., 2012). In addition, CA experienced multiple faults, based on which the region can be subdivided into four major tectonic elements: Maya, Chortis, Chorotega and Chocó blocks (Fig. 1). The blocks have particular climatic, lithologic and tectonic features that are summarized on the physiographic provinces proposed by Marshall (2007) (Supplementary Fig. 1). After this introductory description of the formation of CA, we start by briefly describing the main characteristics of the four blocks with the aim of broadly depicting the development of CA, an essential framework for our further integration of more detailed genetic and geologic information throughout the account of evolutionary patterns.

The Maya block

The limits considered here of the Maya block are the Isthmus of Tehuantepec, Mexico and the Motagua–Polochic–Jocotán fault system (Fig. 1; see Fig. 2 for a timeline depiction of events). The Isthmus of Tehuantepec is a continental strait with a central hill that drops from 2000 to 200 m, ending in two plains: 1) a northern plain that extends through the Gulf of Mexico, approximately 100 km long, and 2) a southern one that extends along the Pacific Ocean, 30 km long. The isthmus central hill decreased in elevation during the Late Miocene–Early Pliocene (ca. 6 Ma) as a result of tectonic movements of the Tehuantepec fracture zone; such complex processes that modeled the isthmus' margins prevail until today and keep it seismically active (Barrier et al., 1998). Also, the suture zone between the Caribbean and North American plates is considered a physiographic province that includes a fault system from the Early Eocene and arranged from north to south along the Polochic, Motagua and Jocotán faults. Mountains from the Motagua valley began uplifting as a result of transpression along the Motagua fault, a process that is still active (Ortega-Gutiérrez et al., 2004; Brueckner et al., 2009).

The Maya block includes the Yucatan platform, the Chiapas complex and the Maya mountains of Belize (Figs. 1, 2). The Yucatan platform consists of two major morphological units, one on the north from the Quaternary with plains of heights of less than 50 m, and an older one on the south from the Oligocene, where plains alternate with hills with heights of up to 400 m. Sedimentary rocks from the Tertiary are evidence of the gradual emersion of the Yucatan platform, at least from the Oligocene, which happened from south to northeast and took millions of years (Lugo-Hubp et al., 1992). The

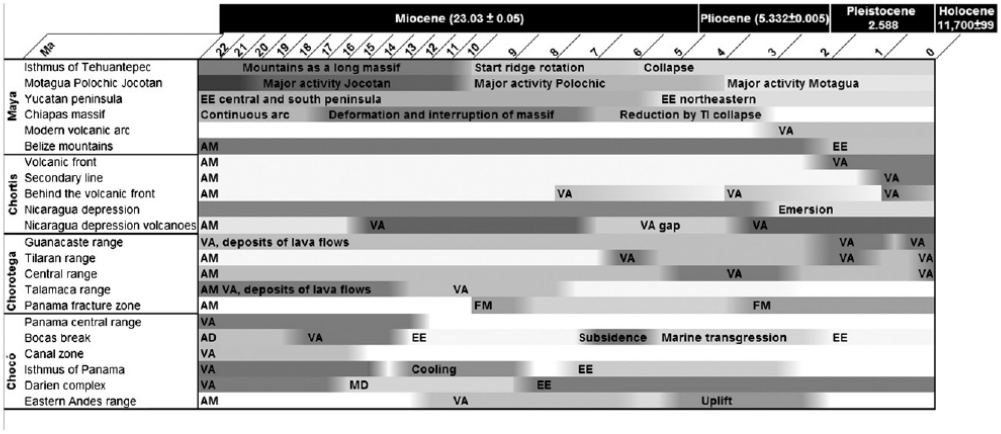

Figure 2. Chronology of the major geologic events described for Central America in the review from the Miocene to the Holocene. Abbreviations correspond to: AD = ancient deposits, AM = event occurring before the Miocene, Cooling = refers to the cooling of submerged volcanoes, EE = emersion and/or exposure above sea level, Ma = million years ago, VA = volcanic activity, TI = Isthmus of Tehuantepec. Geologic periods based on the major chronostratigraphic and geochronologic units (U.S. Geological Survey, 2010).

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Table 1
Summary of the genetic studies that have included geologic information of Central America. The abbreviations are: NA = North America, CA = Central America, SA = South America, IT = Isthmus of Tehuantepec, IP = Isthmus of Panama, MM = Maya mountains, Ch-G H = Chiapas and Guatemala highlands, CR-P H = Costa Rica-Panama highlands, CAVA = Central America volcanic arc, ND = Nicaragua depression, YP = Yucatan platform, DTE = divergence times estimated where Y = considered or estimated in the study, n = not considered or estimated in the study, TR = time range. Information considered in the studies to explain the genetic results: 1) geological event (i.e., closure of IP), 2) specific volcanic events, 3) physiographic provinces, 4) soil distribution, 5) climate event and, 6) climatic zone or data considered (Y = yes, n = no).

Authors	Species/genera/family	Molecular marker	Geographic distribution	CA barriers	DTE	TR	Genetic evidence	Geology				Climate	
								1	2	3	4	5	6
Clade 1: Mayan													
Aguirre-Planter et al. (2012)	*Abies*, Pinaceae	rbcL, rps18-rpl20 and trnL-trnF	Southern NA to northern CA	IT	Y	Eocene–Pliocene	High diversification during Pliocene at both sides of the IT	Y	n	n	n	Y	n
Barber and Klicka (2010)	Passeriformes, Apodiformes and Piciformes	ND2	Both sides of the IT	IT	Y	Pliocene–Pleistocene	Simultaneous intraspecific diversification	Y	n	n	n	Y	n
Cortés-Rodríguez et al. (2008)	*Lampornis amethystinus*	ND2, cyt b	Southern NA to northern CA	IT	N	Miocene–Pliocene	Phylogenetic lineages and genetic differentiation between populations	Y	n	n	n	n	n
Zarza et al. (2008)	*Ctenosaura pectinata*	ND4, GapD, β-fibrinogen, α-enolase	Southern NA	IT	Y	Pliocene–Pleistocene	Genetic distances, phylogenetic trees and genetic structure	Y	n	n	n	Y	Y
Clades 1–2: Mayan and Mid-Central America													
Barrera-Guzmán et al. (2012)	*Ergaticus*, Aves: Parulidae	ND2, cyt b, ATPase	Suthern NA to northern CA	IT/Ch-G H	Y	Pleistocene	High divergence between populations from both sides of the IT and genetic differentiation on Guatemala and Chiapas highlands	Y	n	n	n	Y	N
Bryson et al. (2011)	Colubridae: Pituophis	ND4, tRNAs, ATPase8, ATPase6	Southern NA to northern CA	IT/G H	Y	Pliocene–Pleistocene	Different phylogenetic lineages at both sides of the IT and between the Guatemala highlands	Y	n	n	n	Y	n
Esteva et al. (2010)	*Sorex*	Cyt b	Southern NA to northern CA	IT/ChG H	Y	Miocene–Pleistocene	Genetic distances and phylogenetic lineages	Y	n	n	n	Y	n
Guevara-Chumacero et al. (2010)	*Pteronotus davyi*	Control region	Southern NA to northern CA	IT/YP/Ch-G H	n	Pleistocene	Genetic divergences and distinctive phylogenetic lineages	Y	n	n	n	Y	n
Gutiérrez-Rodríguez et al. (2011)	*Palicourea padifolia*	Chloroplast regions: trnS-trnG and rpl32-trnL	Southern NA to northern CA	IT/Ch-G H	Y	Pleistocene	Major phylogenetic lineages and genetic structure	Y	N	N	N	Y	n
Jardón-Barbolla et al. (2011)	*Pinus* subsection *Australes*	10 microsatellite loci	Northern to central CA and Caribbean islands	MPF/MM/CAVA	Y	Pleistocene–Holocene	Genetic clusters and haplotype network	Y	n	n	n	Y	n
Ordóñez-Garza et al. (2010)	*Peromyscus*	Cyt b	Chiapas to Guatemala highlands	MPF/Ch-G H	n	Pleistocene	Genetic distances and phylogenetic lineages	Y	n	n	n	Y	n
Ornelas et al. (2010)	*Podocarpus matudae*	trnLF anb psbA-trnH	Southern NA to northern CA	IT/Ch-G H	Y	Miocene–Pleistocene	Large scale geographic structuring haplotypes and phylogenetic lineages	Y	Y	n	n	Y	n
Strecker et al. (2004)	*Astyanax* (Teleostei)	Cyt b	Southern NA to northern CA	IT//YP/MM/CAVA	n	Miocene–Holocene	Major phylogenetic, haplotype and nested lineages.	Y	n	n	n	Y	n
Sullivan et al. (2000)	*Rehitrodontomys sumichrasti*, *Peromyscus aztecus*, *Peromyscus hylocetes*	Cyt b	Southern NA to northern CA	IT/Ch-GH	n	Pliocene–Pleistocene	Genetic divergences, concerted topologies	Y	n	n	n	Y	n
Clades 2–3: Mid-Central America and Panamian													
Arrivillaga et al. (2002)	*Lutzomyia longipalpis*	COI	Central CA to SA	ND/IP	n	Pliocene–Pleistocene	Populations from CA form a phylogenetic lineage	Y	n	n	n	Y	n
Poelchau and Hamrick (2011)	*Brusera simaruba*, *Brosimum alicastrum*, *Ficus insipid*	trnG and trnH-psbA	Central CA	CAVA/ND/CR H	Y	Pliocene–Pleistocene	Concordant boundaries across unrelated species by genetic structure and genetic distances	Y	Y	n	n	Y	Y
Clade 3: Panamian													
		ATPase, COI		IP	Y	Miocene–Pleistocene		Y	n	n	n	Y	n

Bermingham and Martin (1998)	Roeboides, Pimelodella, and Hypopomus		Costa Rica to northern Colombia				Similar lineages among species, shared history and recent diversification						
Hauswaldt et al. (2011)	*Oophaga pumilio*	Cyt b and RAG-1, 7 microsatellite loci	Costa Rica and Panama	CR-P H	n	Pleistocene	Genetic structure and main haplotype lineages	Y	n	n	n	n	n
Lessios et al. (1999)	*Eucidaris*	COI	Both sides of the IP	IP	Y	Miocene–Pleistocene	Genetic divergence and cladogenic events	Y	n	n	n	n	n
Lessios et al. (2003)	*Tripneustes*	COI	Both sides of the IP	IP	Y	Miocene–Pleistocene	Genetic divergence, genetic structure and phylogenetic lineages	Y	n	n	n	Y	n
Muss et al. (2001)	*Ophioblennius*	Cyt b	Atlantic and Pacific oceans	IP	Y	Pliocene–Pleistocene	Genetic divergences and genetic partitions	Y	n	n	n	Y	n
Scotti-Saintagne et al. (2012)	*Carapa*	nuSSR, cpSSR and trnH-psbA, trnC-ycf6	Southern CA to central SA	CR-P H/IP	Y	Miocene–Pleistocene	Genetic clusters coincident with phylogenetic lineages and genetic differentiation	Y	n	n	n	Y	n
Stillman and Reeb (2001)	*Petrolisthes*, *Pachycheles*	16S rRNA	Eastern of NA to eastern SA	IP	Y	Pliocene–Pleistocene	Genetic diversity and phylogenetic lineages	Y	n	n	n	Y	n
Wang et al. (2008)	*Pristimantis ridens*	ND2, $tRNA^{MET}$, fragments,COI, WANCY region	Costa Rica and Panama	CR-P H/IP	Y	Miocene–Pleistocene	Highly divergent lineages and phylogenetic trees	Y	n	n	n	Y	Y
Zeh et al. (2003)	*Cordylochernes scorpioides*	COI	Panama to northern SA	IP	Y	Miocene–Pleistocene	Gene flow, genetic structure and phylogenetic lineages	Y	n	n	n	n	N
Clades 1–2–3: Mayan, Mid-Central America and Panamian													
Arbeláez-Cortés et al. (2010)	*Lepidocolaptes affinis*, Aves: Furnariidae	Cyt b, ND2	Southern NA to CA	IT/CH-G H/CAVA/ND/CR H	Y	Miocene–Pliocene	Genetic differentiation and bottleneck on Costa Rica population; low divergence at both sides of the IT.	Y	n	n	n	Y	n
Arellano et al. (2005)	*Reithrodontomys* Muridae	Cyt b	Southern NA to CA	IT/Ch-G H/ND	n	Not considered	Genetic distances between populations from Costa Rica, Chiapas and Guatemala highlands and IT	n	n	n	n	n	n
Cadena et al. (2007)	*Buarremon*, Aves:Emberizidae	ND2, cyt b, ATPase6, ATPase8, Z chrom fragments of ACOI, MUSK	Southern NA to northern SA	IT/CR H/IP	Y	Pre-Pleistocene	Phylogenetic lineages at both sides of the barriers	Y	n	n	n	n	n
Castoe et al. (2003)	*Atropoides*	ND4, cyt b	Southern NA to CA	IT/CAVA/ND/CR H	n	Pliocene–Pleistocene	Phylogenetic lineages along CA	Y	n	n	n	Y	n
Cavender-Bares et al. (2011)	*Quercus*	11 nuclear SSR, chloroplast trnT-trnD, ITS, ITS2, 5.8S, NIA-i3	Southern NA to CA	IT/CAVA/ND/CR H	Y	Miocene–Pleistocene	Significant genetic differentiation, unique and rare haplotypes across the distribution	Y	Y	n	n	Y	Y
Cavers et al. (2003)	*Cedrela odorata*	RFLP's chloroplast	CA	CAVA/ND/CR-P H	n	Pleistocene	Lack of mixing at the contact zone, three different lineages	Y	n	n	n	Y	Y
Crawford and Smith (2005)	Leptodactylidae: *Eleutherodactylus*	ND2, $Trna^{MET,TRP,ALA,\ ASN,CYS,TYR}$, O_LCOI fragment, c-myc nuclear gene	NA,CA,SA	IT/CAVA/IP	Y	Paleocene–Pliocene	Phylogenetic relationships and divergence times between species	Y	n	n	n	Y	n
DaCosta and Klicka (2008)	*Trogon*	ND2	Southern NA to northern SA	IT/ND/IP/Darien	Y	Pliocene–Pleistocene	Areas of origin, time of divergence of phylogenetic lineages	Y	n	n	n	Y	n
Daza et al. (2010)	Viperids and elapids	NADH subunit 4 and cyt b	CA	IT/MPF/ND/TR/IP	Y	Miocene–Pleistocene	Shared patterns of divergence	Y	Y	n	n	Y	n
Eizirik et al. (2001)	*Pantera onca*	Control region, 29 microsatellite loci	Southern NA to central SA	MPF/IP Darien	Y	Pliocene–Pleistocene	Levels of genetic differentiation, divergence between haplotypes	Y	n	n	n	n	n
Eizirik et al. (1998)	*Leopardus pardalis*, *L. wedii*	Control region	Southern NA to central SA	MPF/IP	n	Pliocene	Genetic divergences and phylogenetic lineages	Y	n	n	n	n	n
Hasbún et al. (2005)	*Ctenosaura quinquecarinata* complex	ND4	Suthern NA to central CA	IT/CAVA/ND/CR H	n	Pliocene–Pleistocene	Major phylogenetic lineages and nested lineages	Y	n	n	n	Y	n
Hoffmann and Baker (2003)	*Carollia*: Phyllostomidae	Cyt b	Southern NA to northern SA	MPF/CAVA/ND/IP	Y	Pliocene–Pleistocene	Phylogroups and genetic distances	Y	n	n	n	Y	n
Mulcahy et al. (2006)	*Bufo valliceps*, *B. nebulifer*	16S, cyt b	Southern NA to central CA	IT/G H/CAVA/ND	Y	Pliocene–Pleistocene	Genetic divergences between phylogenetic lineages and topology test	Y	n	n	n	Y	n
Novick et al. (2003)	*Swietenia macrophylla*	7 microsatellite loci	CA	MPF/CR H	n	Pleistocene	Genetic divergence, major clusters based on genetic distances	Y	n	n	n	Y	n

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Table 1 (*continued*)

Authors	Species/genera/family	Molecular marker	Geographic distribution	CA barriers	DTE	TR	Genetic evidence	Geology				Climate	
								1	2	3	4	5	6
Parkinson et al. (2000)	*Agkistrodon*	ND4, tRNA-HIS-SER, 16S and 12S rDNA	Southern NA to central CA	IT/YP/Ch-G H/CAVA/ND	n	Miocene–Pliocene	Significant genetic structure and lineage differentiation	Y	n	n	n	Y	n
Perdices et al. (2002)	*Rhamdia*	Cyt b, ATPase	CA to central SA	IT/CAVA/CR-P H/IP	Y	Miocene–Pleistocene	Phylogenetic trees, genetic distances and genetic structure	Y	n	n	n	Y	n
Perdices et al. (2005)	Teleostei: Synbranchidae	Cyt b, ATP8/6, nuclear RAG-1. RFLP's	CA	IT/MPF/ER-P H/IP	Y	Miocene–Pliocene	Phylogenetic lineages and high genetic distances	Y	n	n	n	n	n
Vázquez-Miranda et al. (2009)	*Camphylorhynchus rufinucha*	ND2	Southern NA to central CA	IT/Ch-GH/CAVA/ND	Y	Pliocene–Pleistocene	Similarities in phylogenetic trees, haplotype groups and sequence divergences	Y	n	n	n	Y	n
Wüster et al. (2005)	*Crotalus durissus*	ND2, ND4, cyt b	Southern NA to central SA	IT/MM/IP	Y	Miocene–Pleistocene	Sequence divergence and consistent phylogenetic lineages	Y	n	n	n	Y	n

Chiapas complex includes a central depression and two mountain chains, the Chiapas massif originated during the Late Permian and is located on the southeast of the Isthmus of Tehuantepec and northwest of Guatemala, where it is bounded by the Motagua and Polochic faults (Manea and Manea, 2005; Weber et al., 2007). The other mountain chain is the Modern volcanic arc (Manea and Manea, 2005), located east of the Central depression of Chiapas, which was formed during the last 3 Ma by intensive volcanic activity. The Central depression of Chiapas (Ferrusquía-Villafranca et al., 2000) is located between the Chiapas massif and the Modern volcanic arc, mostly developed by Quaternary deposits and marine sediments from different geologic periods. Finally, the Maya mountains of Belize are an uplifted block of Paleozoic metasediments and Triassic intrusives with three ancient granitic intrusive complexes (230 Ma) and bounded on the north and south by major faults striking east–west. The Maya mountains experienced minor tectonic activity during the Tertiary (Miller, 1996; Steiner, 2005; Solari et al., 2010).

The Chortis block

The Chortis block is located between the Motagua–Polochic–Jocotán fault system and the Hess escarpment (Figs. 1, 2) and includes seven physiographic provinces (Marshall, 2007), the most outstanding being the Central American volcanic arc, a highly active volcanic chain originated from different activity stages of the Middle American trench, responsible of most earthquakes and volcanic activity along CA (e.g., more than 39 active volcanoes) (García-Palomo et al., 2004, 2006; Kutterolf et al., 2007). Historically, levels of volcanic activity widthwise along the Chortis block decrease from SE to NW, which can be recognized as volcanic lines: Volcanic front, Secondary line and Behind the volcanic front (Figs. 1, 2) (Rogers et al., 2002; Ortega-Gutiérrez et al., 2007). The Volcanic front is located along the Pacific coast and made up of calderas and volcanoes from the Quaternary, many active with eruptions and lava flow during the Holocene (Siebert and Simkin, 2002). The Secondary line consists of few volcanoes with cones forming a parallel line with the Volcanic front, with their origin from the Pliocene to the Holocene. The last line, Behind the volcanic front, was formed by widespread volcanism and overlaps with both the Volcanic front and Secondary line, extending more than 200 km (Carr et al., 2003). At the southern end of the Chortis block stands the Nicaragua depression, which is a tectonic graben that probably began to subside in the Late Miocene, forming a lowland corridor that runs from the Caribbean to the Pacific near the border between Costa Rica and Nicaragua. The Nicaragua depression includes two large lakes, Nicaragua and Managua, from the Early Pleistocene, and twelve major volcanic complexes from the Volcanic front that were active during Miocene and Pliocene, six of which are currently active, and with a gap in activity between 7 and 4 Ma (Kutterolf et al., 2007; Janoušek et al., 2010; Saginor et al., 2011). At the beginning of the Pleistocene, the lowlands of the Nicaragua depression were flooded.

The Chorotega and Chocó blocks

The northern and southern limits of the Chorotega block are the Hess escarpment and the North Panama fracture belt, respectively, while the Chocó block starts on the latter and ends along the subduction zone between the Nazca and the South America plates on the western Andes (Figs. 1, 2) (Pindell and Kennan, 2009). The Chorotega block encompasses from Costa Rica to the northern Panama highlands (Giunta and Oliveri, 2009). Within Costa Rica, four major ranges define the southern volcanic arc: Guanacaste, Tilarán, Central and Talamanca. The Guanacaste and Central ranges include volcanoes that have been active at different periods during the Quaternary. Contrastingly, the area between those regions, the Tilarán range, was formed during a gap of volcanic activity and is located above Miocene–Pliocene volcanic

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and sedimentary rocks, with some modern active volcanoes (Soto and Alvarado, 2006; Carr et al., 2007). The Talamanca range on the south includes mainly Miocene complexes (Vogel et al., 2004), which rapidly uplifted during the Plio-Pleistocene. This range extends to northern Panama, where volcanic activity during the Quaternary was minimal (Johnston and Thorkelson, 1997).

The Chocó block was the latest region to emerge transforming CA into a continuous landmass area (Figs. 1, 2). The Cocos-Nazca ridge became active approximately 25 Ma, this activity resulted in its subduction and convergence with the Caribbean and South America plates. This collision uplifted the isthmian volcanic arc from eastern Panama as an archipelago, the entire Panama region and the northern Andes area 12 Ma (Coates et al., 2004), while during the Quaternary there was restricted subduction causing limited volcanic activity in Panama (Harmon, 2005). It has been suggested that the basement complex of central Panama was an uninterrupted chain above sea level from late Eocene until at least late Miocene times. Central and southern Panama are less mountainous than the north, with flooded areas and rivers along the narrowest region in CA, the Panama channel zone fracture (Escalante and Astorga, 1994; Montes et al., 2012). Collision between the Panama and South America blocks initiated at 23–25 Ma (Farris et al., 2011), followed by continual emergence, cooling and marine sedimentation (Montes et al., 2012). Later, this area fractured in two extension zones (Canal zone and Bocas break) and a contraction zone (Isthmus of Darien) (Farris et al., 2011). The Isthmus of Darien, in southern Panama, was emerging as early as 8.6 Ma and its most recent deposits are from 4.8 Ma (Coates et al., 2004). Geologic evidence indicates that some ranges of the Darien emerged before the Miocene, for example the San Blas range was above sea level on the Eocene and until the Miocene when it acted as a peninsula with South America (Montes et al., 2012). Finally, the southern tip of the Chocó block is a coastal range, the western range of the Andes, which is part of an ophiolitic complex that extends from Costa Rica to Ecuador formed 12 to 6 Ma (Kellog and Vega, 1995).

Molecular genes in biogeographic studies

Complex and dynamic tectonic geological events have shaped CA with valleys, mountain ranges, basins, lakes, and rivers and so forth, which have acted both as barriers and corridors for dispersal, thus also shaping the genetic structure and diversification of the biota. Geological reconstructions can be understood as an evolutionary sequence of events at different spatial and temporal scales, and are mainly based on abiotic information or fossil remains. However, they can also be explored using biotic information (see following sections), spatially using the distribution of genetic variation of species and temporally using genetic tools and molecular dating (Bermingham and Martin, 1998; Daza et al., 2010). This approach that involves evaluating the genetic structure, distribution and diversification of individuals within natural populations throughout their area of distribution, using molecular markers like DNA sequences, has evidenced concordant patterns among different species, indicating that these dynamic processes have been a major force in lineage diversification. On the other hand, intrinsic factors like dispersal ability and ecological features have undoubtedly played an important role and must be considered.

Genetic studies of species from CA have used an ample array of molecular markers (Table 1), which highlight the importance of selecting an adequate marker with sufficient resolution to detect evolutionary patterns from approximately the Miocene onwards. Accordingly, different biogeographic, evolutionary and ecological processes have been deciphered depending on the molecular marker used. That is, to detect a particular genetic event one should consider specific characteristics of the marker (e.g., diversity rate, variability level, saturation of informative sites; Zeh et al., 2003). For instance, Eizirik et al. (1998) found that the control region of the mitochondrial gene (mtDNA) was adequate to detect genetic structure at the intraspecific, but not interspecific, level in ocelots and margays when evaluated throughout their entire distributional range. Likewise, demographic expansions and reductions have been found with both nuclear and mitochondrial markers (Eizirik et al., 2001).

Another characteristic to consider is the potential to estimate times of diversification with the genetic marker used, as some studies have done (Table 1 and the references therein). In particular, when the molecular clock can be applied it is possible to use the rate of change of DNA sequences over time to estimate diversification times and lineages' ages. Divergence time estimation has been controversial due to the fact that rates are different between species and among genes (Nee et al., 1992; Martin and Palumbi, 1993). Nonetheless, using an adequate evolutionary model and the correct calibration, it is possible to date a divergence episode with a temporal resolution of millions to thousands of years, and then relate it to the most plausible geological event of known age and associate it with an evolutionary process, e.g., vicariance, radiation, migration (e.g., Gutiérrez-García and Vázquez-Domínguez, 2012; Martínez-Solano et al., 2012). It is important to consider that different calibrators (e.g., taxa, gene) can generate different dating for the same event, an uncertainty that needs further calibration. For example, Stillman and Reeb (2001) found that the divergence estimated for a clade in two genera of Porcelain crabs (*Petrolisthes* and *Pachycheles*) could be either before or after the closure of the Isthmus of Panama, depending on the rate of change used for the calibration. On the contrary, some studies have used as calibrators accurately dated geological events (e.g., the emersion of the Isthmus of Panama) to estimate divergences (Lessios et al., 2003; Zeh et al., 2003). It is best, when possible, to use a combination of different calibrators (e.g., taxa, fossil, geological events).

Stones and genes: geological and evolutionary patterns in the biota of Central America

Barriers and corridors: historic migrations in Central America

Terrestrial bridges or connections between North America and South America, such as the intermittent island emersions between Central and North America and the formation of the Isthmus of Panama previously described, have been considered central factors in studies about phylogenetic relationships and genetic structure of taxa distributed throughout the Antilles and North, Central and South America (e.g., Zeh et al., 2003; Cadena et al., 2007; Daza et al., 2010; Miura et al., 2012). These connections and reconnections were characterized by dispersal events involving all kinds of taxa, processes that have been frequently corroborated genetically and that we group in three main events, briefly explained in the following.

First migration, the Arc-island and Proto-Antilles-GAARlandia models

Under these models, land continuity or proximity between northwestern South America and Central America allowed species exchange between the two areas during the end of the Cretaceous and the beginning of the Paleocene (Supplementary Fig. 2), as shown by diverse phylogeographic and biogeographic studies. For example, Marshall (1988) described large mammal interchanges during the Miocene, a pattern also suggested for some marsupials (Case et al., 2005) and for the dispersal of Menispermaceae (Herrera et al., 2011). The Paleocene origin of the cichlids from the Great Antilles and Yucatan peninsula (Chakrabarty, 2006; and the examples therein) supports the arrival of fauna to Yucatan by an arc during the Cretaceous and a posterior dispersal via the Chortis block. Crawford and Smith (2005) used the Arc-island model to generate hypotheses about ancestry and dispersal for one of the most ubiquitous, diverse and abundant taxa of Neotropical amphibians from the Antilles (frog, *Eleutherodactylus*).

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The authors describe three independent dispersal events for the origin of this group, where the first event is identified as the migration of an ancestor from South America into northern Central America in the early Paleocene, supporting that the Arc-island model accounts for the present distribution of *Eleutherodactylus* subgenera. In another study with the pitviper *Agkistrodon*, Parkinson et al. (2000) show that two biogeographic hypotheses explain the species migration through these islands before the formation of the Isthmus of Panama: 1) an initial divergence along the east coast of Mexico (Yucatan-east Mexico or gulf-arc), followed by trans-continental divergence, and 2) an initial trans-continental divergence followed by differentiation on the Pacific coast and Yucatan peninsula via subhumid corridors (Yucatan-west Mexico). The first one explains the colonization of the Yucatan peninsula across a set of islands via "island hopping" (GAARlandia or Proto-Antilles), while the second suggests that it happened through a Central American corridor (Parkinson et al., 2000). Wang et al. (2008) also found evidence of a wet corridor that allowed the dispersal of the pygmy rain frog (*Pristimantis ridens*) along the Pacific coast from Panama to Costa Rica. Such a corridor has also been proposed for different species of plants: Cavers et al. (2003) studied populations of the Neotropical tree *Cedrela odorata* and found a pattern of unordered genetic distance that suggests a prior connection between populations from the Yucatan peninsula and southern Central America. Also, Novick et al. (2003) show that populations of the big-lead mahogany (*Swietenia macrophylla*) expanded throughout Central America prior to the uplift of the Talamanca range. Although genetic studies do not describe the geologic features of this corridor (archipelago), other studies suggest that instead of a corridor it was a peninsula (e.g., Kirby and MacFadden, 2005). Geologic studies indicate that emergent islands exist since the Eocene in Honduras (Viland and Henry, 1996), Nicaragua (Darce et al., 1989), Costa Rica (Escalante and Astorga, 1994) and Panama (Montes et al., 2012), not mentioned in genetic studies, and which evidence that a corridor was available and that "island hopping" was possible prior to the closure of the Isthmus of Panama.

Second migration, colonization of highlands and lowlands

Studies by Bermingham and Martin (1998; freshwater fishes), Zeh et al. (2003; beetles), Cadena et al. (2007; finches), Wang et al. (2008; frogs) and Woodburne (2010; mammals) show genetic support for the existence of a terrestrial corridor along CA 7–4 Ma (Supplementary Fig. 2), preceding the formation of the Isthmus of Panama (Pre-GABI, before the Great American Biotic Interchange). The corridor allowed some species to colonize highland and lowlands in Costa Rica and Nicaragua, features that in turn acted as barriers and/or corridors for dispersal. The uplift of the middle region in CA occurred between 10 and 4 Ma (Rogers et al., 2002), a time in which some mammalian fauna already inhabited the region (Webb and Perrigo, 1984), indicating a Miocene dispersal. Likewise, Perdices et al. (2005) studied the distribution and genetic differentiation of freshwater fishes along CA and, based on the observed restricted gene flow between lowland populations, concluded that dispersal of taxa should have been a Pre-GABI event. In another example, the observed population structure of the bird genus *Trogon* (DaCosta and Klicka, 2008) indicates a CA origin and posterior diversification in the region. The authors conclude that such a model would imply an ancient, pre-isthmian colonization.

The above studies agree in terms of an early colonization that was followed by flooding events and posterior extinction of lowland populations, allowing genetic differentiation, through millions of years, of the populations restricted to the highlands. The absence of a geographic barrier for many lowland taxa suggests that there was a second contact between the populations already established, as exemplified by the study of Perdices et al. (2005) in which the Polochic–Motagua–Jocotán fault-system suture zone in the Chortis block was identified as an area of sympatry for synbranchid eels.

Third migration, Great American Biotic Interchange

The most significant geologic event after the emersion of CA was the formation of the Isthmus of Panama, for which the timing of emergence and closure has been suggested between ca. 7 and 3.5 Ma (Coates et al., 1992; Newkirk and Martin, 2009). The emergence of the isthmus triggered a major continental large-scale dispersion known as the Great American Biotic Interchange encompassing approximately 3.1 to 2.5 Ma (GABI; Webb, 1991; Coates and Obando, 1996; Webb, 2006; Cody et al., 2010) (Fig. 2, Supplementary Fig. 2). Woodburne (2010) describes the GABI migration patterns for different taxa along Central America as a set of pulses that happened in concert with climatic and geological events, supporting this region as a center of cladogenesis for diverse species. In agreement with the fossil record and with phylogenetic and phylogeographic studies, this migration was bidirectional, with an asymmetrical number of taxa moving northwards and southwards and in which not all taxa from both landmasses participated. Also, replacement of native taxa (extinctions) and diversification of colonizing taxa (speciation) occurred at both North America and South America during the GABI (Webb, 1991, 2006). In addition, the GABI caused demographic changes that can be linked to complex genetic structure patterns in taxa now present on CA. Research evidence associated with the GABI dynamics includes high genetic variability (new lineages and/or haplotypes) at different taxonomic levels, absence of genetic admixture along contact or suture zones and no correlation between geographic and genetic distance, among others. For instance, DaCosta and Klicka (2008) found high genetic diversity values in their study with *Trogon*, attributable to the high lineage diversification within the genus as a result of the processes related to the GABI, i.e., dispersal into, and subsequent diversification within, South America.

The formation of assemblages of trans-isthmus species after the GABI (1.5 Ma to present; Webb, 1991) demonstrates that the cessation of gene flow was a gradual process that took millions of years and involved entire communities (Zeh et al., 2003). Different post GABI migration patterns such as isolation, secondary contacts and repeated colonization in populations from CA have been mainly associated to the Pleistocene climatic fluctuations (Hewitt, 2000). It has been suggested that local volcanic activity also had an effect on the biota's genetic structure (Vandergast et al., 2004). An example of the effect of volcanic activity after the GABI is the study of Cavender-Bares et al. (2011) with some species of the genus *Quercus*: they suggest that a peak of volcanic activity starting 0.6 Ma on the Guanacaste range (Fig. 1), created a physical barrier and altered local climate causing genetic divergences between populations from Costa Rica and Honduras. Today, faunas of CA are a rich and complex mixture of North and South American components, a clear evidence of the processes associated with the GABI, including the migration and differentiation of endemic and morphologically distinctive species (Orellana et al., 2003; Strecker et al., 2004; Vázquez-Domínguez and Arita, 2010).

The history of Central America told by the genetic configuration of extant species

The geological and evolutionary patterns described above have left a signature on the genetic configuration of a diverse array of extant taxa in Central America. Specifically, this genetic arrangement coincident with the geological history of the region encompasses, in our view, three major evolutionary groups or clades (Fig. 3). Concordant patterns within these groups have been observed—with minor modifications given taxa sampling and distribution—in fishes (Perdices et al., 2005), mammals (Eizirik et al., 1998, 2001), amphibians (Mulcahy and Mendelson, 2000), reptiles (Hasbún et al., 2005; Wüster et al., 2005) and plants (Cavers et al., 2003; Novick et al., 2003). In the following, we describe the main genetic features and patterns of those clades.

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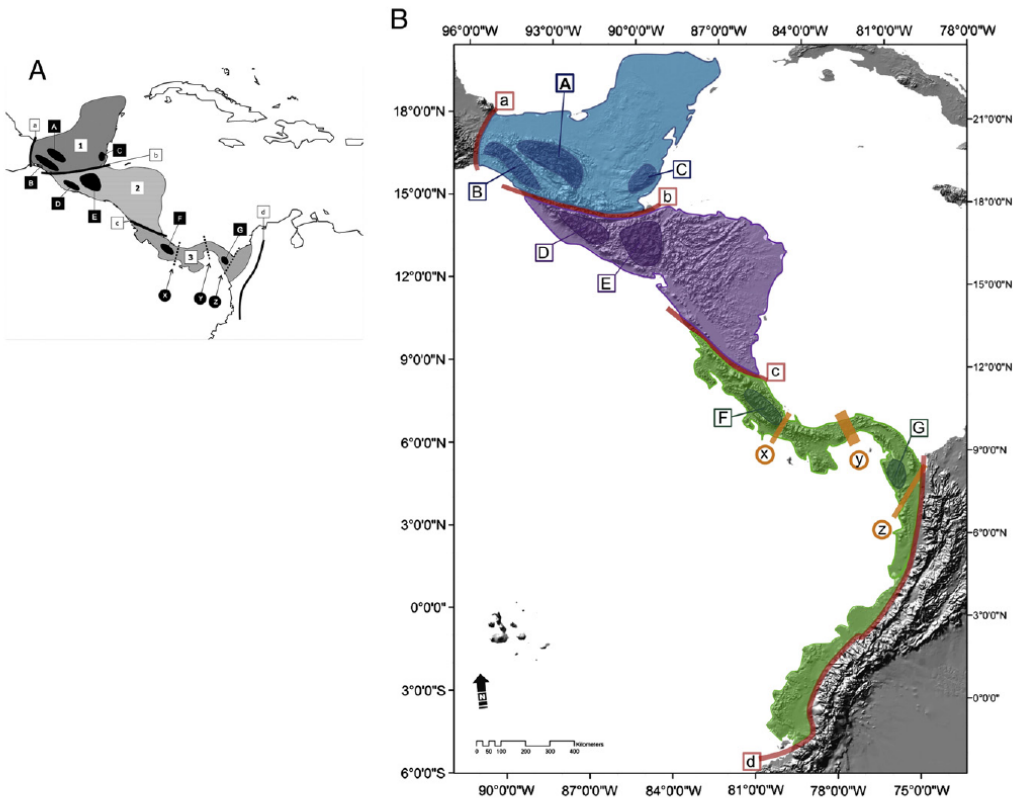

Figure 3. Schematic representation of the genetic configuration of Central America. (A) Numbers indicate the three evolutionary groups (see text): 1 = Mayan clade, 2 = Mid-Central America clade, 3 = Panamian clade. Black squares with white capital letters represent genetically distinctive zones with high endemism, high differentiation and/or relict species: A = Chiapas modern volcanic arc, B = Chiapas mountains, C = Belize mountains, D = Pleistocene refuge, E = Volcanic lines on the Chortis block, F = Talamanca range, G = Panama divergence center. Black lines with white squares indicate geological features that delimit the three clades: a = Isthmus of Tehuantepec, b = Motagua–Polochic–Jocotán fault system, c = Hess escarpment, d = Andes range. Dashed lines with black circles are genetic barriers identified only for some taxa: X = Bocas break, Y = Panama channel, Z = Darien strait. (B) Clades, zones and features are shown with color.

Clade 1: the Mayan clade

The Mayan clade is located between the Isthmus of Tehuantepec and the Motagua–Polochic–Jocotán fault system in an area that comprises southern Mexico (Yucatan peninsula), Guatemala and Belize (Fig. 3). Within this clade, taxa show a marked genetic structure and divergence associated with the Modern Chiapas volcanic arc (Fig. 3, A) and other mountains in Chiapas (Fig. 3, B). A high rate of genetic differentiation has been observed within this clade for populations distributed near a geologic or geographic barrier. For instance, Strecker et al. (2004) suggest that populations of cave fish (*Astyanax*) arrived to Belize during a first expansion, where they persisted in refugia, a pattern evidenced today by a high number of endemisms and by geographically close populations that are markedly different genetically (Fig. 3, C). These refugia are partially located on the contact zone between the Chortis and Maya blocks (Fig. 1), supporting the inference that the Motagua–Polochic–Jocotán fault system acted as a key barrier for gene flow. Also, this fault system is composed by some of the oldest formations along the Chortis and Maya contact zone, associated with its early emersion and uplift before the remainder of CA (Ortega-Gutiérrez et al., 2007). The later is clearly shown by the frog complex *Eleutherodactylus*, where multiple dispersal events divided the Chortis block (northern) from lower Central America species groups (Crawford and Smith, 2005). In a study of the Mesoamerican rodent *Ototylomys phyllotis*, results show that this fault system acted as a strong historical barrier during the Pliocene (3.2–2.3 Ma), shown by high divergence and genetic distance values between the lowlands and the highlands of Guatemala and Chiapas. Moreover, the fact that the mountains in southeastern Mexico, among others, acted as refuges during the cold Pleistocene, is supported by morphological differences shown by *O. phyllotis*, where the morphologically largest members are found at high altitudes of Guatemala and Chiapas mountains (Gutiérrez-García and Vázquez-Domínguez, 2012). A gap in volcanic activity during the Late Miocene at the Chiapas massif was ended by more recent volcanism along the Modern Chiapas volcanic arc (Keppie and Morán-Zenteno, 2005). Because of this lack of volcanic activity, some isolated communities on the highlands differentiated genetically. This triggered speciation processes within these highland communities, given their limited or null dispersal toward the north due to the formation of the Isthmus of Tehuantepec 6 Ma (Manea and Manea, 2005), and toward the south because of the intensification of volcanic activity in Guatemala.

Although the genetic structure of species from Guatemala is poorly studied, unique haplotypes have been found for different taxa within this clade, along the Chiapas massif and northern Guatemala (Mulcahy and Mendelson, 2000; Parkinson et al., 2000; Ornelas et al., 2010). Also,

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different cryptic species of a digenean parasite (*Crassicutis cichlasomae*) diversified between different freshwater bodies of northern Guatemala and southern Mexico (Razo-Mendivil et al., 2010). As described for the Maya block, the Belize mountains were uplifted during the Cretaceous, rising up to 1100 m asl, a process parallel to the Motagua–Polochic–Jocotán fault system (Miller, 1996). As a consequence, species could only have arrived into this region via the fault system and remained isolated until the emersion of the Yucatan platform that made colonization of the lowlands possible. Evolutionary processes in the Mayan clade include the expansion of taxa toward the Yucatan peninsula, following the peninsula's gradual emersion over millions of years and from south to north, which are evidenced by processes of restricted gene flow with isolation by distance and long distance colonization. Results for the rodent *O. phyllotis* (Gutiérrez-García and Vázquez-Domínguez, 2012) suggest that Belize was the unique source of individuals for the colonization of the Yucatan peninsula, with genetic flow and migration events happening during the last 500,000 yr. Strecker et al. (2004) describe that at least two invasions of surface fish *Astyanax*, from Belize to Yucatan, occurred during this time.

At the northern limit of the Mayan clade, on the Isthmus of Tehuantepec (Fig. 3, A), populations on both sides of the isthmus show high species richness and high genetic diversity levels, together with cryptic lineages, which also suggest the existence of refugia. Indeed, Sullivan et al. (2000) show how the isthmus acted as a barrier for highland rodent species, evidenced by a genetic trans-isthmic differentiation at the intraspecific level of 5–8%, high enough to consider those species as evolutionary distinct units. Other studies have found that colonization toward both sides of the isthmus resulted from long distance colonization and/or fragmentation from an ancestral population (García-Moreno et al., 2004; Gutiérrez-Rodríguez et al., 2011; Barrera-Guzmán et al., 2012). Such dynamic history of the Isthmus of Tehuantepec includes not only an east–west differentiation, but also between the north and south: Zarza et al. (2008) found a marked and recent divergence (1.5 Ma) between the Pacific and Atlantic sides of the isthmus in the iguana *Ctenosaura pectinata*.

The barriers distributed within the Mayan clade facilitated genetic differentiation but also allowed populations to maintain ancestral features within refugia (Figs. 3, A, B and C) (Medinilla et al., 2006; Guevara-Chumacero et al., 2010), that is, some of these populations are ancient with respect to those located far from the barriers, a genetic pattern that resulted from multiple dispersion events from the highlands to the lowlands. A combination of factors occurring in this clade contributed to those patterns, like volcanism in the Modern volcanic arc of Chiapas, the absence of recent volcanism in the mountain complex from the Chiapas massif and the isolation of the Belize mountains until the emersion of the Yucatan platform during the Quaternary.

Clade 2: the Mid-Central America clade

Limits of this clade coincide with the Motagua–Polochic–Jocotán fault system (Fig. 3, B) and the Hess escarpment (Fig. 3, C), including southern Guatemala, Honduras, El Salvador and Nicaragua. The configuration observed for this clade includes highly differentiated populations associated with the Central American Volcanic Arc, the Nicaragua depression, the Hess escarpment, active volcanoes and the Nicaragua and Managua lakes, geological barriers that influenced migration and diversification events toward the south of CA (Crawford and Smith, 2005). At the north of the Mid-Central America clade, the Guatemala highlands show a pattern of high levels of genetic differentiation and high endemism with local genetic structure of populations (e.g., *Peromyscus* rodents; Ordóñez-Garza et al., 2010); it also includes a genetic boundary between the Guatemala highlands and the Chiapas massif highlands, as evidenced by strong genetic structure within oak species (*Abies*; Jaramillo-Correa et al., 2008).

The less studied area within this clade, from a genetic perspective, is El Salvador. Hasbún et al. (2005) studied the Mesoamerican spiny-tailed lizard species complex (*Ctenosaura quinquecarinata*) and observed high genetic variability and genetic similarity between the populations from El Salvador and those from northern Honduras. However, they also found a population in Honduras with remarkable genetic, ecological and morphological differentiation, a region located along the Volcanic front, Secondary line and Behind the volcanic front (Fig. 3, E), for which the authors describe this population as an Evolutionary Significant Unit (Fraser and Bernatchez, 2001) (Fig. 3, E). The Central American volcanic arc near western El Salvador was a zone with active volcanism during the Quaternary, whereas the eastern El Salvador and north-central Honduras lack volcanic activity, which made this area both an ancient corridor and a recent barrier for dispersal. The effects of this volcanic activity were markedly strong in plants (Graham, 1989), shown for instance by significant genetic structure within this clade along the Pleistocene refugia for some members of the genera *Bursera* and *Ficus* in El Salvador (Fig. 3, D; Poelchau and Hamrick, 2011) or strong genetic differentiation for populations of tropical trees (Dick et al., 2003) and oaks (*Quercus*; Cavender-Bares et al., 2011) since the Pleistocene. Speciation rates in taxa distributed on the lowlands within the Mid-Central America clade were higher during the Miocene and have decreased since, whereas species divergence from the highlands increased during the last million years. In their study with pitvipers, Daza et al. (2010) found that some boundaries show great synchrony among diverse lineages (breaks in Talamanca, Motagua–Polochic and Tehuantepec), in which two lineages of highland species show early divergences over this area (across the Motagua–Polochic faults estimated between 3.8 and 6.8 Ma), which support the lower volcanic activity for some time along the northeastern region of the Chortis block. A third highland pitviper lineage and the lowland lineage show substantially later divergences (across north and south areas of the Talamanca range, between 2.5 and 3.9 Ma). Regarding the south region of the Chortis block, studies have shown consistent genetic results for populations from western Nicaragua and southern Honduras, which frequently form well-differentiated clades, as seen for the Neotropical sand fly *Lutzomyia longipalpis* that showed 5% divergence between populations from these regions (Arrivillaga et al., 2002). At the beginning of the Pleistocene, Costa Rica was inaccessible because the lowlands of the Nicaragua depression were flooded. It has been suggested that this depression harbored a marine shelf less than 200 m deep at 3 Ma (Harmon, 2005), whereas genetic patterns support that, by that time, it represented a water barrier for many species. Although we do not know when dispersal was possible, Gutiérrez-García and Vázquez-Domínguez (2012) found a split between populations of the rodent *O. phyllotis* from Costa Rica and Nicaragua, dated at approximately 1.85 Ma, a result that suggests that the Nicaragua depression was dry at the time.

Clade 3: the Panamian clade

The northern limit of this clade is on the Hess escarpment (Fig. 3, C), while on the south it is limited by a fragment of the Andes range (Fig. 3, D). To understand evolutionary patterns related with this clade, it is important to consider that the Isthmus of Panama acted as a barrier for marine taxa before it became a bridge for terrestrial taxa (ca. 7 Ma), as examples with crustaceans, echinoderms and fish have been identified (Lessios et al., 1999; Muss et al., 2001; Stillman and Reeb, 2001). Regarding terrestrial taxa, in a study with pseudoscorpions Zeh et al. (2003) found three highly variable and divergent clades in Panama (Fig. 3, G), which are explained by colonization and recolonization processes based on the direction of genetic migration. The authors suggest that highlands in Costa Rica, like the Talamanca range, were colonized by individuals from South America (7–5 Ma) before the closure of the Isthmus of Panama. Flooding events followed

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this colonization, isolating populations from the Talamanca range (Fig. 3, F), and later, with the closure of the Isthmus of Panama (Driese et al., 2006; Webb, 2006; Woodburne, 2010), new dispersal into the lowlands from both highland and South American populations occurred. Finally, they also identify a population with high genetic diversity that likely behaved as a center of dispersal. Bermingham and Martin (1998) observed a striking genetic structure for fish species in this region, even between localities with no geographic barriers. They also confirmed that some populations acted as dispersal centers around 2 Ma. In another study with the frog *P. ridens*, Wang et al. (2008) found two older lineages distributed in central and western Panama, and a much younger one from Costa Rica and Honduras. Their results support a colonization event into Panama during the Miocene (12 Ma), predating the isthmus formation, and a recent and rapid dispersal toward Costa Rica across the Caribbean lowlands via a Pacific route (Pliocene and Pleistocene). The Central range in the Chorotega block, in contrast with the other major ranges, had active volcanoes at the beginning of the Quaternary, which might have limited dispersal via the highlands but facilitated it through the lowlands, although movement of individuals along eastern Costa Rica must also have been conditioned by the drying of the Nicaragua depression during the Pliocene–Pleistocene.

Evolutionary processes within this clade show that the limits between the Chorotega and Chocó blocks are less clear than those between other main geological forms in CA. Populations in this clade are genetically differentiated from those of the Mayan and Mid-Central America clades, and also show a marked genetic structure both between the north and south and the Pacific and Atlantic limits, resulting from independent colonization histories. For example, Hoffmann and Baker (2003) show that, for some species of fruit-eating bats in the genus *Carollia*, the combined effect of the uplift of the Andes and the Panamian land bridge was significant for their diversification. They found two lineages, with a deep subdivision within each, corresponding to differences across the Andes. In another study, Cadena et al. (2007) found that populations of the *Buarremon torquatus* finch complex in Panama are divided into two lineages, a western and a central-eastern one, as a result of different colonization routes from Central to South America. Also, Scotti-Saintagne et al. (2012) studied rainforest trees from the *Carapa* genus and found that the Costa Rica and western Andes populations diversified from the Amazon ones between 9.4 and 26.2 Ma, with later and local diversification events during the Pliocene and Pleistocene. Other barriers have been recognized along the southern limit of this clade, like the Bocas break near the border between Panama and Costa Rica (Wang et al., 2008), a plausible relict of a geological barrier and a more recent ecological break due to climatic features (Fig. 3, X). Likewise, other major geographical barriers such as the Amazon River and the Isthmus of Darien (Fig. 3, Z) between northern South America and Central America appear to have restricted gene flow, producing measurable genetic differentiations. This pattern is observed in the differentiation of jaguar populations along this region (Eizirik et al., 2001) and in a highly divergent clade for finch species (Cadena et al., 2007); however, for other species the Isthmus of Darien was not a barrier (sand fly; Arrivillaga et al., 2002). In fact, given that some ranges of the Darien emerged before the Miocene (Montes et al., 2012), they could have later acted as a corridor for different taxa. The lowlands near the Panama channel (Fig. 3, Y) remained flooded even long after the closure of the Isthmus of Panama, which limited gene flow and defined genetic patterns between populations of aquatic (e.g., freshwater fishes, Bermingham and Martin, 1998) and terrestrial (e.g., frogs, Hauswaldt et al., 2011) species.

Interestingly, the different phases, cycles and clades described in the present review can sometimes be witnessed as a comprehensive history in the evolution and diversification of individual species, whether from South America to Central America, or vice versa, from North America to the south. The harlequin beetle-riding pseudoscorpion, *Cordylochernes scorpioides*, had an early wave of colonization out of South America to Central America at the close of the Miocene, a migration consistent with a transitory proto-isthmus, followed by sea-level rise and flooding of the terrestrial corridor, with a second wave of colonization that occurred with the isthmus closure (Zeh et al., 2003). Likewise, in another example three major phylogeographic lineages have been identified for the rodent *O. phyllotis*, which coincide with some of the main geological features that shaped CA. The species origin and initial presence in CA occurred before 3.35 Ma, prior to the GABI, from where it dispersed following a series of GABI pulses: an initial northward dispersal and posterior diversification within the Mayan clade (2.27 Ma), afterwards dispersal (1.82 Ma) toward both the south and north influenced by the Modern Chiapas volcanic arc and the Central American volcanic arc, respectively, and encompassing two clades, the Mayan and the Mid-Central America, with a later colonization of the Yucatan peninsula that involved radiation and range expansion events (0.8–0.125 Ma) throughout its distributional range (Gutiérrez-García and Vázquez-Domínguez, 2012).

Climate in the evolutionary history of CA, a challenge

Some of the primary sources of climatic and paleoecologic data during the Miocene to Pleistocene in CA have been gathered from moraines and lake sediment cores (e.g., isotopes, fossilized pollen, carbon and diatoms) (Bush et al., 2009). CA climate is associated with a complex system that includes droughts, hurricanes, cyclones, monsoons and tropical storms, which are caused by the interaction of factors mainly like sea-surface temperature, ocean currents and changes in sea level (Hughen et al., 1996). The climatic event that has been most frequently referred to in genetic studies for CA species is the last glacial maximum (LGM) that took place 26,500 to 19,000 years ago (according to Clark et al., 2009). Changes in ocean temperature and sea level following, the LGM have been associated with the melting of the poles and with ocean currents (Clark and Mix, 2002), a time when there was a difference of up to 8 degrees relative to current temperatures and significant variation between wet and dry periods. It is evident that climate, more than geological processes, was the driving force for the exposure and flooding of lowlands in this region (Hillesheim et al., 2005). Moreover, during the last 2.6 Ma, 18 cooling and 19 warm-temperature episodes took place in the North Hemisphere (Lowe and Walker, 1997), which undoubtedly had an effect on climate and sea level (Supplementary Table 1). For example, Hillesheim et al. (2005) suggest, based on the flora and fauna present in lake sediments from lowlands in Guatemala, that three specific periods followed the LGM: 1) Deglaciation (12,600–11,250 yr ago) when temperature and humidity steadily increased, 2) Preboreal (11,250–10,350 yr ago) with four dry episodes and an important succession of different taxa but with almost unchanged vegetation throughout cold areas and, 3) Boreal (10,350–7800 yr ago) when climate became more homogeneous, albeit slowly, with two dry episodes that lasted 100–300 yr, and with increased vegetation diversity due to the onset of seasonality. Finally, cooling stopped on the northern region of the North Hemisphere, causing the cessation of dry episodes on the lowlands as a result of the correlation between cold (e.g., deglaciation) and warmer water from the oceans. Notwithstanding, despite most genetic studies from CA consider that climate in some way modeled the species genetic structure, there are only a few with specific ancient climatic information (Cavers et al., 2003; Cavender-Bares et al., 2011; see Table 1). Some phylogeographic and evolutionary patterns, particularly from the GABI, can more readily be associated with local, fine-scale geologic features (Hoffmann and Baker, 2003; DaCosta, and Klicka, 2008; Gutiérrez-García and Vázquez-Domínguez, 2012).

Conclusions

The present review shows that we have made considerable gains in our understanding of the influence of past events on current patterns of genetic diversity and on the geographic distribution of

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species. The information we integrated in Table 1 demonstrates the kind and frequency of geologic information that genetic studies have used for the interpretation of results. It is evidence that in most CA phylogeographic studies, because of the large temporal and spatial scales encompassed by such surveys, some geologic factors are more frequently correlated with the evolutionary history of species, in particular the role of geomorphology, topographic barriers, volcanic chains, volcanic activity, large climatic changes, intermittent connections and corridors. In geologic studies most of those factors are combined as specific geographic regions like the physiographic provinces (Marshall, 2007). On the other hand, finer-scale factors are mostly lacking in genetic studies, which can be analyzed on specific cases where, for instance, local ecological and geologic information is available, more often found for cases encompassing the time after the closure of the Isthmus of Panama. Plausible reasons for the latter include the fact that one of the most significant geologic events after the emersion of CA is the Panamian bridge, which resulted in a major continental large-scale biologic interchange 3.1–2.5 Ma. A proof of this is the seminal work of Woodburne (2010), which describes in detail the tectonics, climate, sea-level changes and dispersal of mammals during this biotic interchange.

We believe that the present review contributes with a thorough description of Central America geologic history and processes, one that provides a more comprehensive framework that can serve as a basis for the interpretation and correlation of the evolutionary patterns obtained with genetic and phylogeographic studies of flora and fauna. Given that particular climatic events and oscillations and ecological changes that have influenced the distribution of CA taxa can, in most cases, be associated with dating records like fossils or known geologic events, we suggest that this information should be integrated in genetic studies that include dating of events (e.g., diversification, cladogenesis) based on molecular markers. We also suggest that we need to consider biological and ecological characteristics when trying to decipher the evolutionary history of species, because those fine-scale factors ultimately define if a species can disperse, migrate and diversify. The latter could in turn be related with correspondingly more spatially and temporally local geologic factors. Lastly, molecular and geologic evidence proves that the biogeographic history of Central America prior, during and after the emergence of the Isthmus of Panama, influenced the evolutionary history of the biota in a significant, vast and complex manner.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yqres.2012.12.007.

Acknowledgments

We thank Gerardo Rodríguez Tapia for technical advice for map outlines and Eduardo Espinosa-Medinilla for reviewing an earlier version of the manuscript. Jaime Urrutia Fucugauchi and two anonymous reviewers made helpful comments that greatly improved our manuscript. E. Vázquez-Domínguez acknowledges financial support from the Instituto de Ecología, UNAM, and from Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (grants IN215205-2, IX238004 and IN219707). This paper constitutes a partial fulfillment of the Graduate Program in Biological Sciences of the National Autonomous University of México (UNAM). T. A. Gutiérrez-García acknowledges the scholarship and financial support provided by the National Council of Science and Technology (CONACyT 175434) and UNAM.

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Please cite this article as: Gutiérrez-García, T.A., Vázquez-Domínguez, E., Consensus between genes and stones in the biogeographic and evolutionary history of Central America, Quaternary Research (2013), http://dx.doi.org/10.1016/j.yqres.2012.12.007

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Please cite this article as: Gutiérrez-García, T.A., Vázquez-Domínguez, E., Consensus between genes and stones in the biogeographic and evolutionary history of Central America, Quaternary Research (2013), http://dx.doi.org/10.1016/j.yqres.2012.12.007

Supplementary Table 1. Climatic events in CA, suggested as relevant to explain the genetic structure of taxa. The event refers to: C=cooling, Wa=warming, W=wet, D=Dry and V=period with minimal variations between wet and dry. Interval is measured as Ma=million years ago, ka=thousand years ago and *=thousand years

Event	Location of event	Interval/time of event	References
C	Savanna in Bogota	2.7–2.4 Ma	De Porta, 2003
C	Glacier advances in Mexico	151–126,19–18 15–14,10 ka	Martinson et al.,1987; Vázquez & Givnish, 1998
C	Starting of the LGM	34–21 ka	Miranda, 1997
C	Last Glacial Maximum (LGM)	21±2 ka	Mix et al., 2001; Bush et al., 2009
W D	Petén-Itzá lake formation in Guatemala Petén-Itzá lake partially drying	23 ka 18–11 ka	Bush et al., 2009
W	La Yegua lake formation in Panama	16 ka	Bush et al., 1992
V	Lake sediments, Panama	14,350–11,050 ka	Bush et al.,1992
Wa	Deglaciation of Costa Rica ranges	12,360–11,240 ka	Horn, 1990
D	Lake sediments in Costa Rica	14,1–13,5 ka	Fairbanks et al., 2005
W	Lake sediments in Nicaragua	2–6 ka	Kutterolf et al., 2007
V	Punta Laguna lake formation in Mexico	3,310–1,391*	Curtis et al., 1996
C	Little Ice Age (LIA)	1,400*–1,500*	Hodell et al., 2005

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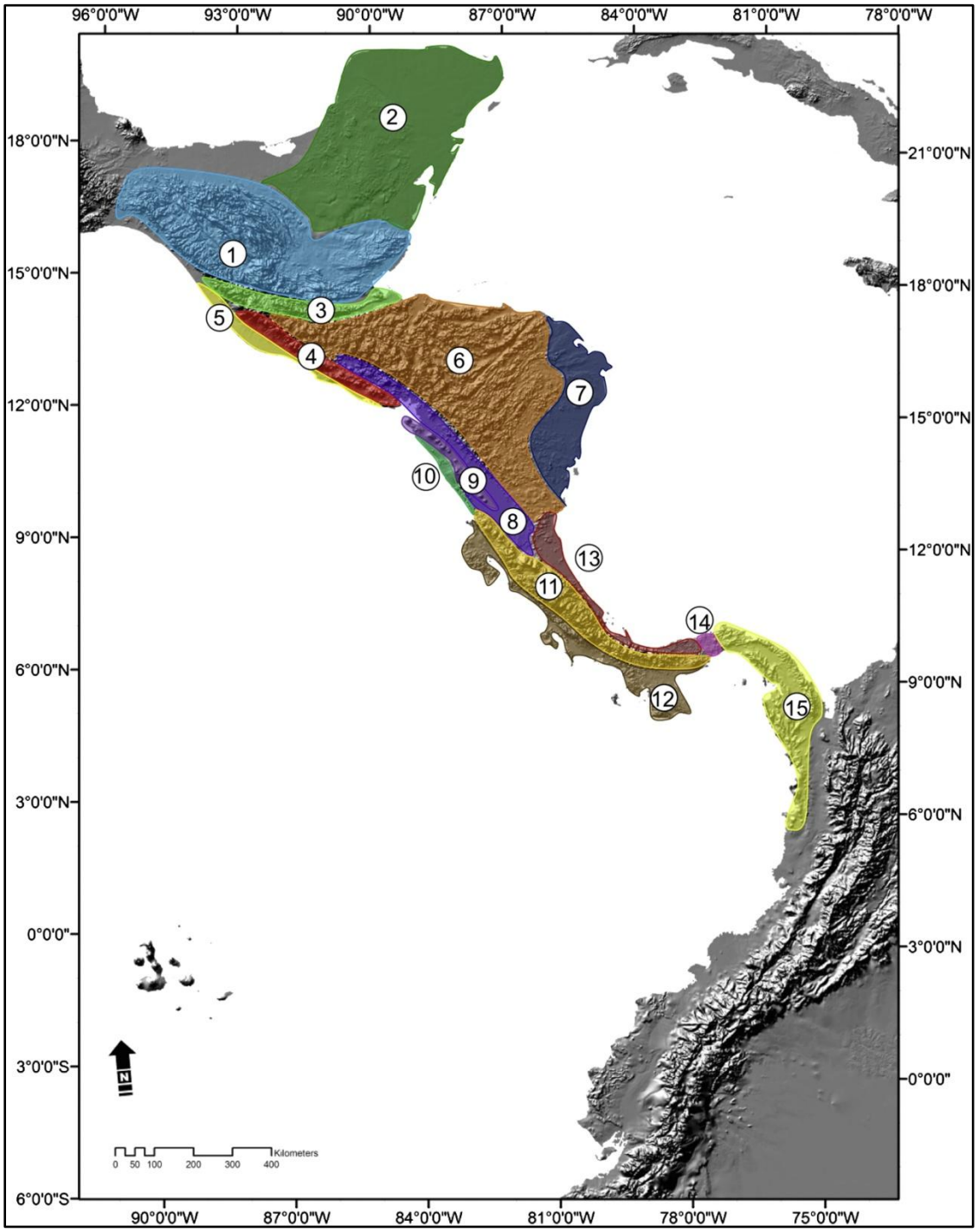
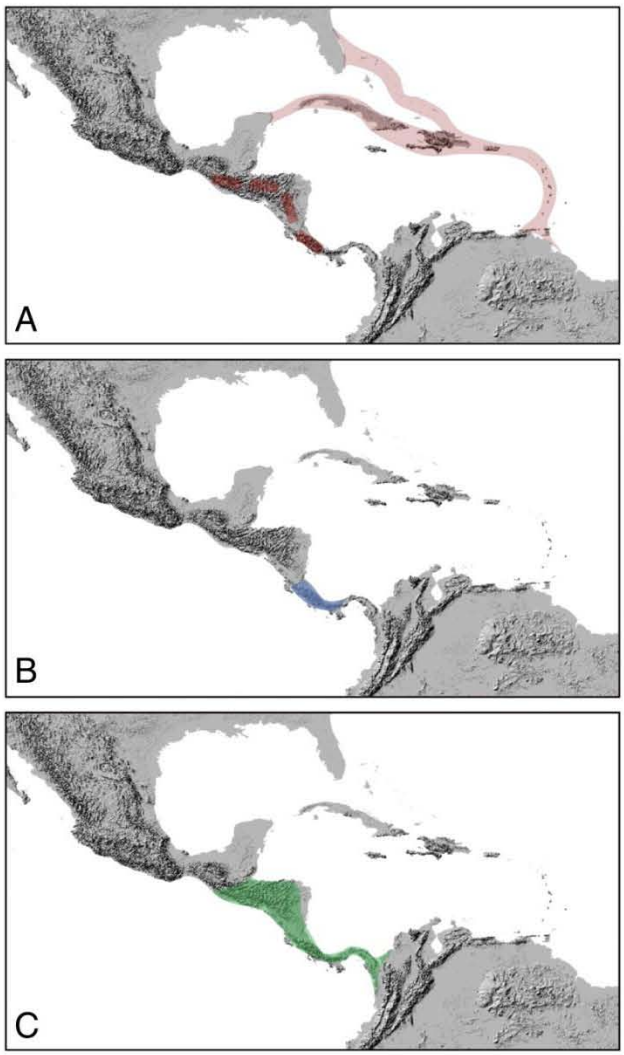
Supplementary Figure 1. *Physiographic provinces of Central America, modified from Marshall (2007). Numbers refer to: 1=Maya highlands, 2=Yucatán platform, 3=Motagua fault zone, 4=Chortis volcanic front, 5=Chortis fore arc, 6=Chortis highlands, 7=Mosquito Coast lowlands, 8=Nicaraguan depression, 9=Nicaraguan volcanic front, 10=Sandino fore arc, 11=Chorotega volcanic front, 12=Chorotega fore arc, 13=Chorotega back arc, 14=Canal Zone lowlands, 15=Darién isthmus.*



Supplementary Figure 2. *Schematic representation of the main paths of the three migrations across CA described in the review, which were inferred based on the genetic structure and phylogeography of different taxa. The migrations are showed as follow: A=First migration, the Arc-island and Proto-Antilles-GAARlandia that occurs before the Miocene (in red); B=Second migration, colonization of highlands and lowlands during the Miocene (in blue), C=Third migration, Great American Biotic Interchange during the Plio-Pleistocene (in green).*



Capítulo 3

Artículo: **Biogeographically dynamic genetic structure bridging two continents in the monotypic Central American rodent *Ototylomys phyllotis***

Publicado en: **Biological Journal of the Linnean Society**

Biological Journal of the Linnean Society, 2012, **107**, 593–610. With 4 figures

Biogeographically dynamic genetic structure bridging two continents in the monotypic Central American rodent *Ototylomys phyllotis*

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Received 2 April 2012; revised 4 May 2012; accepted for publication 4 May 2012

Central America is an ideal region in which to study patterns of historical divergence and population genetic differentiation, because of its extraordinarily dynamic biogeographical, tectonic, and climatic history. The rodent *Ototylomys phyllotis* is the only extant species of the genus *Ototylomys* and is distributed within this region from the Isthmus of Tehuantepec, Mexico, to central Costa Rica, offering an excellent opportunity to study spatial and temporal patterns of population structure of the species and to explain the ecological and evolutionary processes responsible for those patterns. We estimated the genetic diversity and structure within and between populations of *O. phyllotis*, times of divergence, and migration patterns using mitochondrial DNA and a comprehensive combination of phylogenetic and phylogeographical computational analyses. Our results support monophyly of the genus *Ototylomys*. We identified three major phylogeographical lineages within *O. phyllotis* that are linked to its diversification and coincide with the main geological features that shaped Middle America. The origin of the genus was before 3.35 Mya, prior to the Great American Biotic Interchange (GABI), and its initial occurrence was near the centre of its current distribution (Honduras/El Salvador), from which it later spread (3.20–2.84 Mya) following a series of GABI pulses. The species showed an initial northward dispersal to the Chiapas and Guatemala highlands (2.27 Mya) followed by diversification. A later dispersal (1.82 Mya) occurred toward both the south (Nicaragua, Costa Rica) and the north (Belize). The Yucatan peninsula was colonized (0.8 Mya) by individuals from Belize. Extremely high radiation and range expansion occurred throughout the entire range, the highest of which was in the Yucatan peninsula (0.125 Mya). *Biological Journal of the Linnean Society*, 2012, **107**, 593–610.

ADDITIONAL KEYWORDS: endemic species – evolutionary history – GABI (Great American Biotic Interchange) – Middle America – Muridae – phylogeography.

INTRODUCTION

Phylogeography is the study of the geographical distribution of the genetic variation of natural populations in a historical context. As such, it helps to decipher spatial and temporal patterns of population structure (i.e. genetic differences within and among populations) and to explain the ecological and evolutionary processes responsible for those patterns (Avise *et al.*, 1987; Avise, 2000; Hickerson *et al.*, 2010). Moreover, it allows us to understand how specific historical processes and biogeographical boundaries have differentially impacted lineages or biotic assemblages. The recent development of comprehensive phylogeographical studies of various groups of organisms has led to important insights on the history of diversification in different regions of the Earth, which improve our understanding of the genetic structure of populations, the timing of population differentiation, and the role of geological features (e.g. rivers, mountains, faults) as barriers or corridors to gene flow

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(Moritz *et al.*, 2000; Gutiérrez-García & Vázquez-Domínguez, 2011).

Central America is an ideal region in which to study patterns of historical divergence and population genetic differentiation and structuring of endemic biota, mainly because of its extraordinarily intricate geological and biogeographical history, diversity of habitats, and dynamic climatic and tectonic history (Iturralde-Vinent, 2006; Arbeláez-Cortés, Nyári & Navarro-Sigüenza, 2010). The gradual emergence of Central America from a set of islands to a terrestrial block facilitated not only the migration and colonization of species of flora and fauna originating from both the north and the south of the continent, but also contributed to the origin of numerous endemic species. Geological complexity, e.g. mountain ranges, lowlands, lakes, rivers, and volcanic activity, together with a highly dynamic historical climate (hurricanes, droughts, flooding), shaped a set of corridors and barriers that affected the biota's dispersion, migration, and diversification at different time and spatial scales. For the present study and in accordance with temporal and geological features, we define Central America – the Middle American region – as a land area located between the Isthmus of Tehuantepec, Mexico, including the Yucatan peninsula, and Colombia (Webb, 2006; Daza, Castoe & Parkinson, 2010; Hulsey & López-Hernández, 2011).

The formation of the Panamanian land bridge that led to the final closure of the Central American Seaway, approximately 3 Mya, marked the start of the processes that have configured the modern floras and faunas of the Middle American region, known as the Great American Biotic Interchange (GABI) (Coates & Obando, 1996; Webb, 2006; Woodburne, 2010). Before the closure of the Panama Isthmus, the composition of all Mexican mammal faunas was completely North American (Ferrusquía-Villafranca, 2003; Webb, 2006). Today, faunas of Middle America are a rich and complex mixture of North and South American components, clear evidence of the processes associated with the GABI, including the migration and differentiation of endemic and morphologically distinctive species (Orellana, Islebe & Espadas, 2003; Strecker, Faúndez & Wilkens, 2004; Vázquez-Domínguez & Arita, 2010). Good examples of the latter are provided by some mammalian families: Tapiridae, Felidae, Sciuridae, and Muridae that are of North American origin but are represented in Central America by species typically considered tropical (e.g. Baird's tapir, *Tapirus bairdii*; jaguar, *Panthera onca*, and Deppe's squirrel, *Sciurus deppei*). On the other hand, lineages of South American origin are represented by primates (black howler monkey, *Alouatta pigra*; Geoffroy's spider monkey, *Ateles geoffroyi*), marsupials, bats, and hystricognath rodents (Central American agouti, *Dasyprocta punctata*; spotted paca, *Cuniculus paca*) (Vázquez-Domínguez & Arita, 2010).

Within the class Mammalia, the rodent family Muridae is the most diverse family with 16–20 subfamilies. The Muridae has experienced several events of rapid differentiation (radiations), from which the extant subfamilies originated; an example is the Sigmodontinae with 79 genera of rodents. The species distributed in America today radiated from the Sigmodontinae a few million years ago (Musser & Carleton, 1993). Some authors separate the latter into three subfamilies: Neotominae, Tylomyinae, and Sigmodontinae (Steppan, Adkins & Anderson, 2004). The phylogenetic relationships of the genera *Ototylomys* and *Tylomys* have been controversial, considered to belong to the subfamily Tylomyinae or to the tribe Tylomyini within the Neotomine–Peromyscine complex (Musser & Carleton, 1993; Reeder & Bradley, 2004). The big-eared climbing rat *Ototylomys phyllotis*, Merriam 1901, is the only extant member of the genus *Ototylomys*. Its distribution ranges from the Isthmus of Tehuantepec, Mexico, in the north, including the states of Tabasco, Chiapas, and the Yucatan peninsula, to central Costa Rica in the south. It is found mainly in tropical wet forest, from sea level to 1900 m. Lawlor (1969, 1982) suggested that *Ototylomys* has three morphologically distinct groups throughout its distribution, which he recognized as the subspecies *O. p. australis*, *O. p. connectens*, and *O. p. phyllotis*.

Despite the fact that *O. phyllotis* has been included in studies about the phylogeny and divergence of different rodent genera (Edwards & Bradley, 2002; Reeder & Bradley, 2004; Steppan *et al.*, 2004), data about its ecology and genetics are rather limited, and no studies exist regarding its genetic diversity and structure, historical divergence, or phylogeography. Considering the complex geological and climatic history of Central America and the lack of genetic information for the species, our aim was to evaluate the genetic diversity, genetic structure, and phylogeographical patterns of *O. phyllotis* throughout its range of distribution, particularly: (1) to estimate the genetic diversity and structure within and between populations of *O. phyllotis* using mitochondrial DNA (cytochrome *b*), (2) to decipher its origin, divergence time, and historical migration patterns, and (3) to propose the possible diversification events associated with the present distribution of the species.

MATERIALS AND METHODS

Sampling and DNA extraction

We analysed 129 *O. phyllotis* museum and field samples, together with three *O. phyllotis* sequences

from GenBank (AY009788, AY009789, DK179814), from 47 localities covering most of the species' range of distribution (Supporting Information, Table S1 and Fig. S1). Forty individuals were live-trapped during different field trips between 2003 and 2004, as part of an ongoing study on the ecology, phylogeography, and conservation of vertebrate fauna from southern Mexico (Zambrano *et al.*, 2006; Vega *et al.*, 2007; Vázquez-Domínguez *et al.*, 2009). Tissue samples for molecular analyses were ethically obtained, with the corresponding collecting permits, and stored in labelled Eppendorf tubes with ethanol 97% until DNA extraction. We also included 89 museum samples from liver, dry skin, and bone from different biological collections (Supporting Information, Table S1). We isolated whole genomic DNA from fresh and museum samples using the AquaPure Genomic DNA (Biorad Laboratories) and Fujifilm QuickGene DNA tissue kits following the manufacturer's protocol. We quantified DNA by spectrophotometry and confirmed its integrity with UV light in 1% agarose gels stained with 0.5 mg mL^{-1} ethidium bromide.

Amplification and sequencing

We amplified a fragment of the mitochondrial cytochrome *b* (cyt *b*) region using eight primers specifically designed for *O. phyllotis* during this study, which combine 1 with 2, 3 and 4 and so forth (*Oty1*: 5′-CACATTCGAAAAAATCACCCCCT-3′, *Oty2*: 5′-TAGATTCCTCGGCCTACGTGAAGG-3′, *Oty3*: 5′-GCTACCTCCACGCCAATGGAGC-3′, *Oty4*: 5′-GGTGGACTAGGACAAGGGCTGTA-3′, *Oty5*: 5′-CTGTGTAGACAAAGCGACCTT-3′, *Oty6*: 5′-GTGTTKAGTGGATTTGCAGGAGTA-3′, *Oty7*: 5′-CAGATATTCTCGGRGACCCGGA-3′, *Oty8*: 5′-GCTRATTGAAGCTAGTTGACCGAT-3′). PCR reactions were performed in a 25-μL reaction volume containing 50–100 ng template DNA, 0.2 μL of *Taq* DNA polymerase (Invitrogen), 2.5 μL of each primer (10 μM), 2.5 μL of dNTP mix (2 mM), 1 μL $MgCl_2$ (50 mM), and 2.5 μL 10× reaction PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl). Amplifications were performed on a PTC-100 Programmable Thermal Controller (MJ Research, Inc.) as follows: 90 s at 92 °C, followed by 39 cycles of 15 s at 92 °C, 60 s at an annealing temperature of 50 °C, and 70 s at 72 °C, with a final extension at 72 °C for 10 min. Every appropriate precaution was taken when working with historical bone and skin samples, which were amplified by small 200–250-bp fragments with sufficient overlap with adjacent fragments for correct concatenation. Moreover, all PCRs included negative controls to check for contamination and random samples were sequenced twice to ensure reproducibility and correct readings. PCR products were purified using the QIAquick PCR purification Kit (Quiagen Inc.) and both strands were sequenced with the same primers used for the amplification. Sequencing was carried out on a 3730xl DNA analyser (Macrogen). We manually aligned and edited DNA sequences using the BioEdit Sequence Alignment Editor program (Hall, 2005). All sequences were deposited in GenBank (accession nos. JX020572–JX020700).

Phylogenetic analysis and estimation of time of divergence

We used the complete set of 132 sequences from *O. phyllotis* to perform phylogenetic analyses. For the selection of the species as outgroup, we considered the conflict and inconsistencies that exist regarding the phylogenetic relationships among Sigmodontinae, Tylomyinae, and Neotominae: first, although *Tylomys* is a sister species to *Ototylomys*, it has been suggested that *Ototylomys* is the primitive form (Helm, 1975). Second, despite Neotominae (*Peromyscus*, *Reithrodontomys*, and *Neotoma*) has been identified as a sister clade to Tylomyinae (*Tylomys* and *Ototylomys*) by Steppan *et al.* (2004) – with very low bootstrap support – Matocq, Shurtli & Feldman (2007) found that *Tylomys* and *Ototylomys* were basal to *Peromyscus* and *Neotoma*. Finally, other authors report different phylogenetic relationships among the species (e.g. Edwards & Bradley, 2002; Bradley *et al.*, 2004). In accordance, we selected seven genera as outgroups, five within the family Muridae and two from the families Sciuridae and Dipodidae (sequences from GenBank), which include both consistently reported basal species and species that allowed us to have fossil calibration points for the estimation of time of divergence: *Gerbillurus* (AJ430558, AJ430559), *Taterillus* (AJ430563, AJ0430564), *Mus* (AB033699), *Rattus* (AB033713), *Spalax* (AF155871, AJ389537, AJ311138), *Sciurus* (AB292677, AB292678, AB292680), and *Jaculus* (GU433440, GU43341). Tajima's *D* (Tajima, 1989) and Fu and Li's *D* and *F* (Fu & Li, 1993) indexes were used to examine the selective neutrality of mitochondrial fragments. The best-fit model of evolution was estimated under the Akaike Information Criterion (AIC) as implemented in jModeltest v.0.1.1 (Guindon & Gascuel, 2003; Posada, 2008). We performed the Bayesian Inference method with MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003), which consisted of two independent Markov chain Monte Carlo runs, each comprising four differentially heated chains of 5×10^6 generations with a sampled tree every 200 generations. Trees generated prior to achieving stationarity were discarded as burn-in, and stationarity was identified by log-likelihood plots, similarity in topologies and branch support (posterior probabilities), as estimated with

MrBayes (Ronquist & Huelsenbeck, 2003). Phylograms topologically identical to the maximum *a posteriori* tree (MAP) were recovered using PAUP4.0 (Swofford, 2002), and from these, the 25 most resolved at the intra-population level were selected to conduct dating analyses.

The timing of phylogenetic divergence was estimated with r8s v1.71, a program for estimating absolute rates of molecular evolution and divergence times on a phylogenetic tree (Sanderson, 2006). Its starting point is a given phylogenetic tree and a given set of estimated branch lengths (numbers of substitutions along each branch). In addition, one can add calibration points that permit scaling of rates and times to real units. Considering that using accurate dates renders higher precision for the analysis, we performed the calibration on the 25 phylograms identical to the MAP tree by fixing the origin with fossils of the *Mus–Rattus* complex at 12.45 Mya, an average estimated from the origin of *Antemus* and *Karnimata* (Flynn *et al.*, 1990; Benton & Donoghue, 2007), of *Spalax* at 1.4 Mya (Tchernov, 1987), and of *Jaculus* at 2.45 Mya (Behrensmeyer *et al.*, 1997). Another advantage of this program is that, through a cross-validation test, it allows the user to explore the fidelity with which any of the implemented methods explains the branch-length variation (Sanderson, 2002). This procedure removes each terminal branch in turn, estimates the remaining parameters of the model without that branch, predicts the anticipated number of substitutions on the pruned branch, and reports the performance of these predictions as a cross-validation score, which allows the user to select the method that best explains the branch-length variation. Accordingly, we identified the optimal smoothing parameter for each data set through the cross-validation procedure for model selection by means of penalized likelihood (Sanderson, 2002), which uses the internal consistency among the fossil ages to select the appropriate level of rate smoothing. Point estimates of age from each of the phylograms were used to obtain mean and standard deviations of ages of nodes across the tree. This was done by generating a series of phylograms based on a single tree (i.e. the same topology but different sets of branch lengths), reading these into r8s, estimating ages for all trees, and then using the profile command to summarize age distributions for a particular node. The central 95% of the age distribution then provides a confidence interval (Sanderson, 2002, 2006). Finally, for the analysis of divergence times it is necessary to test the MAP tree for congruence with the molecular clock model, and thus we compared the cross-validation criterion with the smoothing parameter gamma with r8s. When data are clocklike, the cross-validation criterion decreases with increasing gamma (Sanderson, 2002).

Genetic diversity, population structure, and demographic history

We conducted all the following analyses at two levels, for the different groups obtained with the phylogenetic analysis (Phylogenetic Major Groups; see Results) and for those obtained with the SAMOVA (phylogeographical groups; see Results). We estimated genetic diversity as the number of haplotypes, number of segregating sites (S), haplotype (h) and nucleotide diversity (π) values, and mean number of pairwise differences (k), using DnaSP v.5 (Librado & Rozas, 2009). To evaluate levels of differentiation, we estimated p-uncorrected genetic distances and net nucleotide divergence (Da) values (Nei, 1978), using MEGA v.4.0 (Tamura *et al.*, 2007) and DnaSP, respectively. We examined the spatial distribution of genetic variation for phylogenetic and phylogeographical groups using an analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992), which characterizes patterns of genetic variation at different hierarchical levels and does not incorporate geographical information; significance level was set at $\alpha = 0.05$ and 10 000 random permutations as implemented in Arlequin v.3.5 (Excoffier & Lischer, 2010). This hierarchical approach considers the genetic structuring among groups (Φ_{CT}), among populations within groups (Φ_{SC}), and within populations (Φ_{ST}), and takes into account the molecular distance between haplotypes (number of pairwise differences).

We conducted a Spatial Analysis of Molecular Variance (SAMOVA v.1.0; Dupanloup, Schneider & Excoffier, 2002), to define groups of local populations that are geographically homogeneous and maximally differentiated, and to identify geographical barriers between these groups. The method is based on a simulated annealing procedure that aims to maximize the proportion of total genetic variance due to differences between groups of populations, measured by the Φ_{CT} coefficient of the AMOVA Φ-statistics. It finds a structure based solely on genetic data and the geographical location of populations (geographical distance), and assigns populations to groups with the constraint that they are geographically adjacent and genetically homogeneous. This approach requires the *a priori* definition of the number (K) of groups, for which we ran SAMOVA successively with K from 2 to 11 using 1000 permutations in each run. The identification of the most probable number of groups was based on the pattern of change of Φ-statistics with K (Dupanloup *et al.*, 2002).

We also estimated the relationships between haplotypes by constructing a minimum spanning network using the median-joining method implemented in Network v.4.5.1.6 (http://www.fluxus-engineering.com; Bandelt, Forster & Röhl, 1999). To test the null

hypothesis that haplotypes are distributed randomly regarding geographical location, and also to explore for plausible processes related to demographic history, we conducted a Nested Clade Phylogenetic Analysis (NCPA; Templeton, Routman & Phillips, 1995). Although the use of NCPA has been controversial (see Templeton 2008, and references therein), it has been proven that when used in combination with other phylogenetic and phylogeographical analyses, NCPA is an adequate tool to statistically infer phylogeographical patterns across species distributions (Mortimer *et al.*, 2012, to mention one example). We nested the network haplotypes using the methods described in Templeton & Sing (1993), Templeton *et al.* (1995), Templeton (1998), and Posada, Crandall & Templeton (2006), and performed the corresponding calculations of statistics and tests of significance with GEODIS v.2.6 (Posada, Crandall & Templeton, 2000). Subsequently, we followed the latest inference key (January 2011; http://darwin.uvigo.es/software/geodis.html) for interpretation of likely processes.

To test whether the groups underwent recent demographic population growth (phylogenetic and phylogeographical groups with $N \geq 5$), we compared the observed frequency distribution of pairwise nucleotide differences between pairs of haplotypes (mismatch distribution) with that expected under the assumption of an expansion model (Rogers & Harpending, 1992; Harpending, 1994) with ARLEQUIN. The goodness-of-fit of the observed data to a simulated model of expansion was assessed using both Harpending's raggedness index (r) (Harpending, 1994) and the sum of squared deviations (SSD), using ARLEQUIN. In addition, we performed Tajima's D-test (Tajima, 1989) and Fu's F_S test (Fu, 1997) of neutrality. Fu's F_S test is highly sensitive to demographic expansions or population bottlenecks, in which significant negative values suggest a signature of population expansion (Ray, Currat & Excoffier, 2003).

RESULTS

PHYLOGENETIC ANALYSIS AND TIME OF DIVERGENCE

The phylogenetic relationships observed suggest that all *O. phyllotis* individuals form a well-supported monophyletic group (posterior probability = 98). The topology obtained shows three groups or lineages (Phylogenetic Major Groups, PMG): Yucatan Peninsula (YP), Chiapas–Guatemala Highlands (CGH), and Central America Nucleus (CAN) (Fig. 1). The first two include haplotypes from the Yucatan peninsula and from the highlands of Chiapas and Guatemala (posterior probability = 97 and 83), respectively; the remaining haplotypes, not clearly resolved in the phylogeny, are from Central American localities.

We analysed 770 bp of the cyt *b* fragment from 132 individuals and 47 localities (Supporting Information, Table S1). Results showed 195 synonymous and 572 non-synonymous sites, with 241 polymorphic (31%) and 146 parsimony-informative (60%) sites. Cyt *b* sequences did not deviate from neutrality under Tajima's test ($D = -1.1345$, $P > 0.10$). Results for the Fu and Li's tests were significant ($D = -4.063$, $P < 0.02$; $F = -3.254$, $P < 0.02$), probably because of an excess of rare alleles or unique haplotypes from populations with one or a few samples. The TrN+I+G model of DNA substitution was selected as the most appropriate for our data (–lnL = 4057.01, $P < 0.001$, AIC = 8652.03): base frequencies were A = 0.2977, C = 0.2896, G = 0.1276, and T = 0.2851; proportion of invariant sites I = 0.3380; gamma distribution $\gamma = 0.6360$. Substitution rates were: A–C = 1.000, A–G = 9.758, A–T = 1.000, C–G = 1.000, C–T = 4.549, and G–T = 1.000. The MAP tree topology was highly consistent, supported by high posterior probabilities (76–99% for most clades; Fig. 1), in which only the haplotype from Costa Rica (CR) showed minor position differences among different trees.

Results showed that the MAP tree was congruent with the molecular clock, and hence we were able to obtain divergence times at the population level (Fig. 2, Table 1). Our data revealed several migrations to the north from the species central distribution; there were probably more migrations to the south but which our data could not detect. There was more or less a constant rate of cladogenesis during the first 2.8 Mya (3.35–500 000), followed by an extremely rapid radiation during the last 500 000 years, and more notorious in the Yucatan peninsula. Results support that *O. phyllotis* originated before 3.35 Mya and that its initial occurrence and divergence in Central America took place from a region near Honduras and El Salvador, as shown by the SMS, LPH, and VCH haplotypes (Fig. 2, insert a; 3.20 ± 0.19 Mya); thus despite the uncertain relationships within the CAN group, the most plausible ancient *O. phyllotis* populations are probably from this region. From there, a range expansion (migration) took place northwards to Guatemala (Fig. 2, insert b; 2.84 ± 0.24 Mya) and to the Chiapas highlands (Fig. 2, insert c; 2.27 ± 0.22 Mya). Subsequently, *O. phyllotis* reached its southern distributional limit in Costa Rica and also migrated northwards to Belize (Fig. 2, insert d; 1.82 ± 0.51 Mya). A later range expansion was observed from its central distribution towards the north-west in Guatemala and north in Honduras (Fig. 2, insert e; 1.14 ± 0.23 Mya). Finally, during the last 800 000 years, the species rapidly expanded its range within the Yucatan peninsula and Nicaragua lowlands (Fig. 2, insert f; 0.68 ± 0.12 Mya). The migration and range expansion events described

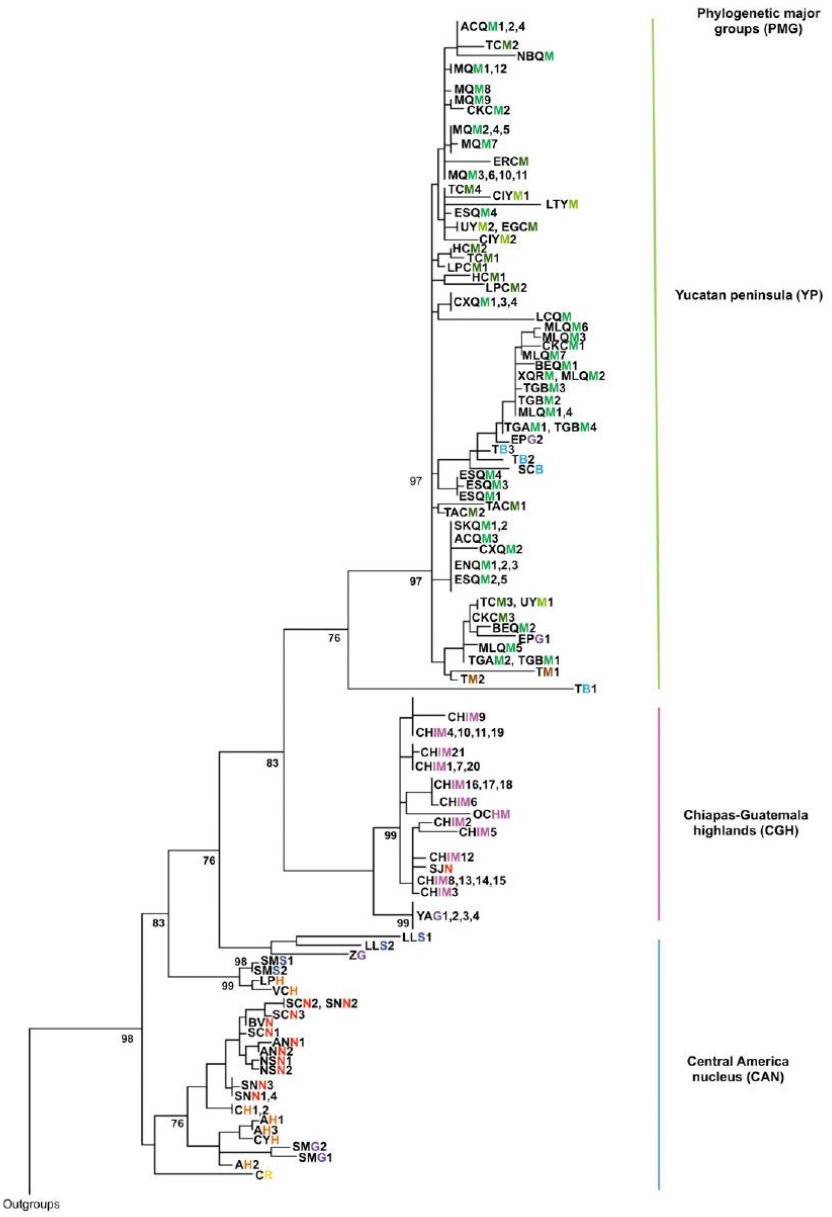

Figure 1. Bayesian phylogenetic estimates of relationships among *O. phyllotis* haplotypes. Numbers below branches are clade support (*a posteriori* Bayesian inference values). Capital letters with numbers depict the Sample ID (see Supporting Information, Table S1), in which different colours refer to localities on the minimum spanning network (see Fig. 3). The three phylogenetic major groups (PMG) are shown: Yucatan Peninsula (YP), Chiapas–Guatemala Highlands (CGH), and Central America Nucleus (CAN).

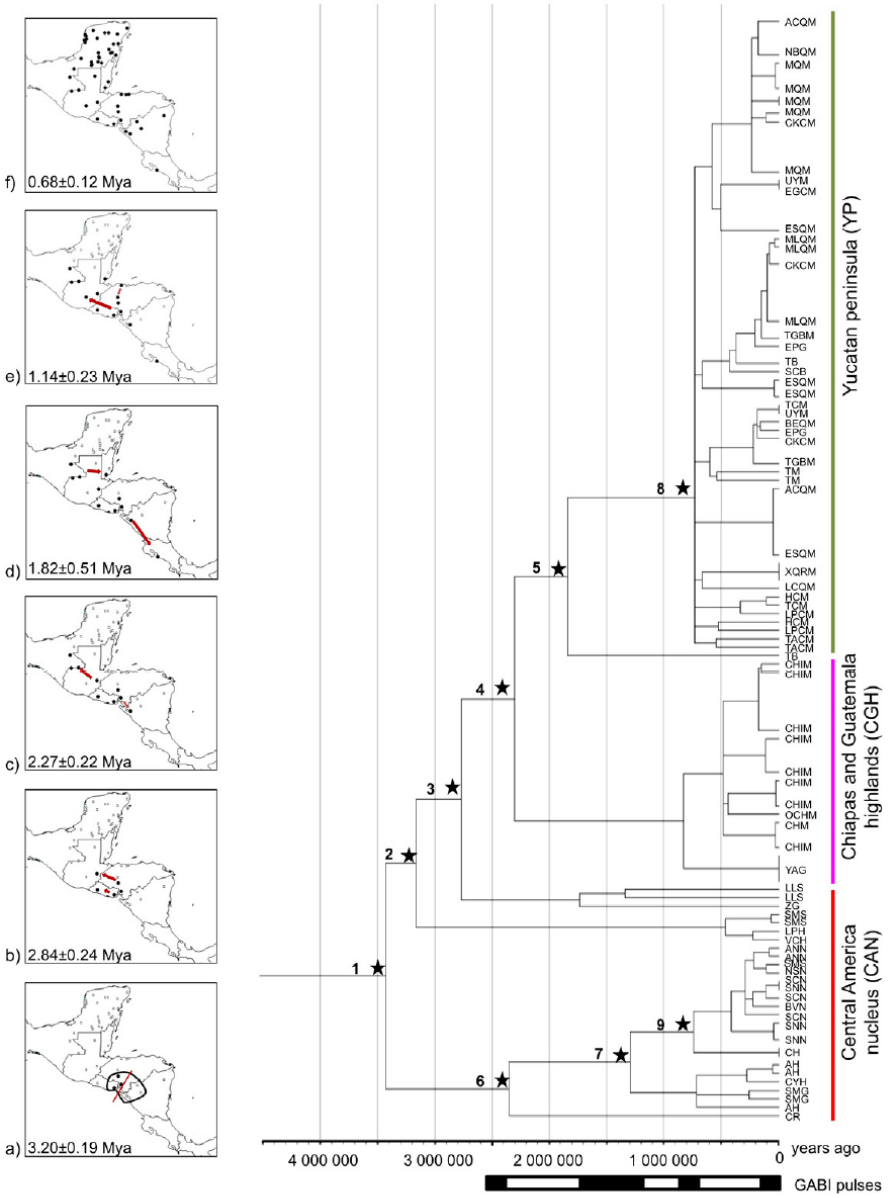

Figure 2. Chronogram derived from the analysis of r8s from the cytochrome *b* MAP tree from *O. phyllotis*. Stars above branches correspond to nodes in which a consensus of divergence time estimates was calculated with the program r8s and numbers correspond to the divergence events from Table 1. Phylogenetic major groups (PMG) from Bayesian inference are shown at the right side of the chronogram. Inserts *a* to *f* depict different dispersal/diversification events and their estimated dating (see text for more detail and Table 1). Major GABI pulses from the study of Woodburne (2010) are shown in the bar below the chronogram, where migration is marked as a filled bar, and a gap in migration as an empty bar.

Table 1. Time of phylogenetic divergence estimated with the program r8s (see Fig. 3) (Mya) (mean ± SD)

MAP*	PMG†	Evolutionary events	Time of divergence
1	CAN	Divergence of the genus *Ototylomys* and occurrence in Central America (Nicaragua, Honduras, Salvador)	3.35 ± 0.21
2	CAN	Divergence from a putative ancestral central distribution (Honduras/Salvador)	3.20 ± 0.19
3	CAN	Range expansion northwards (El Salvador, Guatemala)	2.84 ± 0.24
4	CGH	Range expansion northwards to Chiapas and Guatemala highlands Fragmentation within the ancestral population (Nicaragua)	2.27 ± 0.22
5	YP	Range expansion towards lowlands (Belize)	1.84 ± 0.13
6	CAN	Range expansion southwards (Costa Rica)	1.82 ± 0.51
7	CAN	Fragmentation/range expansion within the central distribution (Honduras, Guatemala)	1.14 ± 0.23
8	YP	Range expansion northwards (Yucatan peninsula)	0.75
9	CAN	Range expansion southwards (Nicaragua)	0.68 ± 0.12

*Number above branches indicate diversification date on the maximum *a posteriori* tree (MAP) shown in Figure 2.
†Phylogenetic major groups (PMG) defined in Figure 1.

are supported by results of the phylogenetic relationships and the processes identified with NCPA.

Genetic diversity, population genetic structure, and demographic history

Cytochrome *b* genetic variability estimated was moderate to high, variation that is strongly distributed among groups. A significant genetic structure was also observed, identified as ten different groups, some of which were concordant with the phylogenetic ones (PMG). Based on the cyt *b* fragment from the total 132 individuals, 90 different haplotypes were obtained, with high and moderate haplotype ($h = 0.990 \pm 0.003$) and nucleotide diversity ($\pi = 0.0448 \pm 0.0019$) values, respectively (Table 2). Results of SAMOVA showed that the best representation of genetic structure, i.e. the highest Φ_{CT} value, was observed at $K = 10$ ($\Phi_{CT} = 0.771$): those ten phylogeographical groups (SAMOVAG) include haplotypes from individual localities such as Costa Rica (group 1), El Salvador (2), Belize (3), and Guatemala highlands (4 and 7), as well as from combined localities such as Honduras and Nicaragua (5), El Salvador and Honduras (6), Chiapas highlands and Nicaragua (8), Honduras and Guatemala (9), and all Yucatan, Campeche and Tabasco haplotypes, together with two from northern Belize and Guatemala (10) (Supporting Information, Fig. S1). Haplotype diversity values estimated for the phylogenetic and phylogeographical groups (PMG and SAMOVAG) showed values of $h = 0.932$–0.980 and 0.929–1.00, respectively, whereas nucleotide diversity values were $\pi = 0.0108$–0.0295 and 0.0067–0.0413 (Table 2). The mismatch distribution was almost unimodal as expected under the sudden expansion model for two of the PMG (YP and CGH) and for one SAMOVAG (group 10) (Supporting Information, Fig. S2). The latter was supported by non-significant SSD and *r* values and significantly negative Fu's F_S (Table 2).

Genetic divergences (*Da*) and p-uncorrected distance values for PMG showed the highest values between YP and CAN (0.0536 ± 0.0041; 0.0763 ± 0.0028, respectively), which are also the furthest apart geographically, whereas the other two comparisons showed lower, similar values (YP–CGH: 0.0481 ± 0.0032; 0.0615 ± 0.002; CAN–CGH: 0.0452 ± 0.005; 0.0653 ± 0.0035) (Supporting Information, Table S2). Regarding the SAMOVAG, the highest values for *Da* ranged from 6.0 to 7.2%, mostly in comparisons between Costa Rica, Guatemala highlands, and Yucatan peninsula and the rest of the haplotype groups. The genetic distance values were 7.0–8.1%, involving similar group comparisons but including Belize (Supporting Information, Table S2). We also estimated divergences in relation to two species from the Tylomyini tribe and results showed a divergence of 15.6% between *Ototylomys* and *Tylomys* (DQ179812, AF307839) and 23.9% between *Ototylomys* and *Nyctomys* (AY195801). AMOVA results were concordant for both PMG and SAMOVAG, in which the highest genetic variation is distributed among groups (69.5 and 76.1%, respectively), followed by that among populations within groups (21.5 and 14.5%); within-groups variation showed the smallest values (8.9 and 9.3%) (Table 3).

Phylogeographical analyses

The minimum spanning network and nested clade analysis results showed three main groups, which are

Table 2. Genetic diversity values and neutrality tests for (a) phylogenetic (PMG) and (b) phylogeographical (SAMOVAG) groups

Group	N	Hp	h (±SD)	π (±SD)	S	k	Tajima's D	Fu's F_S	SSD	r
(a) PMG										
CAN	28	25	0.992 (0.012)	0.0295 (0.0028)	107	22.1	−0.754	−12.57***	0.006	0.016*
CGH	27	14	0.932 (0.025)	0.0108 (0.0013)	45	8.3	−1.101	−23.22***	0.027	0.049
YP	77	51	0.980 (0.007)	0.0159 (0.0016)	163	12.1	−2.176**	−24.35***	0.004	0.006
(b) SAMOVAG										
1	1	1	1.0							
2	2	2	1.0 (0.50)	0.0345 (0.0169)	26	2				
3	3	3	1.0 (0.272)	0.0413 (0.0164)	46	30.7				
4	1	1	1.0							
5	14	11	0.967 (0.037)	0.0097 (0.0012)	23	7.4	0.095	−8.19***	0.033	0.127*
6	4	4	1.0 (0.177)	0.0067 (0.0017)	9	5.2				
7	4	1								
8	23	13	0.929 (0.031)	0.0086 (0.0012)	37	6.6	−1.321	−20.67***	0.022	0.062*
9	6	6	1.0 (0.096)	0.0139 (0.0026)	23	10.6	0.329	−0.868	0.069	0.133
10	74	48	0.978 (0.008)	0.0143 (0.0010)	135	10.9	−2.075**	−24.47***	0.004	0.006

PMG codes: CAN, Central America Nucleus; CGH, Chiapas–Guatemala Highlands; YP, Yucatan Peninsula. Numbers depicting the ten SAMOVAG groups and the localities they include are as follows: 1, CR; 2, LLS; 3, TB; 4, ZG; 5, CH, SNN, SCN, BVN, NSN, ANN; 6, SMS, LPH, VCH; 7, YAG; 8, OCHM, CHIM, SJN; 9, SMG, AH, CYH; 10 = SCB, EPG, TACM, HCM, ERCM, LPCM, EGCM, TCM, CKCM, ESQM, ENQM, SKQM, TGAM, CXQM, MQM, ACQM, TGBM, BEQM, MLQM, NBQM, XQRM, LCQM, TM, UYM, LTYM, CIYM. N, number of samples; Hp, number of haplotypes; h, haplotype and π, nucleotide diversity values; S, number of polymorphic sites; k, mean number of pairwise differences; r, Harpending's raggedness index; R_2, Ramos–Onsins and Rozas index.
Significant at: $^*P = 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

concordant with the three PMGs and are separated by more than 35 mutational steps (Fig. 3). The two most frequent haplotypes were from four different localities each (eight and five haplotypes, respectively) from the Yucatan peninsula (Fig. 3). Three derived haplotypes (tip haplotypes on the network) are separated from their nearest haplotype by more than 20 mutational steps: one from Costa Rica (haplotype number 1), one from El Salvador (4) and one from Belize (80).

The nested clade analysis identified two major haplotype nested clades: clade 5-1, which includes all Central American haplotypes with the exception of those from Belize and northern Guatemala, which are included in clade 5-2, together with all haplotypes from Yucatan peninsula. Twelve associations between clades and geographical location were significant, in which the main phylogeographical structure (total cladogram) was associated with restricted gene flow with isolation by distance (Supporting Information, Table S3). Allopatric fragmentation was the predominant evolutionary process identified for the CAN and CGH groups. The Yucatan peninsula showed restricted gene flow for clades 2-20 and 3-7, encompassing most haplotypes from Quintana Roo and Campeche, allopatric fragmentation for clades 2-21 and 4-4, which include Belize and southern Yucatan, and range expansion/colonization for clade 4-3, which includes all Yucatan peninsula haplotypes (Supporting Information, Table S3).

DISCUSSION

PHYLOGEOGRAPHIC PATTERNS AND THE GABI

Geological background of Central America and pre-GABI origin of O. phyllotis

To understand the biogeographical, genetic, and evolutionary history of *O. phyllotis*, it is important to describe the geological background of Central America. Four main geological features, from north to south, have shaped this region: the Maya, Chortis, Chorotega, and Chocó blocks (Giunta & Oliveri, 2009; James, 2009) (Fig. 4). Tectonic activity along these blocks has been suggested as a dominant force that determined lineage diversification in Middle America (Daza *et al.*, 2010). Moreover, this geological configuration, assembled during a highly dynamic tectonic period, has resulted in concordant phylogeographical and genetic patterns frequently observed when describing some flora and fauna of the region: fishes (Perdices, Doadrio & Bermingham, 2005), plants (Cavers, Navarro & Lowe, 2003), mammals (Eizirik *et al.*, 1998, 2001), amphibians (Mulcahy & Mendelson, 2000), and reptiles (Hasbún *et al.*, 2005).

Table 3. Analysis of molecular variance (AMOVA) for the phylogenetic (PMG) and phylogeographical (SAMOVAG) groups

	PMG				SAMOVAG			
Genetic structure measure	d.f.	Sum of squares	Variance components	Percentage of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	2	1391.480	17.1698	69.50***	9	1654.374	18.14665	76.16***
Among populations within groups	59	710.889	5.1331	21.51***	51	437.673	3.45975	14.52***
Within populations	70	155.464	2.2209	8.99***	70	155.464	2.22092	9.32***

***$P < 0.001$.

Coinciding with these studies, the *O. phyllotis* phylogeographical patterns are strongly associated with the geology of the region.

The Chortis Block, in southern Central America, is located between the Motagua–Polochic–Jocotán fault system (MPJ) and the Hess Escarpment (HE) (Fig. 4); its central area (Central Chortis) has been shaped by a complex of grabens and highlands resulting from diverse plate subduction events from the Cretaceous (Rogers, Kárason & Van der Hilst, 2002; Ortega-Gutiérrez *et al.*, 2007). Some mammalian fossil deposits in Panama (16 Mya) and Honduras (9–6 Mya) (Webb & Perrigo, 1984; Retallack & Kirby, 2007) demonstrate that fauna arrived from North America to this region by crossing the MPJ fault system during the Miocene, suggesting the existence of a suitable corridor between Central and North America (Whitmore & Stewart, 1965). Despite the fact that no broad highway had opened between 9 and 3.6 Mya, palaeontological studies suggest a Miocene–Pliocene diversification of rodents in North America and posterior dispersal of multiple lineages from the north to the south, recognized as a pre-GABI event (Jacobs & Lindsay, 1984; Coates *et al.*, 2004).

Ototylomys phyllotis is considered to be a member of the tribe Tylomyini from the Neotomine–Peromyscine complex, a monophyletic group of controversial origin (Bradley *et al.*, 2004) and with most of its current species widespread in North America and/or Central America. The origin of Tylomyini has been estimated at 8.6 ± 2.1 Mya (Engel *et al.*, 1998), although the tribe has also been placed as a subfamily that diverged from the Sigmodontinae at ~16.2 Mya (Steppan *et al.*, 2004). Notwithstanding the dating incongruence for this tribe/subfamily, the Tylomyini origin and posterior migration and radiation have been suggested to occur from North America to the south 6 Mya (Woodburne, 2010). Initial connections around 3.6–3.1 Mya allowed further movements between North America and South America, until the main interchange began about 3.0–2.6 Mya when the Panamanian land bridge was fully formed (Webb, 2006; Woodburne, 2010). At some stage in the pre-GABI event, the ancestor of *Ototylomys* should have arrived to Central America during the dispersal wave of northern rodents. The latter is in agreement with molecular clock estimates of divergence times among murid rodents that date diversification events before the GABI (Webb, 2006). Indeed, our results identify a pre-GABI origin of *O. phyllotis*, before 3.35 Mya, and its initial occurrence in Central America on a region near the centre of its current distributional range (Honduras/El Salvador), from where it later dispersed and expanded its range (Fig. 2). In addition, the phylogenetic topology obtained for *O. phyllotis* shows

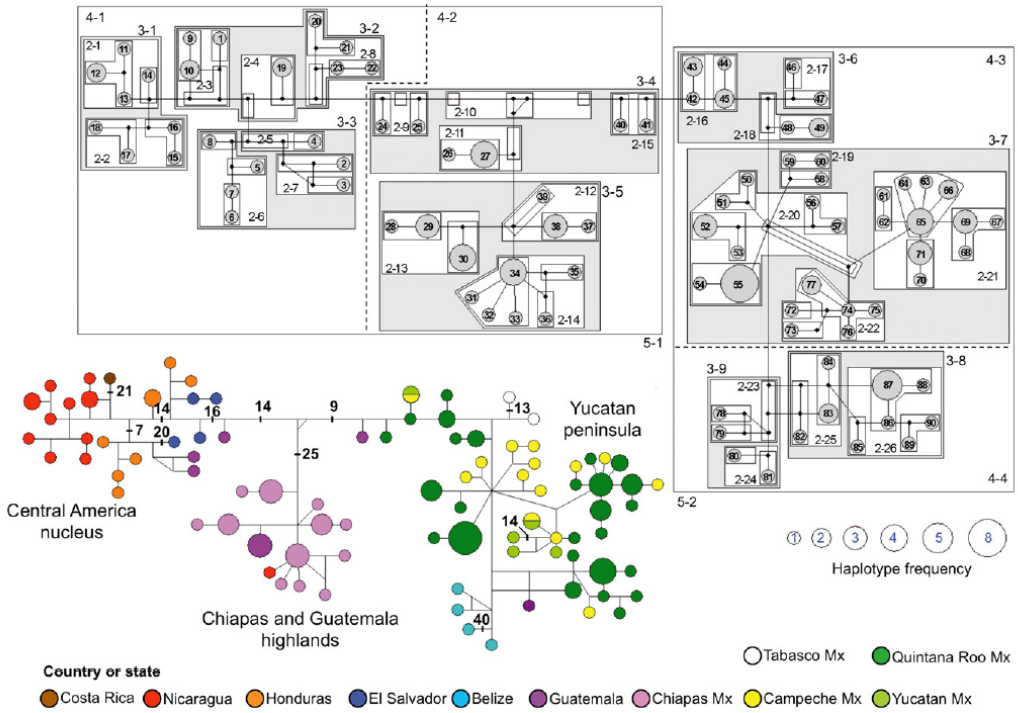

Figure 3. Nested Clade Phylogenetic Analysis (NCPA) and minimum spanning network (MSN) using the median-joining method for *O. phyllotis* cytochrome *b* haplotypes. On the NCPA, black and blue numbers indicate haplotype codes and haplotype frequencies, respectively. Nested clade level is shown by dashed numbers. The three phylogenetic major groups (PMG) are shown on the MSN: Yucatan Peninsula (YP), Chiapas–Guatemala Highlands (CGH), and Central America Nucleus (CAN). Black numbers along lines on the MSN indicate mutational steps.

the Central American haplotypes (Central America Nucleus phylogroup) as an ancient group and the northern haplotypes (Yucatan Peninsula) as a more recent one (Figs 1, 2). A Central American origin and posterior diversification has been documented for other taxa, such as the bird genus *Trogon* (DaCosta & Klicka, 2008). Our results also show that the MPJ fault in fact acted as a strong geographical barrier for *O. phyllotis* during the Pliocene, as indicated by the divergence and genetic distance values between the CAN and GCH phylogroups. Our divergence time estimates show that approximately 500 000 years were needed for populations from Guatemala to traverse the MPJ via Chiapas highlands, and there is no evidence within the phylogenetic tree of an interchange between populations from both sides of the fault system.

Ototylomys phyllotis diversification during the GABI

Climate and topography were key factors for the biota that dispersed and migrated during the GABI (Woodburne, 2010), where vegetation communities established in Central America prior to the arrival of fauna (Cody *et al.*, 2010). It is likely that *O. phyllotis* dispersal and diversification events must have been associated with the warm climate and tropical-like vegetation during some stages of the GABI. Woodburne (2010) describes the GABI migration patterns for different taxa along Central America as a set of pulses that happened in concert with climatic and geological events, supporting this region as a centre of cladogenesis for diverse mammal species. Some of these pulses agree with the divergence and dispersal events suggested by our phylogenetic, phylogeo-

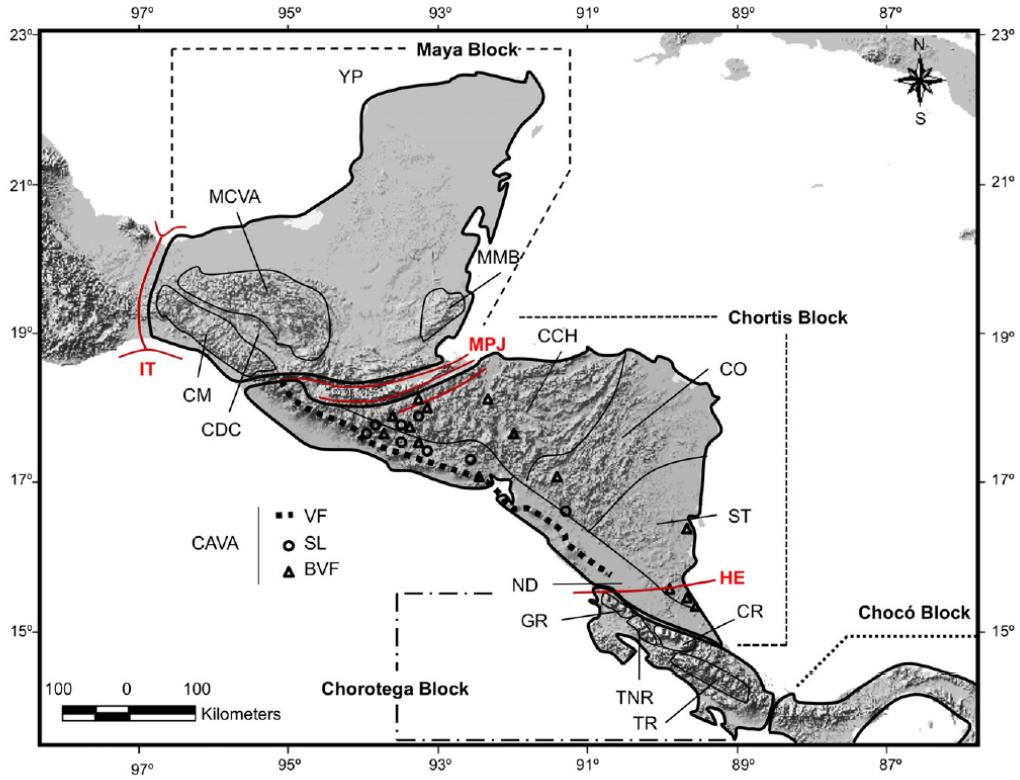

Figure 4. Historical geology of Central America, which includes four main geological features from north to south: Maya, Chortis, Chorotega, and Chocó blocks. These blocks are separated by fault breaks: Isthmus of Tehuantepec (IT), Motagua–Polochic–Jocotán fault system (MPJ), and Hess Escarpment (HE). Abbreviations: YP, Yucatan peninsula; MCVA, modern Chiapas volcanic arc; CM, Chiapas massif; MMB, Maya mountains of Belize; CDC, central depression of Chiapas; CCH, Central Chortis; CO, Chortis Oriental; ST, Siuna Terrain; CAVA, Central America Volcanic Arc; VF, Volcanic Front; SL, Secondary Line; BVF, behind the Volcanic Front; ND, Nicaragua Depression; GR, Guanacaste Range; CR, Central Range; TNR, Tilarán Range; TR, Talamanca Range.

graphical, and demographic results for *O. phyllotis* (Fig. 2; Supporting Information, Tables S2, S3): GABI1 begins at 2.6–2.4 Mya, associated more with climatic conditions than geology and where the migration of savanna-adapted taxa coincided with a period of glaciations in the northern hemisphere. Fossil evidence shows that at this time Central America was an area of savannas with belts of rainforest and warm climate, a tropical environment that was similar to present-day habitat for *O. phyllotis* (Lawlor, 1969, 1982). During this period, *O. phyllotis* reached a north-western limit at the Chiapas highlands, on the Maya Block (Fig. 2, insert c). The latter is a mass of land to the north of the MPJ fault system, bounded on the west by the Isthmus of Tehuantepec (Fig. 4). A further dispersal to the north was probably limited by the dynamics of the Chiapas massif (CM), the recently collapsed Isthmus of Tehuantepec (7–3 Mya), and the central depression of Chiapas (CDC), together with volcanic activity and rivers that acted as barriers for other species (Cortés-Rodríguez *et al.*, 2008).

Our results date the diversification of the CGH lineages at approximately 2.27 Mya, during the uplift of the Modern Chiapas Volcanic Arc (MCVA) (Manea & Manea, 2005). They also indicate that Belize was later colonized from these populations, in which the phylogenetic tree shows a haplotype from Belize that diverged from Chiapas and Guatemala populations; this was a unique invasion event, which has been

documented for species from other groups, such as birds and reptiles (Cavers *et al.*, 2003; Mulcahy, Morrill & Mendelson, 2006). Subsequently, populations from Chiapas and Guatemala highlands were probably isolated and became highland 'islands' or refuges during cooler climates. In fact, different potential refuges have been proposed to exist during the cold Pleistocene, such as the mountains in south-eastern Mexico (Guevara-Chumacero *et al.*, 2010). The latter is supported by morphological differences shown by *O. phyllotis* individuals from highlands vs. lowlands: the morphologically largest members are found at high altitudes. Moreover, our results show that haplotypes from Chiapas and Guatemala form a well-supported group (Chiapas–Guatemala highlands), which is subdivided into two clades by significant allopatric fragmentation encompassing highland (clade 2-13; Fig. 3) and lowland haplotypes (clade 3-4). The GABI1 pulse was followed by an apparent migration gap from 2.4 to 1.8 Mya, accompanied by dramatic decreases in the relative abundance of taxa as a result of environmental changes (Woodburne, 2010). A divergence gap can be appreciated for *O. phyllotis* during this period (2.27–1.82 Mya; Fig. 2), when environmental conditions became wetter and tropical systems developed from Honduras to Mexico.

The diversification of *O. phyllotis* in central and northern areas of the Chortis Block was followed by several dispersal events from north to south and also coincided with the arrival of *O. phyllotis* to Belize lowlands (Fig. 2, insert d). The latter coincides with GABI2 (1.8–0.7 Mya), during which climate cooling increased savanna habitats and a significant reduction of sea level exposed coastal plains. The Chortis Block includes the Central American Volcanic Arc (CAVA; Fig. 4), a highly active volcanic chain that originated from different activity stages of the Middle American trench. Historically, volcanic activity levels widthwise along the Chortis Block decrease from south-east to north-west, which can be recognized as precise volcanic lines (Volcanic Front, Secondary line, and Behind the Volcanic Front; Fig. 4) (Rogers, Kárason & Van der Hilst, 2002; Ortega-Gutiérrez *et al.*, 2007). This particular sequence of geological events resulted in restricted gene flow and isolation-by-distance pattern for *O. phyllotis* CAN haplotypes. In addition, the eastern (Chortis Oriental and Siuna Terrain) and south-eastern region of the Chortis Block (Fig. 4) were steadily exposed, an area also gradually colonized by *O. phyllotis* over a period of 600 000 years (1.14–0.68 Mya; Fig. 2, insert e).

In the south, the Nicaragua Depression (ND) and Hess Escarpment (HE) lines on the Chorotega Block (Fig. 4) are geological barriers that influenced migration and diversification events towards the south of Central America (Crawford & Smith, 2005), and that also affected *O. phyllotis* phylogeographical patterns and its migration towards Costa Rica. Costa Rica was probably inaccessible to *O. phyllotis* because the lowlands of the ND were flooded (Harmon, 2005), which is known to have acted as an important barrier for diverse taxa (Arbeláez-Cortés *et al.*, 2010, and references therein). Our results indicate an estimated time of divergence for the split between Costa Rica and Nicaragua populations approximately 1.85 Mya (Fig. 2, insert d), a migration that was possible because of the drying of the ND. In addition, results show a pattern of restricted gene flow with isolation by distance between these populations, with a 4.57–2.67% divergence, which can be associated with volcanic activity and ecological or climatic barriers during this period that limited gene flow between populations from both sides of the ND and HE lines. Costa Rica is the current southernmost distributional limit of *O. phyllotis*. During the Pleistocene, further southern localities were unattainable as a result of different factors that limited migration of species southwards: the geological components from the Guanacaste, Central, Tilarán, and Talamanca ranges (Fig. 4), with high volcanic activity and uplift processes, could have limited the migration of *O. phyllotis* to southern areas. Climate changes have played an important role in the history of Central America during the last million years, resulting in multiple changes in vegetation structure that would have affected herbivores such as *O. phyllotis*. According to Graham (1988), southern Central America vegetation consisted of mangroves, with ferns and palms and adjacent well-drained slopes (versions of tropical and premontane wet and moist forests), whereas Panama had forested and woodland habitats (MacFadden, 2006); this vegetation cover would probably have limited migration of *O. phyllotis*.

The colonization of the Yucatan peninsula matched the third pulse (GABI3; *c.* 0.8 Mya). Our results suggest that Belize was the unique source of individuals for the Yucatan peninsula colonization, which can be seen in the phylogenetic tree, where Belize haplotypes are found in the Yucatan Peninsula major clade. The latter suggests that some genetic flow happened during the last 500 000 years, and that Belize was a source of individuals that persisted in refugia on the Maya mountains. Strecker *et al.* (2004), in a phylogeographical study of the fish *Astyanax*, describe that at least two invasions of surface *Astyanax* from Belize to Yucatan occurred during this time. Flora and fauna arrivals to the peninsula were gradual and present-day faunas of the northern part of the peninsula are of recent origin. Indeed, most vertebrate faunas of northern Yucatan are subsets of the fauna of the base of the peninsula, which indicates that faunas of the

northern part of the peninsula originated by dispersal of species from the south (Parkinson, Zamudio & Greene, 2000; Vázquez-Domínguez & Arita, 2010). Our phylogenetic results, in which the northern haplotypes (Yucatan Peninsula group) are observed as a derived, more recent lineage, support that *O. phyllotis* arrived to Mexico from the south (Central America).

The last pulse, GABI4 (0.125 Mya), coincides with a period of extremely rapid radiation for *O. phyllotis* throughout its entire distributional range, but which is more evident in the Yucatan peninsula (Figs 1, 2). The observed pattern of range expansion and the signal of population expansion detected for the Yucatan Peninsula group support the latter. This pulse is characterized by a virtual absence of northward dispersal and a strong diversity of savanna-adapted taxa moving southward, probably related to overall cooler conditions (Woodburne, 2010). Dispersal from northern Central America and posterior diversification along the Yucatan peninsula and southern Mexico (Chiapas, Oaxaca, Tabasco) is a pattern documented in other taxa, like the cantil pitviper genus *Agkistrodon* (Parkinson *et al.*, 2000). Likewise, Strecker *et al.* (2004) found a pattern of range expansion in combination with restricted gene flow with isolation by distance and long-distance colonization in this region for *Astyanax*. We are aware that our results are based on one gene and that the standard deviations of the divergence estimates overlap for some events and that this uncertainty should be considered. It has been recognized that confidence intervals of estimated divergence times obtained in molecular dating analyses are usually large, and that the problem is probably more pronounced when no calibration point is present within the group under study, for which age estimates of individual nodes are important for making inferences of historical biogeography (Smedmark, Eriksson & Bremer, 2010). Accordingly, we consider that confidence in our results is supported by: the use of several precise fossil estimates, individual-node age estimations, agreement between the evolutionary processes identified with NCPA, and the phylogenetic relationships and geographical distribution of haplotypes, concordant genetic distance, and demographic analyses.

Potential *O. phyllotis* complex of species

Although evaluating the taxonomy of *O. phyllotis* is beyond the scope of this study, our results, based on the analysis of cytochrome *b*, merit a brief discussion. According to Baker & Bradley (2006), *O. phyllotis* has at least three potential unrecognized species. Our results show three major lineages (PMG) with markedly high genetic divergence values (6–7%) (see also Mouton *et al.*, 2012). They also reveal that *O. phyllotis* has strikingly high divergence in relation to its sister taxa (~16% with *Tylomys* and ~24% with *Nyctomys*), as compared with values reported by Baker & Bradley (2006) for other rodents and mammals (average 4.7%). Templeton (2001) reviewed the use of phylogeographical analyses of gene trees to test species status, as for example the study of Mulcahy (2008) in which he recognizes six species of the colubrid *Hypsiglena* based on phylogeographical results. Moreover, our genetic results are partially coincident with Lawlor's (1969) recognized subspecies of *O. phyllotis*, which he defined based on body size and pelage colour. Accordingly, we suggest that *O. phyllotis* may comprise four differentiated lineages, based on the following combined information: (1) major phylogenetic groups strongly supported (83–99%) by Bayesian inference analysis, (2) concordant Nested Clade Phylogenetic Analysis grouping results, (3) genetic divergence percentages, and (4) morphologically distinctive features (Lawlor, 1969). These groups are (from present to past): (1) Yucatan peninsula, the most recent lineages belonging to the subspecies *O. p. phyllotis*, and encompassing individuals with smaller sizes; (2) Chiapas and Guatemala highland lineages coinciding with the subspecies *O. p. connectens*, individuals at the top of the altitudinal range and with remarkable larger sizes; (3) a Costa Rica lineage belonging to *O. p. australis*, but which needs further phylogenetic evaluation; and (4) a Central America nucleus lineage that, although coinciding geographically with the *O. p. phyllotis* designation, nonetheless shows high genetic divergence in relation to *O. p. phyllotis* from the Yucatan peninsula (5.4%).

ACKNOWLEDGEMENTS

We thank Luisa I. Falcón, Juan C. Gutiérrez-García, and José M. Macías-Preza for their help with phylogenetic analyses. We are immensely grateful to the following collections and their curators for tissue loans: Angelo State Natural History Collection; Colección Nacional de Mamíferos, Instituto de Biolgía (UNAM); El Colegio de la Frontera Sur; The Field Museum of Natural History; Louisiana State University Museum of Natural Science; Museo de Historia Natural de la Universidad de San Carlos, Guatemala; Museo de Zoología Alfonso L. Herrera, Facultad de Ciencias (UNAM); Smithsonian Institute; Texas Tech University Museum; Burke Museum of the University of Washington. Sergio Pérez Consuegra, Camila González, and Robert D. Bradley are thanked for sharing samples and data. We are grateful for constructive comments from Jim Kenagy and three anonymous reviewers that greatly improved the manuscript. Daniel Piñero, Gerardo Rodríguez Tapia, and Enrique

Martínez Meyer provided technical advice. We thank the members of the Macroecology and the Genetics and Ecology laboratories for their help in fieldwork and fruitful discussions, and Jorge Salazar Bravo for hosting a research visit for T.A.G.G. E.V.D. acknowledges financial support from the Instituto de Ecología, UNAM, and a grant from Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (grants IN215205-2, IX238004, and IN219707). T.A.G.G. received scholarships from Consejo Nacional de Ciencia y Tecnología (Conacyt 203129 and 175434); the present work fulfils the requirement for T.A.G.G.'s PhD degree. Scientific collector permit to E.V.D.: Semarnat-FAUT-0168. We would like to dedicate this work to the memory of Timothy E. Lawlor.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Map of *Ototylomys phyllotis* area of study (yellow points represent sampling localities). Colours indicate phylogenetic major groups (PMG; see results) as follows: green, Yucatan Peninsula (YP); purple, Chiapas–Guatemala Highlands (CGH); blue, Central America Nucleus (CAN). Polygons indicate the phylogeographical groups (SAMOVAG; see Results): Costa Rica (group 1), El Salvador (2), Belize (3), Guatemala highlands (4 and 7), Honduras and Nicaragua (5), El Salvador and Honduras (6), Chiapas highlands and Nicaragua (8), Honduras and Guatemala (9), and all Yucatan, Campeche and Tabasco haplotypes, together with two from northern Belize and Guatemala (10). Dotted lines indicate plausible barriers.

Figure S2. Mismatch distribution results estimated for expected frequencies under a sudden expansion model for SAMOVAG groups: (a) group 5, (b) 8, (c) 9, (d) 10, and phylogenetic major groups: (e) Central America Nucleus, (f) Chiapas–Guatemala Highlands, and (g) Yucatan Peninsula.

Table S1. List of *Ototylomys phyllotis* individuals used in this study. Catalog number in the first column corresponds to the sample ID in the last column. GenBank accession numbers are indicated in the table and in the text.

Table S2. Net nucleotide divergence (*Da* ± SD; above diagonal) and p-uncorrected distance (below diagonal) between *O. phyllotis* phylogeographical groups (SAMOVAG; see Table 2). Bold numbers and gray shading indicate highest and lowest divergence values, respectively.

Table S3. Inference of the phylogeographic structure obtained with the Nested Clade Phylogenetic Analysis and following the latest inference key (January 2011; http://darwin.uvigo.es/software/geodis.html) for interpretation of evolutionary processes.

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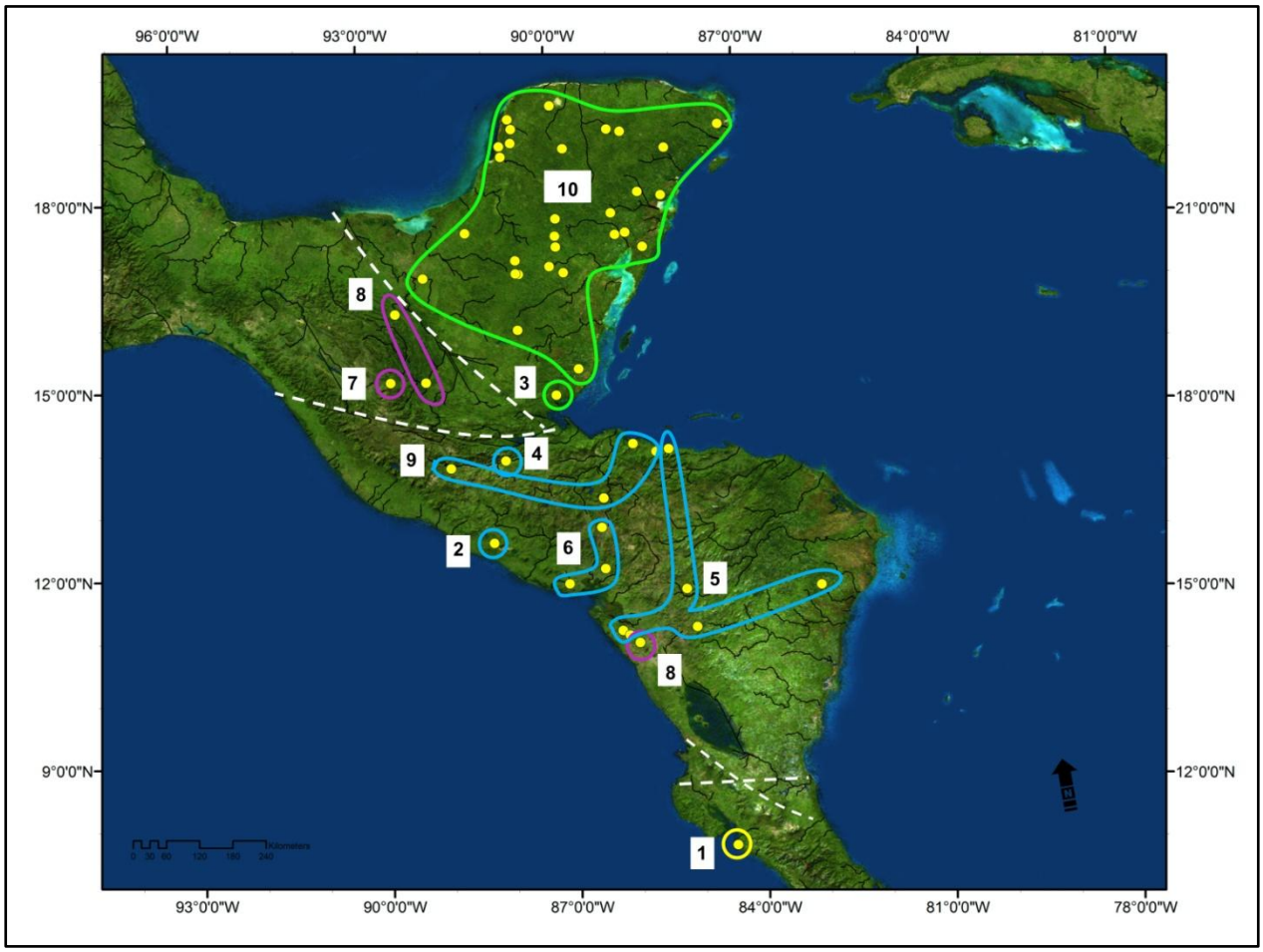
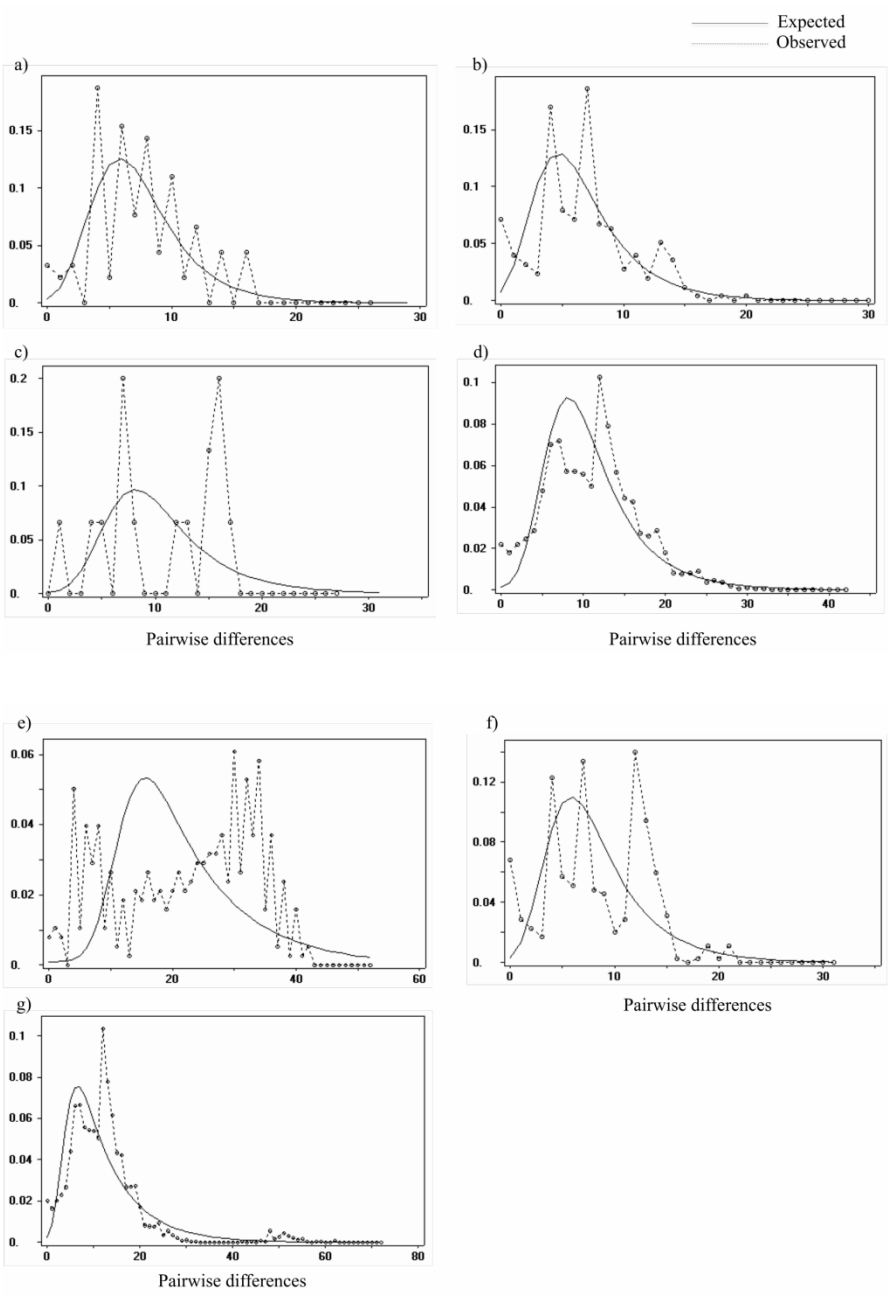
Supporting Information Figure 1



Supporting Information Figure 2



Supplementary material Table S1. List of *Ototylomys phyllotis* individuals used in this study. Catalog number in the first column corresponds to the sample ID in the last column. GenBank accession numbers are indicated in the table and in the text.

Collection / Catalog number	N	Country/ State	Locality (abbreviation)	Coords N	Coords W	Sample ID(haplotype code)
FMNH / 128043, 128045, 128048	3	Belize	Toledo (TB)	16.292008	88.815056	TB1(H80), TB2(H81), TB3(H78)
SI / 583075	1		Stann Creek (SCB)	16.773611	88.532500	SCB(H79)
LSUMNS / 1832	1	Costa Rica	Puntarenas (CR)	9.629522	84.640733	CR(H1)
TTU / 64119, 64121	2	El Salvador	La Libertad (LLS)	13.750000	89.383333	LLS1(H4), LLS2(H24)
TTU / 64128, 64129	2		San Miguel (SMS)	13.316670	88.066670	SMS1(H22), SMS2(H23)
MHNG / 1341, 1343	2	Guatemala	Planta San Miguel (SMG)	14.816972	90.286694	SMG1(H2), SMG2(H3)
SI / 565152, 565482	2		El Peten (EPG)	17.216628	89.616547	EPG1(H40), EPG2(H82)
SI / 565162	1		Zacapa (ZG)	15.097350	89.433269	ZG(H25)
MHNG / 223319, 223320, 223321, 223322	4		Yalambojoch (YAG)	16.007650	91.498900	YAG1(H30), YAG2(H30), YAG3(H30), YAG4(H30)
TTU / 101714, 101715	2	Honduras	Atlantida (AH)	15.733914	87.455753	AH1(H6), AH2(H5)
GenBank / DK179814	1		Atlantida (AH)	15.675428	87.062314	AH3(H7)
TTU / 136064	1		Comayagua (CYH)	14.783333	87.766667	CYH(H8)
TTU / 136829, 136881	2		Colon (CH)	15.754950	86.872308	CH1(H19), CH2(H19)
SI / 565166	1		La Paz (LPH)	14.308438	87.714525	LPH(H20)
SI / 565167	1		Valle del Cauca (VCH)	13.660963	87.533836	VCH(H21)
TTU / 93601, 72923, 113512, 113534	4	Nicaragua	Selva Negra (SNN)	12.997222	85.906944	SNN1(H10), SNN2(H12), SNN3(H9), SNN4(H10)
TTU / 113520, 113598, 113603	3		San Cristobal (SCN)	12.722500	87.083333	SCN1(H14), SCN2(H12), SCN3(H11)
TTU / 113568	1		Bella Vista (BVN)	12.669444	86.954166	BVN(H13)
TTU / 121413, 121419	2		Nueva Segovia (NSN)	13.573611	86.180556	NSN1(H18), NSN2(H17)
TTU / 121426, 121434	2		Atlantico N (ANN)	14.027253	84.037881	ANN1(H15), ANN2(H16)
TTU / 93683	1		San Jacinto (SJN)	12.580556	86.776389	SJN(H31)
MZALH / 8783, 8789	2	Mexico/Campeche	Tachich (TACM)	20.175000	90.268300	TACM1(H56), TACM2(H57)
MZALH / 8787, 8865	2		Hampolol (HCM)	19.926289	90.387344	HCM1(H50), HCM2(H59)
GenBank / AY009789	1		El Remate (ERCM)	20.546944	90.384167	ERCM(H66)
MZALH / 8801, 8797	2		Los Petenes (LPCM)	20.090758	90.440825	LPCM1(H58), LPCM2(H51)
ASNHC / 7242	1		Escarcega (EGCM)	18.606458	90.737803	EGCM(H77)
ASNHC / 7238, 7236, 32806, 7243	4		Tancuche (TCM)	20.400000	90.716667	TCM1(H60), TCM2(H68), TCM3(H42), TCM4(H76)
IE / OpJ4, OpJ6, OpJ7	3		Calakmul (CKCM)	18.315944	89.856500	CKCM1(H85), CKCM2(H61),

						CKCM3(H43)
ECOSUR / 467	1	Mexico/Chiapas	Ocosingo(OCHM)	17.113333	91.622222	OCHM(H39)
UWBM / 778, 779, 780, 781, 782, 784, 785, 786, 787, 789, 791, 792, 793, 794, 795, 796, 799, 800, 801, 802, 803	21		Chajul (CHIM)	16.116111	90.933138	CHIM1(H38), CHIM2(H36), CHIM3(H32), CHIM4(H27), CHIM5(H33), CHIM6(H28), CHIM7(H38), CHIM8(H34), CHIM9(H26), CHIM10(H27), CHIM11(H27), CHIM12(H35), CHIM13(H34), CHIM14(H34), CHIM15(H34), CHIM16(H29), CHIM17(H29), CHIM18(H29), CHIM19(H27), CHIM20(H38), CHIM21(H37)
IE / 12, 14, 20, 28, 67	5	Mexico/Quintana Roo	Ejido Señor (ESQM)	19.768883	88.103314	ESQM1(H49), ESQM2(H55), ESQM3(H48), ESQM4(H74), ESQM5(H55)
IE / 68, 69, 75, 91	4		Ejido Naranjal (ENQM)	19.352900	88.465340	ENQM1(H55), ENQM2(H55), ENQM3(H55), ENQM4(H49)
IE / SK4, SK6	2		Los Lirios 1 (SKQM)	19.784722	87.720556	SKQM1(H55), SKQM2(H55)
IE / Op3, Op4	2		Tres Garantías1 (TGAM)	18.266667	89.043889	TGAM1(H83), TGAM2(H45)
IE / Op9, Op10, Op12, Op15	4		Chemax Cobá (CXQM)	20.551389	87.805000	CXQM1(H52), CXQM2(H54), CXQM3(H52), CXQM4(H52)
IE / Op21, Op22, Op23, Op26, Op27, Op28, Op29, Op31, Op32, Op33, Op34, Op36	12		Mahahual (MQM)	18.908056	87.864722	MQM1(H63), MQM2(H71), MQM3(H65), MQM4(H71), MQM5(H71), MQM6(H65), MQM7(H70), MQM8(H64), MQM9(H62), MQM10(H65), MQM11(H65), MQM12(H63)
IE / Op39, Op42, Op50, Op54	4		Ávila Camacho (ACQM)	19.022222	88.337500	ACQM1(H69), ACQM2(H69), ACQM3(H55), ACQM4(H69)
IE / Op55, Op56, Op57, Op58	4		Tres Garantías2 (TGBM)	18.266111	89.049444	TGBM1(H45), TGBM2(H87), TGBM3(H88), TGBM4(H83)
IE / C1, C2	2		Nvo Becal (BEQM)	18.652120	89.250390	BEQM1(84), BEQM2(41)
IE / C3, C4, C5, C6, C7, C8, C9	7		Mancolona (MLQM)	18.821171	89.293690	MLQM1(H87), MLQM2(H87), MLQM3(H90), MLQM4(H87), MLQM5(H44), MLQM6(H89), MLQM7(H86)
ASNHC / 7244	1		X-Kanha (XQRM)	19.100000	89.333333	XQRM(H87)
ECOSUR / 510	1		Lázaro Cárdenas (LCQM)	21.081944	87.015833	LCQM(H53)
GenBank / AY009788	1		Noh Bec (NBQM)	19.083333	88.183333	NBQM(H67)
CNMA / 33076, 33077	2	Mexico/Tabasco	Acalan (TM)	17.766111	91.281944	TM1(H46), TM2(H47)
ASNHC / 7274, 7273	2	Mexico/Yucatan	Uman (UYM)	20.888889	89.750000	UYM1(H42), UYM2(H77)

CNMA / 18967	1	Oxkutzcab (LTYM)	20.238889	89.418333	LTYM(H72)
CNMA / 30784, IE / OpJ2	2	Chichen Itza (CIYM)	20.680278	88.554722	CIYM1(H75), CIYM2(H73)

Information includes museum collection, number of samples (N), sample's location (country/state and locality), geographic coordinates in decimals and sample code with corresponding haplotypes. Collection code: ASNHC= Angelo State Natural History Collection, CNMA= Colección Nacional de Mamíferos (UNAM), ECOSUR= El Colegio de la Frontera Sur, FMNH= Field Museum of Natural History, IE= Instituto de Ecología (UNAM), LSUMNS= Louisiana State University Museum of Natural Science, MHNG= Museo de Historia Natural de la Universidad de San Carlos, Guatemala, MZALH= Museo de Zoología Alfonso L. Herrera (UNAM), SI= Smithsonian, TTU= Texas Tech University Museum and UWBM = University of Washington Burke Museum.

Supplementary material Table S2. Net nucleotide divergence (*Da* ± standard deviation; above diagonal) and p-uncorrected distance (below diagonal) between *O. phyllotis* phylogeographic groups (SAMOVAG; see table 2). Bold numbers and gray shading indicate highest and lowest divergence values, respectively.

Groups	1	2	3	4	5	6	7	8	9	10
1	-	0.0366 (0.0283)	0.0597 (0.0388)	0.0469 (0.0)	0.0314 (0.0095)	0.0439 (0.0205)	**0.0722 (0.0313)**	0.0614 (0.0211)	0.0330 (0.0150)	**0.0695 (0.0089)**
2	0.0538 (0.0269)	-	0.0334 (0.0339)	0.0214 (0.0215)	0.0235 (0.0136)	0.0187 (0.0189)	0.0471 (0.0270)	0.0391 (0.0178)	0.0135 (0.0157)	0.0413 (0.0109)
3	**0.0803 (0.0379)**	0.0713 (0.0320)	-	0.0416 (0.0308)	0.0537 (0.0178)	0.0509 (0.0274)	0.0474 (0.0256)	0.0447 (0.0180)	0.0463 (0.023)	0.0075 (0.0148)
4	0.0469 (0.0)	0.0386 (0.0196)	0.0623 (0.0298)	-	0.0447 (0.0129)	0.0487 (0.0226)	**0.0679 (0.0294)**	0.0615 (0.0211)	0.0369 (0.0165)	0.0451 (0.0061)
5	0.0362 (0.0095)	0.0456 (0.0104)	**0.0792 (0.0155)**	0.0496 (0.0129)	-	0.0317 (0.0071)	0.0656 (0.0123)	0.0567 (0.0073)	0.0156 (0.0045)	**0.0695 (0.0039)**
6	0.0472 (0.0205)	0.0394 (0.0169)	0.0749 (0.0266)	0.0521 (0.0226)	0.0399 (0.0070)	-	0.0621 (0.0201)	0.0558 (0.0118)	0.0337 (0.0115)	0.0565 (0.0055)
7	0.0722 (0.0313)	0.0644 (0.0256)	0.0680 (0.0247)	0.0679 (0.0294)	0.0705 (0.0123)	0.0654 (0.02010)	-	0.0133 (0.0043)	0.0593 (0.0171)	0.0534 (0.0047)
8	0.0694) (0.0145)	0.0637 (0.0170)	0.0698 (0.0135)	0.0658 (0.0137)	0.0660 (0.0054)	0.0637 (0.0089)	0.0176 (0.0026)	-	0.0525 (0.0099)	0.0499 (0.0034)
9	0.0400 (0.0150)	0.0377 (0.0131)	0.0739 (0.0435)	0.0439 (0.0164)	0.0274 (0.0044)	0.0440 (0.0114)	0.0663 (0.0170)	0.0638 (0.0075)	-	0.0636 (0.0052)
10	**0.0766 (0.0089)**	0.0657 (0.0066)	0.0354 (0.0145)	0.0523 (0.0061)	**0.0816 (0.0038)**	0.0671 (0.0055)	0.0605 (0.0047)	0.0614 (0.0023)	**0.0777 (0.0051)**	-

Supplementary material Table S3. Inference of the phylogeographic structure obtained with the Nested Clade Phylogenetic Analysis and following the latest inference key (January 2011; http://darwin.uvigo.es/software/geodis.html) for interpretation of evolutionary processes.

Clade	Geographic area	Demographic event inferred[a]
2-13	Chiapas and Guatemala highlands	Allopatric fragmentation
2-20	Yucatan peninsula	Restricted gene flow with isolation by distance
2-21	Yucatan peninsula	Allopatric fragmentation
3-1	Nicaragua	Allopatric fragmentation
3-4	Chiapas, Guatemala and southern Yucatan peninsula	Allopatric fragmentation
3-7	Yucatan peninsula	Restricted gene flow with isolation by distance
4-1	Costa Rica, Nicaragua, Honduras, and southern Guatemala	Restricted gene flow with isolation by distance
4-3	Yucatan peninsula	Range expansion/colonization or restricted dispersal/gene flow
4-4	Belice, Guatemala, and southern Yucatan peninsula	Allopatric fragmentation
5-1	Major south Central America clade	Allopatric fragmentation
5-2	Major north Central America clade	Allopatric fragmentation
Total Cladogram		Restricted gene flow with isolation by distance

[a] Interpretation of results from Nested Clade Phylogenetic Analysis based on Templeton's inference key (January 2011)

Capítulo 4

Filogeografía comparada de *Oryzomys couesi* y *Ototylomys phyllotis*

I. INTRODUCCIÓN

América Central es una región con una historia geológica y climática compleja, la cual ha generado patrones de estructuración genética concordantes en poblaciones de especies que se distribuyen parcial o totalmente dentro de ésta. La historia geológica de América Central implica la conexión de dos masas terrestres o regiones (Norteamérica y Sudamérica), a través del origen diferencial de cadenas montañosas y la continuidad de tierras bajas, lo cual permitió el intercambio de la biota residente en ambas regiones. La posterior distribución de especies fue influenciada también por la formación de complejos volcánicos al sur de México y la emersión de la península de Yucatán. Por otro lado, la historia climática de dichas regiones durante su conformación geológica hasta la actualidad incluye fluctuaciones en variables ambientales (e.g. precipitación y temperatura), que afectaron directamente la colonización, la diversificación y el establecimiento de especies y comunidades. Existe controversia sobre la respuesta de los organismos presentes en América Central a dichos cambios, a nivel individual como de comunidades, durante el periodo que va del Plioceno (5.332 ± 0.005 a 2.588 millones de años; USGS, 2011) hasta el presente (Hershkovitz, 1969; Halfter, 2002; Arita y Vázquez-Domínguez, 2003; Orellana *et al.*, 2003; Hasbún *et al.*, 2005; Wüster *et al.*, 2005).

El estudio de los patrones de estructuración genética de las especies codistribuidas en América Central ha permitido evaluar procesos como la dinámica de dispersión, la diferenciación geográfica y la formación de especies nuevas y, sin duda podría facilitar la descripción de fenómenos geográficos, geológicos y/o climáticos asociados a esos patrones (Bermingham y Martin, 1998). Así, en la denominada filogeografía comparada los estudios interespecíficos permiten analizar las características ecológicas compartidas y la cronología de eventos climáticos y geológicos a diferentes escalas espaciales y temporales. En la filogeografía comparada se estudian los patrones filogeográficos de especies codistribuidas y su concordancia (Gutiérrez-García y Vázquez-Domínguez, 2011), y es cada vez más frecuente que este enfoque se aplique en los estudios de taxones de América Central. De acuerdo con la filogeografía comparada, un conjunto de eventos históricos vicariantes o de dispersión estructurará de forma similar a especies que han estado codistribuidas o en asociación (e.g parásito-huésped) por periodos largos, dado que éstas evolucionarían en respuesta a eventos históricos, ecológicos y ambientales similares. Por el contrario, si hay incongruencia en los patrones filogeográficos entre las

especies codistribuidas, ello puede tener diversas explicaciones (e.g. diferentes historias de vida) (Churikov y Gharrett, 2002; Gutiérrez-García y Vázquez-Domínguez, 2011). Por otro lado, los estudios sobre la respuesta de taxones codistribuidos a un mismo evento geológico o climático con frecuencia muestran que pueden responder de manera independiente, por lo que las áreas con historia compleja como América Central, representan un modelo ideal para probar hipótesis sobre las respuestas concertadas, independientes y/o la combinación de ambas (Sullivan *et al.*, 2000).

Dos especies codistribuidas en América Central son los roedores *Oryzomys couesi* y *Ototylomys phyllotis*. Sus distribuciones van desde el norte de México hasta Colombia y del Istmo de Tehuantepec a Costa Rica, respectivamente. *O. phyllotis* es actualmente la única especie dentro del género *Ototylomys*; es considerada semiarborícola pero su preferencia por árboles, zonas bajas de enredaderas, rocas o ramales, varía de acuerdo con el tipo de hábitat; es de tamaño mediano (34-84 g) y se encuentra desde el nivel del mar hasta los 1900 m (Lawlor, 1982). *O. couesi* es de hábitos terrestres y semiacuáticos y habita en zonas de humedal, manglares, marismas, bordes de selva inundable e incluso selva semidecidua; es de tamaño mediano (43-82 g) y se encuentra desde tierras bajas hasta 2000 m (Reid, 1997). Ambas especies se han incluido en estudios sobre filogenia y divergencia de otros géneros de roedores, e.g. *Peromyscus* y *Neotoma* de Bradley *et al.* (2007) y Matocq *et al.* (2007) respectivamente; pero la información sobre su ecología y genética es aún limitada. De *O. pyllotis* se conocen la diferenciación morfológica (Lawlor, 1969) y los patrones filogeográficos de sus poblaciones a lo largo de su distribución (Gutiérrez-García y Vázquez-Domínguez, 2012). En el caso de *O. couesi*, se sabe que existe flujo genético dentro de la península de Yucatán (Vega *et al.*, 2007), que hay diferenciación genética de sus poblaciones en el sureste mexicano (Vázquez-Domínguez *et al.*, 2009), que existe diferenciación morfológica en las poblaciones del noroeste (Carleton y Arroyo-Cabrales, 2009) y, recientemente se publicó un estudio de sistemática para conocer su ubicación filogenética dentro del complejo de *Oryzomys palustris* (Hanson *et al.*, 2010). Sin embargo, los patrones de origen y dispersión tanto de la especie como de sus poblaciones no han sido analizados desde la perspectiva filogeográfica.

De acuerdo con diferentes dataciones moleculares, se sabe que ambos roedores se originaron previamente al Gran Intercambio Biótico Americano que ocurrió entre 3.1 y 2.4 millones de años (Ma) (Webb 1991, 2006; Cody *et al.*, 2010; Gutiérrez-García y Vázquez-Domínguez, 2012). Las dos especies

han estado presentes en América Central durante distintos eventos climáticos (e.g. el último gran glacial que inició hace 2.4 Ma; Wüster *et al.*, 2005) y geológicos (e.g. la formación del arco volcánico moderno de Chiapas hace 3 Ma; Manea y Manea, 2005) que han generado patrones filogeográficos concordantes en otras especies de la región. Específicamente se conocen al menos cuatro barreras geológicas que han tenido una influencia marcada en la estructuración genética de las poblaciones de diferentes taxones de América Central: Istmo de Tehuantepec, Tierras altas de Chiapas, Sistema de fallas Motagua-Polochic-Jocotán y Depresión de Nicaragua (Gutiérrez-García y Vázquez Domínguez, 2013). Sin embargo, las especies tienen estrategias de historia de vida y habilidades de dispersión diferentes, por lo que sus respuestas a los diferentes eventos vicariantes y de dispersión podrían ser distintas.

Los objetivos de este capítulo son: 1) determinar los patrones filogeográficos de la rata arrocera *O. couesi* utilizando un marcador molecular mitocondrial, 2) comparar los patrones de distribución de la variación genética de *O. couesi* y *O. phyllotis*, dos especies de roedores codistribuidas en América Central, 3) identificar si existen barreras geológicas comunes asociadas con la estructura de la variación genética de las dos especies y 4) evaluar algunos factores ecológicos y su relación con la estructura genética a lo largo de la zona donde se codistribuyen las dos especies.

II. MATERIALES Y MÉTODOS

Muestreo y extracción de ADN de Oryzomys couesi

Se analizaron 201 secuencias de *O. couesi*, que incluyeron muestras de museo (125), de campo (29) y de GenBank (47) de 108 localidades a lo largo de su distribución (Tabla 1). Las muestras de campo constaron de fragmentos de tejido conservados en etanol al 97%, de 29 individuos que fueron capturados siguiendo los protocolos de ética (Sikes *et al.*, 2011) durante trabajo de campo que incluyó muestreos entre 2000 y 2008, como parte de un proyecto de fauna del sur de México (Vega *et al.*, 2007; Vázquez-Domínguez *et al.*, 2009). Las muestras de museo fueron donadas por diferentes colecciones biológicas y constaron de piel, hueso, hígado y riñón, almacenadas como tejido fresco o material deshidratado de curación. La extracción de ADN de muestras frescas y de museo se realizó mediante los kit comerciales AquaPure Genome KIT DNA (Biorad Laboratories), FujiFilm Quick gene DNA Tissue, DNA Easy Blood and Tissue (QIAgen Inc.) y QIAmp DNA Investigator KIT (QIAgen Inc.), siguiendo sus respectivos protocolos. La

cuantificación e integridad del ADN extraído se realizó mediante espectrofotometría y observación mediante UV de geles de agarosa al 1% teñidos con 0.5mg ml^{-1} de Bromuro de Etidio.

Amplificación y secuenciación

Se amplificó un fragmento del gen citocromo b de la región mitocondrial con diferentes combinaciones de primers universales MVZ04, MVZ05, MVZ16 y MVZ45, diseñados por Smith *et al.* (1992) y primers específicamente diseñados para *O. couesi* en el Ancient DNA Centre de McMaster University (Tabla 2). Las reacciones de PCR se hicieron en un volumen de 20 µl, incluyendo: 50-100 ng de ADN genómico, 0.2 µl de Taq polimerasa (Invitrogen), 0.8 µl de cada primer (10µM), 5 µl de dNTP mix (2mM), 1.2 µl de $MgCl_2$ (50mM) y 2 µl de buffer de reacción para PCR (200 mM Tris-HCL, pH8.4, 500 mM KCl). Las amplificaciones se realizaron en un termociclador PTC-100 Programmable Thermal Controller (MJ Research, Inc.) con las siguientes condiciones: 90 s a 92˚C, seguido por 39 ciclos de 15 s a 92˚C, alineamiento de 60 s a 50-55˚C y 70 s a 72˚C y una extensión final de 72˚C por 4 min; la temperatura de alineamiento varió dependiendo de la combinación de primers (Tabla 2). Todas las reacciones de PCR incluyeron controles negativos para detectar contaminación y algunas muestras al azar fueron amplificadas repetidamente para asegurar la reproducibilidad y correcta lectura de la secuenciación. Los productos de PCR fueron purificados utilizando el kit QIAquick PCR Purification (QIAgen Inc.). La secuenciación se realizó con los primers usados para la amplificación en un equipo ABI 3730xl DNA Analyzer. Las secuencias fueron alineadas y editadas manualmente con el programa BioEdit Sequence Alignment Editor v7.0.0 (Hall, 2005).

Análisis filogenéticos

Para la reconstrucción filogenética se utilizaron 201 secuencias de *O. couesi*. Además, se realizó un análisis filogenético en conjunto incluyendo las 132 secuencias de *O. phyllotis* del estudio filogeográfico de Gutiérrez-García y Vázquez-Domínguez (2012), con la finalidad de obtener un árbol del gen citocromo b, para seleccionar los filogrupos mayores y hacer el análisis comparado. Para la selección de los grupos externos se consideraron las especies reportadas consistentemente como basales para ambos géneros (Engel *et al.*, 1998; Michaux *et al.*, 2001; Jansa y Weksler, 2004; Steppan *et al.*, 2004). Las especies usadas como grupo externo para ambas especies (secuencias GenBank; Tabla 1) incluyeron siete géneros, cinco

pertenecientes a la familia Muridae y dos a las familias Sciuridae y Dipodidae. Los géneros fueron: *Gerbillurus* (AJ430557 y AJ430559), *Taterillus* (AJ430563 y AJ851263), *Mus* (AB033699), *Rattus* (AB033713), *Spalax* (AF155871), *Sciurus* (SNU10180 y FJ200744) y *Jaculus* (AJ416890). Asimismo, se incluyeron para *O. couesi* los géneros *Sigmodon* (AF425203), *Calomys* (EU5799473) y *Holochilus* (EU579496 y EU579497) (Weksler, 2006; Hanson *et al.*, 2010), mientras que para *O. phyllotis* fueron de los géneros *Nyctomys* (AY195801) y *Otonyctomys* (JQ183060) (Bradley *et al.*, 2004; Corley *et al.*, 2011).

Se estimaron los índices *D* de Tajima (Tajima, 1989) y *D* de Fu y Li (Fu y Li, 1993) para evaluar la neutralidad del fragmento mitocondrial utilizado para cada especie en conjunto y de forma separada para cada grupo filogenético obtenido (ver resultados). Se estimó el modelo de evolución de mejor ajuste a los datos para la reconstrucción fiogenética de acuerdo al Criterio de Información de Akaike (AIC), con el programa jMODELTEST v.0.1.1 (Guindon y Gascuel, 2003; Posada, 2008). Se hizo un análisis de máxima verosimilitud (MV) utilizando tanto el programa PhyML 3.0 (Guindon *et al.*, 2010) como su plataforma disponible en: http://www.atgc-montpellier.fr/phyml/. Para el análisis se utilizó como árbol de inicio un árbol basado en distancia (BIONJ), optimizando la topología y el largo de ramas considerando los parámetros de gamma y proporción de sitios invariantes calculados con jMODELTEST. El soporte estadístico calculado para la certeza de las ramas fue aLRT (Anisimova and Gascuel, 2006), interpretado usando el procedimiento no-paramétrico Shimodaira-Hasegawa. Las reconstrucciones filogenéticas resultantes fueron editadas utilizando FigTree v.1.3.1 (Rambaut, 2009).

Diversidad genética, estructura poblacional y demografía histórica

Los parámetros de diversidad genética estimados incluyeron el número de haplotipos, número de sitios segregantes (*S*), diversidad haplotípica (*h*) y nucleotídica (π), además de la media de diferencias pareadas (*k*), los cuales fueron calculados tanto para la especie como para los grupos filogenéticos con el programa DnaSP v.5 (Librado y Rozas, 2009). Para evaluar la distribución de la variación genética de los grupos se realizó un análisis de varianza molecular (AMOVA; Excoffier *et al.*, 1992) con el programa ARLEQUIN v.3.5 (Excoffier y Lischer, 2010). El AMOVA considera la distancia molecular entre haplotipos para obtener la estructuración genética entre grupos (Φ_{CT}), entre poblaciones dentro de los grupos (Φ_{SC}) y dentro de las poblaciones (Φ_{ST}). Se analizó también la asociación entre homogeneidad geográfica y diferenciación

genética para identificar posibles barreras geográficas utilizando el programa SAMOVA v.1.0 (Dupanloup *et al.*, 2002). SAMOVA realiza combinaciones para la conformación de grupos considerando tanto la variación genética como la distancia geográfica a través de los estadísticos Φ de AMOVA. Para cada simulación de agrupamiento se requiere definir *a priori* un número de grupos *K*, para los cuales se utilizaron de 2 a 10, con 1000 permutaciones en cada una. También se calculó una red mínima de haplotipos para los grupos filogenéticos con N>15 con el programa Network v.4.5.1.6 (Bandelt *et al.*, 1999), el cual permite utilizar el método median-joining para estimar las relaciones entre los haplotipos.

Se determinó la distribución de frecuencias pareadas ("análisis mismatch") con el programa DnaSP, para identificar si existía señal de crecimiento demográfico tanto para *O. couesi* como para los grupos filogenéticos identificados (aquellos con N≥5; ver resultados). Este análisis compara la distribución de las frecuencias observadas de las diferencias nucleotídicas pareadas de los haplotipos con aquellas esperadas de acuerdo a un modelo de expansión (Rogers y Harpending, 1992; Harpending, 1994). Para complementar este análisis también se realizaron las pruebas de neutralidad *D* de Tajima (Tajima, 1989) y *Fs* de Fu y Li (Fu y Li, 1993), ésta última es particularmente sensible tanto a la expansión demográfica como a cuellos de botella; por ejemplo, un valor significativo y negativo sugiere una expansión poblacional (Ray *et al.*, 2003). También se calculó la suma de la desviación de cuadrados (SSD, por sus siglas en inglés) cuyos valores de P altos confirman la similitud a un modelo de expansión (Rogers y Harpending, 1992).

Análisis de agrupación de especies codistribuidas en América Central

Las hipótesis que han sido más estudiadas para explicar la estructuración genética de especies codistribuidas en América Central son aquellas relacionadas con la historia geológica de esta región (Gutiérrez-García y Vázquez-Domínguez, en prensa). Sin embargo, existen también ejemplos donde los factores geológicos no explican la variación genética. Sullivan *et al.* (2000) en su estudio con roedores en América Central sugieren que cuando existe una respuesta diferente de cada especie a las condiciones climáticas y eventos geológicos, es de esperarse que las poblaciones no presenten congruencia filogeográfica o la presenten sólo parcialmente. Por ello, y para determinar si la estructura genética de las especies de este estudio está relacionada con otras características, se evaluaron diferentes hipótesis de

estructuración definidas con base en información morfológica, genética y ecológica disponible para las dos especies. En el caso de *O. phyllotis*, la información utilizada se tomó del estudio de Gutiérrez-García y Vázquez-Domínguez (2012).

El análisis consistió en definir grupos con las secuencias de citocromo b; para cada especie, las agrupaciones fueron consideradas hipótesis de estructuración genética y se utilizó un análisis de varianza molecular (AMOVA) para determinar los valores entre grupos (Φ_{CT}), entre poblaciones dentro de los grupos (Φ_{SC}) y dentro de las poblaciones (Φ_{ST}) con ARLEQUIN v3.5. La primera agrupación (hipótesis taxonómica) se definió de acuerdo con la información morfológica, es decir con base en la taxonomía de cada especie, como sigue: para *O. phyllotis* los grupos se basaron en las subespecies determinadas por Lawlor (1989): *O.p. phyllotis*, *O.p. australis* y *O.p. connectens*; para *O. couesi* se definieron dos hipótesis taxonómicas, la primera basada en el complejo de subespecies y especies descrito por Goldman (1918) y la segunda basada en el trabajo realizado con morfología y genética de Hanson *et al.* (2010) del complejo *Oryzomys palustris*. La segunda agrupación (hipótesis filogeográfica) se basó en los filogrupos definidos para cada especie, obtenidos con el análisis de SAMOVA. La tercera agrupación es la hipótesis filogenética y la definen los grupos observados en la reconstrucción de las relaciones filogenéticas del citocromo b con base en los valores de aLRT y/o bootstrap. Para la cuarta agrupación o hipótesis ecológica, se consideraron variables ambientales.

Para definir estos grupos ecológicos se consideraron los valores de las 19 variables bioclimáticas comúnmente usadas para el modelado de nicho ecológico, junto con la altitud (msnm), mediante la extensión BIOCLIMav 1.2. (Nix, 1986) en Arc View GIS 3.2 (ESRI, 1999). Las variables utilizadas fueron: BIO1=temperatura media anual, BIO2=intervalo diurno medio, BIO3=Isotermalidad, BIO4=temperatura por estacionalidad, BIO5=temperatura máxima del mes más cálido, BIO6=temperatura mínima del mes más frío, BIO7=intervalo de temperatura anual, BIO8=temperatura media del trimestre más húmedo, BIO9=temperatura media del trimestre más seco, BIO10=temperatura media del trimestre más cálido, BIO11=temperatura media del trimestre más frío, BIO12=precipitación anual, BIO13=precipitación del mes más húmedo, BIO14=precipitación del mes más seco, BIO15=precipitación por estacionalidad, BIO16=precipitación del trimestre más húmedo, BIO17=precipitación del trimestre más seco, BIO18=precipitación del trimestre más cálido, BIO19=precipitación del trimestre más frío. Se utilizó el

programa PAWStatistic 18 (SPSS, Inc.) para estandarizar las variables y para determinar, con base en dichas variables, grupos ecológicos diferentes por medio de un análisis de agrupamiento jerárquico y utilizando la distancia de Ward. Con base en estos grupos se realizó un análisis de asignación, de acuerdo a K-medias, para determinar a qué grupo se asignaban las secuencias.

Una vez conformadas las agrupaciones y con la finalidad de identificar las variables ecológicas explicativas de cada agrupación, se realizó un análisis de discriminantes utilizando un método paso a paso con la Lambda de Wilks. Con ello se obtiene un valor de Lamdba de Wilks, un valor de F y su significancia estadística, de tal forma que la combinación de valores menores de Lambda y valores mayores de F indican que la influencia de la variable es más significativa y útil para la función discriminante.

Prueba de hipótesis de barreras geológicas de América Central

De acuerdo con Gutiérrez-García y Vázquez-Domínguez (en prensa), ciertos patrones genéticos comunes a múltiples taxones en América Central se relacionan con las principales barreras geológicas de la región; con base en lo cual los autores definen cuatro "clados" principales: 1) montañas altas de Guatemala y Chiapas, 2) península de Yucatán, 3) América Central y 4) Panameño. Así, si las barreras geográficas que originan la conformación de estos clados determinan el grado de estructuración genética para las especies de este estudio, se podrán observar similitudes entre ellas en cuanto a niveles de estructuración basados en la variación y divergencia genética. Para probar esta hipótesis, se utilizaron todas las secuencias de *O. phyllotis* (132) y únicamente las secuencias de *O. couesi* que comparten distribución con *O. phyllotis*, es decir aquellas de poblaciones localizadas entre el Istmo de Tehuantepec y la cordillera de los Andes. Se agruparon las secuencias de cada especie de acuerdo a los cuatro clados mencionados y se calculó: a) el valor de porcentaje de variación e índices de fijación de AMOVA y b) los valores de divergencia entre ellos (*Da* y *Dxy*). Diferentes estudios filogenéticos de roedores de América Central que incluyen organismos de ambos lados del Istmo de Tehuantepec han sugerido que éste funciona como barrera a la dispersión para poblaciones tanto en dirección este/oeste (Sullivan *et al.*, 1997; Arellano *et al.*, 2005; Sullivan *et al.*, 2000) como norte/sur (Castoe *et al.*, 2003; Vázquez-Miranda *et al.*, 2009). Para *O. phyllotis* el istmo es el límite de su distribución al norte, sin embargo para *O. couesi* se desconoce la forma y medida en que el istmo funciona como barrera. Para obtener una aproximación de qué tanto representa una barrera o promotor

de divergencia genética para *O. couesi*, se agruparon los individuos cercanos al istmo como una barrera norte/sur y otra este/oeste, y se estimaron valores de divergencia (*Da* y *Dxy*) entre ellas.

III. RESULTADOS

Análisis filogenético de Oryzomys couesi

Se analizó un fragmento de 733 pares de bases del citocromo b de 201 individuos de *O. couesi* de 108 localidades. Los resultados mostraron un total de 506 sitios sinónimos y 227 sitios no sinónimos, con 67 polimórficos (29.5%) y 160 (70.5%) parsimoniosamente informativos. Las secuencias del citocromo b analizadas se desvían de la neutralidad de acuerdo a la prueba de *D* de Tajima (*D*=-1.6479). Las pruebas de *D* y *F* de Fu y Li (1993) resultaron negativas y significativas (*D*=-4.2738, P<0.05; *F*=-3.5723, P<0.05), probablemente a causa de un exceso de alelos raros o haplotipos únicos. El modelo de sustitución de ADN más adecuado seleccionado fue TIM2+I+G (-lnL=9634.0619,P>0.001, ACI=20144.18), la frecuencia de las bases fue A=0.3344, C=0.3142, G=0.0643 y T=0.2872; la proporción de sitios invariables I=0.4060 y la distribución de gamma γ=0.6560. Las tasas de sustitución fueron: A-C=0.5810, A-G=6.2697, A-T=0.5810, C-G=1.0000, C-T=4.9110 y G-T= 1.000. Los valores de aLRT de las ramas de la topología del árbol de MV fueron de 75 a 97% para la mayoría de los clados (Figura 1). El análisis filogenético muestra que los individuos de *O. couesi* representan un grupo monofilético, con valores de soporte aLRT altos, tanto en la topología del árbol de la especie (97.5%), como en la del árbol del citocromo b que incluye ambas especies (95.7%). Además, ambas topologías (Figuras 1 y 2, anexo 1) muestran siete filogrupos bien definidos: Golfo de México (G), Pacífico (PA), Península de Yucatán (PY), Península Basal (PB), América Central (AC), América del Sur (AS) y Costa Rica (CR).

Diversidad genética, estructura genética de las poblaciones e historia demográfica de O. couesi

La variabilidad genética del citocromo b estimada para este roedor fue alta (h=0.994 ± 0.002) y la diversidad nucleotídica baja (π=0.02597 ± 0.0015), efecto que se observa incluso dentro de los grupos filogenéticos (Tabla 3) y que sugiere una rápida expansión demográfica a partir de una población de tamaño efectivo pequeño (Avise, 2000). El análisis de distribución mismatch (Figura 3, inciso f) y la

significancias de *Fs* de Fu (*Fs*=-3.5723, P<0.05) sugieren para *O. couesi* una población en expansión, mientras que el índice R_2 significativo (R_2=0.0389, P<0.05) corrobora el crecimiento poblacional (Ramos-Onsins y Rozas, 2002); de manera similar, la prueba de SSD mostró una probabilidad alta para crecimiento: $P(SSD_{Sim.} \geq SSD_{Obs})$=0.8261. El análisis de cada grupo filogenético (Figura 3, incisos a-e) mostró que los grupos G y PY también presentan modelos poblacionales en expansión, apoyado por los resultados de los índices *Fs*, R_2 y SSD (Tabla 3). PB fue la única agrupación con valores no significativos. La forma de la curva mismatch del grupo G fue de crecimiento poblacional unimodal (Slatkin y Hudson, 1991) y la del grupo PA fue bimodal, ésta última normalmente se observa después de un cuello de botella (Weber *et al.*, 2004).

Los valores de divergencia genética se muestran en la Tabla 4. Los valores más altos son aproximados al 10% y se obtuvieron al comparar el grupo de CR con el resto de los grupos (e.g. CR vs AS, 0.119 ± 0.047). Los siguientes valores altos resultan de comparar el grupo AS con dos grupos, PA (0.0719 ± 0.0074) y PY (0.0628 ± 0.0134). Por otro lado, los valores más pequeños de divergencias fueron 0.0133 ± 0.00152 y 0.0078 ± 0.0013, que resultan de la comparación entre PA y dos grupos, AC y PB. Se estimaron los valores de distancia genética entre *O. couesi* y las secuencias de los géneros utilizados como grupos externos *Sigmodon* y *Holochilus*, que resultaron en valores entre 15 y 20%. También se calculó la divergencia entre el set de secuencias de *O. couesi* y las secuencias de una especie del mismo género *O. palustris* (EU074636, EU074637 y EU074638), con un resultado cercano al 10% (0.1017 ± 0.0071). Los valores de AMOVA indicaron que el mayor porcentaje de la variación genética está distribuida entre los grupos (50%), seguida de los valores entre las poblaciones dentro de los grupos (31%); la variación dentro de las poblaciones mostró el menor valor (19%); mientras que los índices de fijación que resultaron de estas jerarquías de variación fueron Φ_{SC}=0.6181, Φ_{ST}=0.8100 y Φ_{CT}=0.5026 respectivamente. Los resultados de SAMOVA mostraron que la mejor representación de la estructura genética (e.g. el valor más alto de Φ_{CT}), de acuerdo a la distancia geográfica, se observó con K=3 (Φ_{CT}=0.7211). Estos grupos son 1) Costa Rica, 2) Panamá y 3) el resto de las poblaciones.

Se realizó una red mínima de haplotipos para los grupos G, AC, PA y PY (Figuras 4-7). La red del grupo PY (Figura 4) mostró una configuración en estrella, normalmente indicativo de una población en expansión, en la que hay un haplotipo ancestral con alta frecuencia y compartido entre localidades, con

muchos haplotipos terminales (punta), de los cuales los de El Salvador son los más alejados por 6 a 12 pasos mutacionales. La red para el grupo PA (Figura 5) mostró los haplotipos de Nayarit y Guerrero aislados por 11 y 17 pasos mutacionales respectivamente. Los haplotipos de Colima son los mayormente distribuidos en la red y existe alta división (hasta 5 pasos mutacionales) dentro del conjunto de haplotipos pertenecientes a las localidades de Chiapas, El Salvador y Guatemala. En la red de AC (Figura 6) se observó que los haplotipos más alejados tienen de 4 a 10 pasos mutacionales entre ellos y se encuentran en Honduras, Chiapas y Guatemala. Finalmente, la red del grupo G (Figura 7) estuvo organizada de zona norte a zona sur y los haplotipos de Veracruz son los mayormente distribuidos en la red. En la zona norte las poblaciones de Hidalgo son las más alejadas por 10-11 pasos mutacionales, a éste se unieron haplotipos únicos de localidades alejadas geográficamente, tales como de Honduras y Guatemala. En la zona sur los más alejados son haplotipos únicos de Guerrero, Puebla y Michoacán.

Análisis filogeográfico comparado de O. couesi y O. phyllotis

La topología del árbol de citocromo b de las especies en conjunto corrobora los grupos mayores de *O. phyllotis* obtenidos mediante el árbol de inferencia bayesiana (IB) de Gutiérrez-García y Vázquez-Domínguez (2012), con excepción de la posición de tres muestras de localidades de Costa Rica, Guatemala y El Salvador. El modelo de sustitución de ADN que mejor se ajusta a los datos de ambas especies y sus grupos externos fue GTR+I+G (-lnL=13389.12, P>0.001, ACI=28194.24), la frecuencia de las bases fue A=0.3316, C=0.3242, G=0.0622, T=0.2820; la proporción de sitios invariables I=0.2970; distribución de gamma γ=0.7100. Las tasas de sustitución fueron: A-C=0.4150, A-G=4.6948, A-T=0.4299, C-G=0.6007, C-T=2.6871 y G-T=1.0000. La topología del árbol de MV presentó valores altos de aLRT (70-99%) para los filogrupos mayores (Figura 2), donde *O. couesi* y *O. phyllotis* fueron monofiléticos y ambos coincidieron en la formación de dos filogrupos: América Central y Península de Yucatán.

Los análisis con las variables ecológicas resultaron en una diferenciación en siete grupos para *O. couesi* (Figura 8) y 10 para *O. phyllotis* (Figura 9); los detalles de las agrupaciones por especie se muestran en las Tablas del anexo 2 y anexo 3, respectivamente. En términos de los análisis de AMOVA estimados para las diferentes agrupaciones (hipótesis) evaluadas para cada especie (Tablas 5 y 6), el porcentaje de variación entre grupos (Φ_{CT}) para *O. couesi* (Figura 10A) mostró que el grupo con más alta diferenciación

fue el basado en la hipótesis filogeográfica (SAMOVA), seguido del de la filogenética, mientras que la que tuvo el menor porcentaje de explicación fue la ecológica. Los resultados en función del porcentaje de la variación entre poblaciones dentro de los grupos (Φ_{SC}) y dentro de las poblaciones (Φ_{ST}) mostraron que la agrupación que explica mejor esta variación fue la ecológica, seguida de la taxonómica basada en la morfología de Goldman (1819) y, lo valores menores fueron los basados en la hipótesis filogeográfica. Por otro lado, los resultados para *O. phyllotis* (Figura 10B) fueron con mayor porcentaje de variación entre grupos (Φ_{CT}) para la hipótesis filogeográfica seguido de la taxonómica. En cuanto a la variación entre poblaciones dentro de los grupos (Φ_{SC}), el porcentaje más alto fue el de la hipótesis taxonómica seguida de la ecológica. Similar a *O. couesi*, los valores más bajos a este nivel fueron los de la hipótesis filogeográfica. Finalmente, la variación dentro de las poblaciones (Φ_{ST}) se explica en porcentajes similares para todas las agrupaciones.

Respecto a los índices de fijación (Figura 11), de todas las agrupaciones de *O. couesi*, el valor más cercano a cero fue Φ_{CT} para el grupo basado en la hipótesis ecológica y el valor más cercano a 1 fue para Φ_{ST} de los grupos filogeográficos. De todas las agrupaciones de *O. phyllotis*, el valor más cercano a cero fue Φ_{CT} de la hipótesis taxonómica y el valor más cercano a 1 para Φ_{ST} de la misma hipótesis.

Los análisis estadísticos mostraron que las cinco variables climáticas que influyeron significativamente en cada agrupación para cada especie de acuerdo con Lambda y F se muestran en las Tablas 7 y 8. En ambas especies la variable más significativa fue la precipitación, aunque para *O. couesi* además de la precipitación hubo relación con la temperatura y la altitud. De acuerdo a las agrupaciones con base en la hipótesis taxonómica, la variable que afecta a ambas especies fue la precipitación por estacionalidad (BIO 15), seguida por la temperatura por estacionalidad (BIO 4). Para la hipótesis filogenética éstas son la precipitación del trimestre más frío (BIO 19) y la temperatura por estacionalidad (BIO 4), mientras que para la filogeográfica son cuatro de las variables principales: precipitación del trimestre más frío (BIO 19), precipitación del mes más seco (BIO 14), precipitación del trimestre más seco (BIO 17) y precipitación anual (BIO 12). Finalmente, para la hipótesis ecológica coinciden en tres variables ambas especies: precipitación anual (BIO 12), precipitación del mes más húmedo (BIO 13) y precipitación del trimestre más húmedo (BIO 16), mientras que la altitud fue significativa sólo para la ecológica en *O. couesi*.

Hipótesis de barreras geológicas

Los resultados del análisis de varianza molecular, divergencia y diversidad genética de los clados geológicos en los que se agruparon las especies para analizar el efecto de la geología (Figura 12) se muestran en la Tabla 9. Los valores de AMOVA mostraron que el porcentaje más alto de variación para *O. phyllotis* fue a nivel de entre grupos y para *O. couesi* fue en las poblaciones dentro de los grupos. Los valores más altos y más bajos de divergencia y diversidad genética entre los clados geológicos no coinciden entre las especies. Finalmente, respecto al Istmo de Tehuantepec (Figura 13), los valores de divergencia fueron relativamente bajos: norte/sur *Da*=0.017±0.005, *Dx*=0.031±0.005, mientras que las este/oeste fueron *Da*=0.003±0.004 y *Dx*=0.021±0.004. La divergencia más alta fue la comparación entre norte/sur por ≈1%.

IV. DISCUSION

O. couesi, el origen de la especie

El árbol filogenético del citocromo b de *O. couesi* muestra que la especie es monofilética y los haplotipos cercanos a los grupos externos sugieren ancestros provenientes del límite sur de su distribución actual, cerca de Colombia (Figura 14). La dispersión sugerida por la topología es en dirección sur a norte, tanto por vía el Pacífico hasta alcanzar Nayarit o vía Atlántico hasta Texas, así como la posterior recolonización de América Central. La dirección de la dispersión coincide con el de la hipótesis descrita por Hershkovitz (1966) para el complejo de *Oryzomys palustris*, en la que sugiere un primer conjunto de migrantes del sur al norte que posteriormente regresaron por rutas costeras a América Central durante el Plioceno, donde diversificaron. Aunque de acuerdo con esta hipótesis el origen de estas especies sucedió antes del Gran Intercambio Biótico Americano (GABI), Weksler (2006) señala que podría haber ocurrido como parte del GABI y en condiciones más secas que las sugeridas por Hershkovitz (1966). Para el origen y la diversificación de los Oryzominos, Engel *et al.* (1998) también sugirieron dos modelos previos al GABI: el modelo 1 en el que la diversificación es en América del Norte y el modelo 2 en el que la diversificación es cerca del norte de los Andes. De haber ocurrido esta diversificación Pre-GABI y de acuerdo con los resultados de este estudio, el origen de *O. couesi* concordaría con el modelo 2 de Engel *et al.* (1998), ya

que nuestros resultados sugieren un origen entre Costa Rica y los Andes, con una dispersión hacia el norte como lo señala Hershkovitz (1966); la posterior diversificación de la especie pudo haber ocurrido durante o después del GABI.

La topología también muestra la existencia de individuos altamente divergentes en Costa Rica y que comparten un ancestro común con el resto de los grupos de *O. couesi*. Los valores de distancia genética obtenidos al comparar estas muestras de Costa Rica (N=2) y los diferentes grupos dentro de *O. couesi*, son de ≈11%. Estos valores son similares a los calculados entre *O. couesi* y su especie hermana *O. palustris* 9-10%, pero son menores a los valores obtenidos al comparar *O. couesi* con especies de otros géneros, como *Holochilus,* de ≈15%. Estas muestras de Costa Rica están identificadas como *O. couesi* pero la distancia genética corresponde a la que hay entre individuos de especies distintas, tal como describe Hanson *et al.* (2010). Por lo tanto, sin duda es necesario un estudio morfológico detallado de estos individuos y de las poblaciones actuales de la región para, si fuera el caso, su correcta asignación a nivel de especie. No todas las poblaciones de Costa Rica pertenecen a este grupo altamente diferenciado, otra muestra del sur de Costa Rica a la que se tuvo acceso está en el grupo de América Central (Figura 1), con valores de divergencia similares a los observados en las poblaciones dentro de los grupos de la especie, no mayores de un 7%. Por lo tanto, de acuerdo a los valores de soporte en el árbol filogenético y a los de divergencia no mayores al 7%, *O. couesi* tiene una estructura genética definida por seis linajes o grupos filogenéticos, los cuales se detallan a continuación.

Dispersión y diferenciación de los grupos de O. couesi

La primera divergencia y la distancia genética más alta dentro de la especie definen al linaje o grupo América del Sur (AS), que está localizado al límite sur de la distribución e incluye las poblaciones de Panamá y Colombia. Aunque se utilizó un número reducido muestras (N=4) y cada una representa un haplotipo único, se observa que las poblaciones están diferenciadas del resto de los linajes por distancias genéticas de entre 6 y 7%. Es el linaje genéticamente más cercano a las muestras de Costa Rica que conforman el grupo altamente diferenciado y con el cual comparten un ancestro común en la base de la topología del citocromo b (Figura 1). De acuerdo con lo anterior, el origen de este linaje pudo haber sido hace entre 19 y 12 millones de años (Ma), cuando las cordilleras de Panamá formaban un archipiélago

emergente (Coates *et al.*, 2005), que algunas especies de Oryzominos del sur pudieron colonizar y posteriormente diversificar. Una vez originadas las primeras poblaciones de *O. couesi* (linaje AS), la migración al sur podría haber estado limitada por la alta actividad volcánica en la región este de la cordillera de los Andes, entre 12 y 5 Ma, y por la rápida elevación posterior de la cordillera (5-2 Ma; Gregory-Wodzicky, 2000). Por lo tanto, es probable que *O. couesi* se haya dispersado hacia el norte, ya sea a través del archipiélago emergente o a través de una península que se cree que ya conformaba América Central a finales del Mioceno (23.03 ± 0.05 a 5.332 ± 0.005 Ma; USGS, 2011; Kirby y MacFadden, 2005) y en la cual la vegetación dominante era de manglar, helechos y palmas (Graham, 1988), hábitat similar al que habita *O. couesi* actualmente. Asimismo, también había actividad volcánica en Costa Rica, que es el límite sur del Arco Volcánico de América Central (CAVA) y que pudo limitar la migración del grupo AS hacia el norte,. Las barreras geográficas actuales entre las poblaciones del linaje AS y las poblaciones de los otros cinco linajes son los complejos montañosos de Panamá y Costa Rica, cuya actividad fue mayor durante el Cuaternario (Carr *et al.*, 2007), que incluye los últimos 2.6 Ma (USGS, 2011). Estos complejos están asociados con valores altos de divergencia para otras especies además de *O. couesi* (Gutiérrez-García y Vázquez-Domínguez, en prensa).

El linaje genéticamente más cercano a AS es Península basal (PB), e incluye poblaciones del centro y sur de la península de Yucatán. Las localidades de PB y AS más cercanas entre ellas están separadas por una distancia lineal aproximada de 1,000 km, es decir, las poblaciones de PB no son las poblaciones geográficamente más cercanas a AS. El linaje más cercano en términos de distancia geográfica es América Central (AC), pero AC no es el siguiente linaje en la topología. Esto permite sugerir que hubo una primera migración de sur a norte facilitada por la emersión de las tierras bajas, zonas inundadas y bordes de América Central hasta alcanzar el centro y sur de la península de Yucatán para conformar el grupo PB. Posteriormente, las poblaciones a lo largo de esta primera migración desaparecieron o disminuyeron su tamaño a tal grado que desapareciera la evidencia genética de la ruta seguida durante esta migración, por lo que no se detectan haplotipos ancestrales en las poblaciones que actualmente se distribuyen en el espacio geográfico entre AS y PB. La disminución poblacional y/o extinción de las poblaciones de AC podría ser resultado del incremento en el nivel del mar que afectaría a las poblaciones de las zonas bajas y las conexiones terrestres. Un evento así ocurrió entre 7.2 y 5.3 Ma y está registrado en Bocas del Toro, al

sur de América Central (Coates *et al.*, 2005). A finales de Mioceno y principios del Plioceno, América Central recibió un mayor número de especies desde el sur hacia el norte, de especies adaptadas a bosques húmedos, mientras que durante el Pleistoceno (2.59 Ma a 11,7 ± 99 años; USGS, 2011), la migración fue mayor en sentido norte a sur y por especies de vegetación más seca, similares a las sabanas (Vermej, 1991). La invasión durante grandes intercambios bióticos induce extinciones (Vermej, 1991, Lessa *et al.*, 1997), ya que la presencia de algunas especies puede restringir el establecimiento de otras, como *O. couesi* a lo largo de América Central, y explicar así la ausencia de haplotipos de esta primer migración en las poblaciones actuales. Por otro lado, la sugerencia de incrementar el número de individuos y/o poblaciones prevalece, con la finalidad de corroborar que la ausencia de estos haplotipos no es un resultado tan sólo del muestreo de este trabajo para la región.

Posterior a la divergencia de los grupos AS y PB, se observa en la topología de *O. couesi* la divergencia casi simultánea de cuatro linajes a partir de una rama con alto valor de soporte (92.3%; flecha amarilla en figura 1). El primer grupo es Península de Yucatán (PY), que incluye individuos de las poblaciones de la península de Yucatán e Isla Cozumel, así como algunos de Guatemala y El Salvador. Además de los análisis de neutralidad y de mismatch de este grupo, la configuración de la red de haplotipos en forma de estrella apoya un modelo de expansión poblacional reciente (un haplotipo ancestral que genera nuevos haplotipos con reducido número de pasos mutacionales entre ellos; Ferreri *et al.*, 2011). Estos resultados coinciden con los de otros estudios de genética de *O. couesi* del sureste de México con ADN nuclear; por ejemplo Vega *et al.* (2007) muestran que las poblaciones de *O. couesi* de la península de Yucatán y de Isla Cozumel mantuvieron flujo genético entre ellas. Sin embargo, también muestran que dicho flujo genético es restringido ya que ha permitido la estructuración de las poblaciones de Isla Cozumel, lo que se ve reflejado en los valores de alta diversidad genética. Asimismo, de acuerdo con el estudio de Vázquez-Domínguez *et al.* (2009), las poblaciones de *O. couesi* de la península de Yucatán muestran un exceso de heterocigotos, un alto grado de diversidad genética estructurada principalmente a nivel intrapoblacional y un número alto de migrantes entre sus poblaciones que sugiere que históricamente ha existido flujo genético entre ellas. La distancia genética entre las secuencias de Isla Cozumel y la península de Yucatán del presente estudio es baja (*Da*=0.00023±0.0011 y *Dx*=0.0064±0.0011), y es menor a la que existe entre otra especie de roedor de la isla, *R. spectabilis*, y su

equivalente en el continente *R. gracilis* que es de1.2-1.3% (Arellano *et al.*, 2005). La distancia entre las poblaciones de la península de Yucatán y de Isla Cozumel es menor al 1%, por lo que genéticamente no sugiere que sean especies distintas o que sean un linaje diferente como son, por ejemplo, AC o PA.

El siguiente linaje de acuerdo con el árbol es Pacífico (PA) e incluye localidades de la costa del Pacífico y del centro de México. Tiene su origen en poblaciones de El Salvador y la migración fue de sur a norte. La reticulación de los haplotipos en la red a manera de bifurcaciones sencillas sugiere que dicha colonización sucedió de forma más o menos rápida. La topología también sugiere que este patrón de colonización sur a norte ocurrió en dos ocasiones, ambas con origen en El Salvador, la primera en el borde sur de la Faja Volcánica Trans-Mexicana (FVTM) hasta Jalisco y la segunda por la costa del Océano Pacífico hasta Nayarit (Figura 1). La historia demográfica de este linaje corrobora esta recolonización, ya que la forma de la gráfica de mismatch coincide con la de una población que ha pasado por un cuello de botella. Este cuello de botella y el límite norte de la distribución del grupo PA podrían estar asociados a los eventos geológicos de la región a finales del Mioceno y a las variaciones climáticas del Pleistoceno. La primer colonización de PA debió ocurrir previa conformación del Istmo de Tehuantepec (IT) hace 6 Ma (Barrier *et al.*, 1998), cuando aún era una cadena montañosa continua y podía funcionar como barrera de dispersión al norte. Esta migración inicial de PA no sucedió a lo largo de la costa del Pacífico sino por el sur de la Faja Volcánica Trans Mexicana (FVTM), probablemente porque las regiones de la Sierra Madre del Sur (SMS) que incluyen Oaxaca y Guerrero tenían alta actividad volcánica durante principios del Mioceno. LA FVTM está conformada por vulcanismo con edades que van del Mioceno (16 Ma) hasta el presente y, que va desde Veracruz hasta Jalisco. De acuerdo a los resultados de este trabajo, *O. couesi* alcanzó el extremo noroeste de su distribución avanzando por el borde sur de la FVTM, como se ha observado para otras especies (e.g. serpientes del género *Pituophis*; Bryson*et al.*, 2011). Una vez ahí podría haber limitado su migración al norte debido a: 1) las tierras altas de la Sierra Madre Occidental (SMO), cuyo magmatismo cesó aproximadamente entre 27-25 Ma (MacDowell y Clabaugh, 1981; Moran-Zenteno *et al.*, 2000) y, 2) el vulcanismo asociado al FVTM, que fue sucesivamente alto (11 y 8 Ma), reducido (8 y 5 Ma) y nuevamente intenso desde hace 5 Ma hasta la actualidad (Ferrari *et al.*, 1999). Con base en la ubicación en la topología de la muestra más norteña de Nayarit, se sugiere que es durante la segunda migración que este roedor logró extenderse más allá de estas barreras geológicas para alcanzar su límite noroeste en el sur de

Sonora. Esta migración fue a lo largo de la costa del Pacífico, por lo que coincide con el cese de la actividad volcánica en la SMS durante la formación del arco volcánico moderno de Chiapas y los volcanes del sur de Veracruz en el Plioceno (Manea y Manea, 2005). El límite sur de PA se encuentra en El Salvador, restringido al este por el CAVA, el cual estaba activo desde finales del Mioceno (Gans *et al.*, 2003; Soto and Alvarado, 2006; Carr *et al.*, 2007) La migración de las poblaciones de PA al sur podría haber sido limitado por el conjunto que incluye las islas volcánicas de frente volcánico en El Salvador, el vulcanismo de la segunda línea del CAVA en Honduras (Rogers *et al.*, 2002; Ortega-Gutiérrez *et al.*, 2007) y la Depresión de Nicaragua que aún estaba sumergida a finales del Mioceno (Harmon, 2005). Este conjunto de barreras geográficas incluso ha generado diversificación a nivel de especie para grupos, como anfibios (Hasbün *et al.*, 2005), reptiles (Parkinson *et al.*, 2000), y otros (Gutiérrez-García y Vázquez-Domínguez, en prensa).

La distribución geográfica del linaje PA es similar al de otras especies y, dada la geología de la región, se sugieren diferencias morfológicas y diferenciación genética dentro del grupo, como ocurre para especies del género *Ctenosaura* (Zarza *et al.*, 2008). Carleton y Arroyo-Cabrales (2009) analizaron la morfología de las subespecies del complejo *O. couesi* del noroeste de México que incluía, entre otras, a *O.c. albiventer*, *O.c. mexicanus*, *O.c. regillus* y *O.c. lambi*, de los que distinguieron dos grupos morfológicos bien diferenciados: *O.c. mexicanus* y *O.c. albiventer*, entre los que observaron variación morfológica significativa en proporción de tamaño y la forma del cráneo, en ambos casos relacionada con la edad y el sexo de los individuos incluso a pequeñas escalas geográficas. De acuerdo con la filogeografía de *O. couesi* con citocromo b, la distancia genética entre estas subespecies no es mayor al 2%, por lo que se evidencia el grado de plasticidad morfológica al menos dentro del linaje PA. Además, los autores también sugieren que a esta zona llegó un "stock" ancestral de Oryzominos que colonizó incluso las islas cercanas, a partir del cual se diferenciaron algunas especies (e.g. *O. peninsulae*). Aunque los resultados obtenidos utilizando citocromo b no explican el origen a partir de un "stock" ancestral de Oryzominos, sí muestran que en el área se conservan haplotipos de los grupos PB, PA y G, por lo que la región podría tener haplotipos de una primera y antigua migración de *O. couesi* a la región. Por otro lado, el estudio de sistemática de Hanson *et al.* (2010) muestra la división Golfo de México/Pacífico de *O. couesi*; división este/oeste que también se observa para otros grupos de vertebrados (e.g. aves; Vázquez-Miranda *et al.*, 2009 y Weir, 2009) y que también se corrobora con los linajes P y G de este estudio. No obstante,

Carleton y Arroyo-Cabrales (2009) no observaron diferencias morfológicas entre las poblaciones este/oeste en su estudio al comparar individuos del centro con los del noroeste de México, lo cual se puede deber a que los límites exactos de esta división no están definidos geográficamente.

El siguiente linaje observado es América Central (AC) e incluye las localidades de Centroamérica, con excepción de algunas ya mencionadas dentro de los grupos PB y PY. La dirección de colonización que se aprecia con base en la topología es de norte a sur, la mayor en esta dirección para todos los linajes de *O. couesi*. Los análisis demográficos de AC apoyan un modelo de expansión poblacional que se sugiere fue reciente ya que no se observa en la red de haplotipos. La red de haplotipos de AC es tipo "reticulada", lo que indica que hubo intercambio de haplotipos entre poblaciones; asimismo, el alto número de pasos mutacionales entre algunos de estos haplotipos sugiere que hubo aislamiento geográfico posterior al intercambio. La barrera geológica que marca el límite norte de AC es el sistema de fallas Polochic-Motagua-Jocotán cuya conformación y emersión precede al Mioceno (Keppie y Morán-Zenteno, 2005). Las tierras altas del centro de Honduras tienen yacimientos con fósiles de mamíferos de entre 9 y 6.7 Ma (Webb y Perrigo, 1984), lo que sugiere que para el Mioceno ya era una región expuesta con condiciones adecuadas para la presencia de comunidades de mamíferos. La dirección de colonización de *O. couesi* en AC coincide con el patrón filogeográfico de otras especies, como plantas tropicales para las que se ha sugerido una expansión en la región favorecida por los cambios climáticos del Pleistoceno (Cavers *et al.*, 2003). El linaje AC tiene su límite suroeste en Costa Rica, es decir *O. couesi* se dispersó a través de la Depresión de Nicaragua y por los límites de los complejos volcánicos hasta la base de la cordillera de Talamanca sin llegar a Panamá. Se ha sugerido que el vulcanismo de esta región formó una barrera física que modificó el clima, impidiendo el movimiento de especies a través de Costa Rica desde hace 1.5 Ma (Cavender-Bares *et al.*, 2011). Las cordilleras del norte de Panamá representan la barrera geológica del sur para AC, como es el caso para especies de reptiles de la región, las cuales presentan diferenciación intraespecífica entre las poblaciones de esta zona (Castoe *et al.*, 2003).

Finalmente, el linaje G incluye las poblaciones cercanas al Golfo de México, del extremo norte de la distribución de la especie en Texas hasta la península de Yucatán incluyendo Isla Cozumel. De acuerdo con la topología, las poblaciones que dieron origen a G son del centro de México, las cuales se dispersaron primero por el borde continental al sur y luego se expandieron al norte hasta alcanzar Texas. Estas

poblaciones coinciden con el modelo de expansión, y cuya red de haplotipos muestra más reticulaciones en las localidades del norte, lo que sugiere un mayor flujo genético entre éstas que entre las del sur. Hay poblaciones dentro de este linaje que han quedado "aisladas" y entre ellas se encuentran las cercanas al grupo del PA (e.g. haplotipos únicos de Michoacán y Guerrero) y AC (e.g. haplotipos únicos de Honduras y Guatemala), que son los límites de distribución del linaje. Dado que el linaje G incluye las poblaciones del borde continental del Golfo de México, su dispersión en el área podría estar condicionada a la emersión de la plataforma continental y la zona costera del Golfo de México. De acuerdo con Padilla y Sánchez (2007), la planicie costera del Golfo de México inició su emersión durante el Mioceno y emergió antes de la región norte de la península de Yucatán. Durante el Pleistoceno, las únicas áreas expuestas para la dispersión de *O. couesi* eran los límites (bordes) tanto de la Sierra Madre Oriental como de la FVTM, hasta llegar a la base de las tierras altas de de Guatemala. En el noroeste, la barrera geológica es la Sierra Madre Oriental, cuya formación finalizó entre los 42 y 39 Ma (Gray y Lawton, 2011), sin embargo no existe una barrera geológica clara que limite la migración del linaje G al norte y tampoco una que explique por qué su migración inicial es hacia el sur. Respecto al norte, el estudio de Hanson *et al.* (2010) muestra que existe una sucesión de especies del complejo de *Oryzomys palustris* en el norte: en el límite de la distribución de *O. couesi* comienza la distribución de *O. texensis* y en el límite de distribución de *O. texensis* inicia la distribución de *O. palustris*, pero que no existe una barrera geográfica evidente entre los límites de sus distribuciones. Lo anterior nos sugiere que otros factores, ya sea climáticos, ecológicos, de vegetación, afectaron la distribución de este linaje del Golfo de México. Aunque se desconoce la composición de las comunidades vegetales durante el Plio-Pleistoceno, de acuerdo a Moreno-Cassasola y Espejel (1986) las condiciones actuales de precipitación, temperatura, tipo de suelo y vegetación dividen la región costera del Golfo de México en dos regiones: Golfo y Caribe. La región del Golfo se divide en la zona Norte que incluye Tamaulipas y el norte de Veracruz, y la zona Centro-Sur que incluye el resto de Veracruz y Tabasco. Mientras que la región Caribe divide a la península de Yucatán en la región semi-seca del norte y húmeda del este. Esta regionalización coincide con la división y la forma de migración observadas en las poblaciones del linaje G, así como lo observado para otras especies (e.g. género *Bufo*; Mulcahy *et al.*, 2006).

Filogeografía comparada de *O. couesi* y *O. phyllotis*

El origen de las especies y sus patrones de dispersión

De acuerdo con la filogeografía comparada, las especies codistribuidas responden a los cambios geológicos y climáticos de una región de manera independiente (individual) o de manera concertada (como comunidad) (Sullivan *et al.*, 2000; Gutiérrez-García y Vázquez-Domínguez, 2011). Las especies también pueden coincidir, en más de una ocasión, en la forma de respuesta a los cambios de su entorno, lo que se denomina "respuesta mixta", y que es el patrón observado para *O. couesi* y *O. phyllotis*. Aunque en este estudio no se estimó el tiempo de divergencia entre las especies, un estudio previo (Steppan *et al.*, 2004) sugiere que la divergencia Tylomyinae/Sigmodontinae ocurrió hace aproximadamente 16.2 Ma y que el origen de *O. couesi* ocurrió entre 8.8 y 6.0 Ma, durante la diversificación de los Oryzominos previa al GABI. En otro estudio se calculó el origen de los Neotominos, que incluyen a *O. phyllotis*, hace 8.6 ± 2.1 Ma y de los Oryzomyine, que incluyen a *O. couesi*, entre 8.4 y 5.1 Ma (Engel *et al.*, 1998). Por lo tanto, de acuerdo a dichas estimaciones, las dos especies coincidieron y experimentaron los cambios geológicos y climáticos de la región por lo menos durante los últimos 6 Ma, desde finales del Mioceno. Por lo tanto, se esperarían patrones concordantes (e.g. divergencia coordinada; Daza *et al.*, 2010) entre ambas especies, como se ha demostrado para otros roedores, incluso si cohabitaron tan sólo los últimos 2 Ma (Riddle *et al.*, 2000). Sin embargo, las concordancias entre las especies de este estudio son menores a las observadas en los trabajos mencionados, iniciando con el patrón de origen y la forma de dispersión. Aunque ambos géneros se diferenciaron a partir de un ancestro común de América del Norte, el origen de *Ototylomys* fue en el centro geográfico de su distribución actual (en Centro América, Gutiérrez-García y Vázquez-Domínguez, 2012), mientras que el de *Oryzomys* fue en el sur, por lo que sus patrones de migración a lo largo de América Central, desde su origen, son distintos (Figura 15). Además, *O. phyllotis* muestra un patrón de migración, ya sea al norte o al sur, sin cambios de dirección, mientras que *O. couesi* presenta variaciones en el sentido de colonización, del sur al norte, y de regreso al sur. Ambos patrones se han observado en especies de América Central asociados tanto a la geología como a los cambios climáticos desde el Mioceno (Gutiérrez-García y Vázquez-Domínguez, en prensa)

Respuestas concordantes e individuales

Geología

La hipótesis de que las barreras geológicas mayores de América Central estructuraron la variación genética de las dos especies estudiadas, independientemente de sus diferencias ecológicas, se ha observado en estudios de filogeografía de otros mamíferos y otras especies como aves, reptiles, anfibios, independiente del potencial de dispersión, historia de vida y preferencia de hábitat que son distintos entre las especies (Gutiérrez-García y Vázquez Domínguez, en prensa). Al mismo tiempo, aunque existe un efecto de la configuración geológica de la región sobre la estructuración de las dos especies de roedores, varios de los patrones encontrados son diferentes. De acuerdo con el patrón filogeográfico de *O. couesi*, la depresión de Nicaragua, el Istmo de Tehuantepec en dirección este-oeste y las tierras altas de Chiapas y Guatemala no representan barreras para la migración, mientras que sí lo son para *O. phyllotis*. Las únicas barreras geológicas concordantes entre estas especies son la región del centro al este del Sistema de fallas Motagua-Polochic-Jocotán y las cordilleras del Costa Rica y del norte de Panamá (Figura 14), las cuales son también de las más antiguas, previas al Mioceno. En efecto, estos elementos geológicos han generado divergencia entre y dentro de especies con afinidades tanto a tierras altas como de tierras bajas (Daza *et al.*, 2010).

Demografía

Oryzomys couesi y *Ototylomys phyllotis* coinciden en dos grupos filogenéticos, el de América Central y el de la península de Yucatán. Los resultados muestran también, además de esta divergencia concertada, que las poblaciones de estos grupos experimentaron procesos de expansión, con la diferencia de que para *O. phyllotis* sugieren que pasaron por cuellos de botella. Según Lawlor (1982), ambas especies tienen una asociación ecológica y, aunque no se ha descrito el grado de esta asociación, se ha encontrado evidencia en el registro fósil del Pleistoceno/Holoceno en la cueva de Loltún en la península de Yucatán (Arroyo-Cabrales y Álvarez, 2003). El registro fósil muestra que en algunas capas de suelo con diferente datación, cuando la abundancia de fósiles de *O. couesi* se incrementa, la abundancia de *O. phyllotis* disminuye ligeramente, y viceversa cuando *O. phyllotis* incrementa su abundancia la de *O. couesi* disminuye (Álvarez, 1983). Esta diferencia en el patrón de abundancia sugiere, entre otras cosas, que aunque en aquel

entonces las especies compartían hábitat y los factores ecológicos asociados en Yucatán, sus patrones demográficos eran distintos. Por lo tanto, *O. couesi* y *O. phyllotis* tuvieron una respuesta concertada al clima, pero con una respuesta independiente a nivel de sus poblaciones locales, lo cuál es común como resultado a los cambios climáticos y dadas las características ecológicas de las especies (Stewart, 2009). Una explicación sería que al haber cambios en el clima, si éstos fueran muy breves el efecto en cuanto a disminución del tamaño poblacional se registraría sólo en las poblaciones de la especie más susceptible a los cambios en la vegetación, en este caso las de *O. phyllotis*, que es la especie que habita menos tipos de vegetación que en los que se encuentra *O. couesi*. Estas diferencias en la tolerancia a cambios climáticos ha sido observada para otras especies de América Central (*e.g.* género *Bursera*, Poelchau y Hamrick, 2011). Otra posible explicación podría estar relacionada al marcador molecular utilizado, aunque los estudios genéticos que incluyen a ambas especies hasta ahora sugieren que el citocromo b evoluciona con una tasa similar y los modelos de mejor ajuste obtenidos en este estudio son idénticos para cada especie. El uso de múltiples loci, tanto nucleares como mitocondriales, podría mostrar a más detalle la dinámica poblacional y facilitar la comparación de su historia demográfica (Bell *et al.*, 2012).

Hipótesis de estructuración genética de roedores codistribuidos, la morfología no concordante

La definición taxonómica de especies basada en la morfología no siempre explica o coincide con la variación genética (e.g. Pastorini *et al.*, 2003; Mukherjee *et al.*, 2010), tal como se observa para *O. couesi*. Se ha sugerido que una clasificación taxonómica que contrasta con la estructuración genética es consecuencia de plasticidad fenotípica o de la convergencia de caracteres. En mamíferos es frecuente que las especies tengan variación genética no representada a nivel morfológico, como en las especies crípticas, por lo cual se ha sugerido el concepto de especie genética para una clasificación independiente de las características morfológicas (Baker y Bradley, 2006). Los resultados del citocromo b en *O. couesi* muestran que el dimorfismo sexual, las diferencias morfológicas entre individuos en diferentes edades e incluso la variación del tamaño asociadas al gradiente altitudinal reportadas por ejemplo, por Carleton y Arroyo-Cabrales (2009), no explican ni están relacionadas con la estructuración genética que se detecta con este gen. Por un lado, esto puede deberse incluso a que algunas especies de *Oryzomys* pudieron haberse clasificado basándose únicamente en la altitud en la que se encontraron y no en las características

morfológicas (como mencionan Carleton y Arroyo-Cabrales, 2009). Por otro lado, podría deberse a que variables climáticas y ambientales influyen significativamente la estructuración genética de *O. couesi*. Por ejemplo, se reconoce que hay factores ecológicos que causan diversificación fenotípica (e.g. diferenciación morfológica como resultado de la combinación de cambios en el clima y expansiones poblacionales; Hellberg *et al.*, 2011), y que el clima puede ocasionar una respuesta independiente, tanto en las especies como en sus poblaciones (Stweart, 2009). Esto coincide con los resultados observados en este estudio para *O. couesi*. En cuanto a *O. phyllotis*, los tres grupos morfológicos sugeridos como subespecies por Lawlor (1982) coinciden con los grupos obtenidos con base en las barreras geológicas en este estudio, aunque los resultados asimismo sugieren que puede existir una subespecie o, un linaje más (Gutiérrez-García y Vázquez Domínguez, 2012).

La variables climáticas, su influencia en la variabilidad genética de roedores codistribuidos en América Central

El estudio de la distribución de especies de acuerdo con las características ecológicas que las definen (e.g. el modelado de nicho), ha revelado que la ecología tiene un papel importante en cuanto a la diferenciación y estructuración genética de las mismas (Mukherjee *et al.*, 2010). Aunque para ninguna de las dos especies de este estudio la agrupación ecológica fue significativa entre grupos, sí lo fue a nivel de variación entre poblaciones y entre individuos. Los resultados sugieren que para ciertas especies de roedores, incluso aquellos con plasticidad fenotípica como *O. couesi*, se pueden observar grupos genéticos 'mayores', o linajes, asociados a grandes barreras geológicas, distancia geográfica o filogenia, los cuales a su vez tienen diferencias internas asociadas más con aspectos y factores ecológicos. Vázquez-Domínguez *et al.* (2009) observaron que algunas poblaciones de *O. couesi* del sureste de México tienen diferenciación genética relacionada a gradientes en el tipo de vegetación y el clima; dicho resultado coincide con el de este estudio pero, en nuestro caso, a una escala geográfica amplia, a nivel de la distribución completa de la especie. Respecto a *O. phyllotis*, la ecología influye en la estructura genética a nivel de las poblaciones también, pero en menor grado que la morfología. Sin embargo, si la ecología explica la variación a nivel de individuos, podría estar promoviendo la variación a nivel morfológico, por lo que la variación morfológica observada podría ser resultado más bien de una respuesta histórica, que

inicialmente fue modificada por variables tales como la precipitación y la temperatura, como se ha observado para otros roedores (Wolf *et al.*, 2009). En este estudio se hicieron grupos de acuerdo con variables climáticas, pero también se determinó cuáles de esas variables son más importantes para las agrupaciones que se hicieron con base en la morfología y la genética. Los resultados muestran que la variable que define significativamente las agrupaciones de ambas especies es la precipitación, la cual se sabe está relacionada significativamente con la morfología en roedores (e.g. heterómidos; Wolf *et al.*, 2009). En el caso de *O. phyllotis* las variables son las mismas para las diferentes agrupaciones, mientras que para las agrupaciones de *Oryzomys* son distintas. En relación con las agrupaciones filogeográficas, filogenéticas y ecológicas de *O. phyllotis*, la variable más importante es la de precipitación del mes más frío, mientras que para los grupos morfológicos, es la precipitación del mes más húmedo. Por otro lado, para *O. couesi* las variables que influyen en las agrupaciones son más y más diversas, incluyendo además la temperatura y la altitud. En específico para las agrupaciones morfológicas, filogenéticas y filogeográficas las variables más importantes son de precipitación, mientras que para la agrupación ecológica se incluye la temperatura por estacionalidad y la altitud. De hecho, en otras especies de roedores se ha asociado la altitud con la estructuración genética (e.g. para *Peromyscus*; Ordóñez-Garza *et al.*, 2010), como lo observado para la agrupación ecológica de *O. couesi*.

En un estudio donde se realizó la predicción y modelado de distribución geográfica de estas dos especies de roedores de acuerdo con diferentes escenarios de cambio climático (Calixto, 2009), se encontró que la distribución de *O. phyllotis* es la que se vería mayormente afectada por los cambios en el clima. De ser así, el hecho de que encontramos un mayor número de variables ambientales que definen la estructuración genética de *O. couesi* podría asociarse con un mayor potencial de esta especie para enfrentar fluctuaciones climáticas. Lo anterior coincide con lo observado por Vega *et al.* (2007): que la especie mantiene su variabilidad genética alta aún después de cuellos de botella debido a cambios climáticos fuertes como huracanes (e.g. poblaciones de Isla Cozumel). En roedores se sabe que la amplitud de respuesta a cambios climáticos está limitada por la plasticidad fenotípica (Auffray *et al.*, 2009), la cual es una característica observada en *O. couesi* (Carleton y Arrollo-Cabrales, 2009, entre otros). Por otro lado, el hecho de que la precipitación fue una variable significativa en relación con las agrupaciones

de *O. phyllotis*, sugiere que cambios en el clima asociados con el régimen de lluvias serían los más importantes para esta especie, en comparación con la temperatura o la altitud, por ejemplo.

V.CONCLUSIONES

América Central es una región ideal para probar patrones filogeográficos de respuesta mixta de especies codistribuidas. Los patrones filogeográficos de *O. couesi* y *O. phyllotis* son en su mayoría no concordantes debido especialmente a sus particularidades ecológicas. El principal factor que está modelando la estructura genética a nivel de grupos es la distancia geográfica, mientras que para el nivel de poblaciones son la ecología y la morfología, que a su vez tienen diferente importancia para cada especie. Así, las características ecológicas de *O. couesi* le permitieron colonizar regiones y atravesar barreras geológicas que fueron, por el contrario, limitantes para la distribución de *O. phyllotis*. Los estudios comparados entre especies de grupos taxonómicos similares, como estos dos roedores, permiten identificar cuáles son los factores importantes (además de la geología y el clima) para el estudio de la historia y evolución de las comunidades.

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Tabla1. Lista de individuos de *O. couesi* utilizados para este estudio

Especie	Voucher/ GenBank	Museo	País	Latitud	Longitud	ID Muestra
O. c. couesi	583074	NMNH (SI)	Belice	16.265800	-88.870000	1Bel
O. c. gatunensis	125408	FMNH	Colombia	8.757500	-75.890000	1Col
O. c. gatunensis	127250	FMNH				2Col
O. sp.	M-1831	LSUMZ	Costa Rica	9.084742	-83.574672	1CoRi
O. sp.	EU074670	RMT		10.694628	-84.099322	2CoRi
O. sp.	EU074671	RMT				3CoRi
O. c. couesi	98754	MVZ	El Salvador	13.583330	-88.066670	1ElSalv
O. c. couesi	98759	MVZ		13.766670	-88.216670	2ElSalv
O. c.	FJ360634	CMNH		13.507889	-89.115844	8ElSalv
O. c.	FJ360635	CMNH				9ElSalv
O. c.	FJ360636	CMNH				10ElSalv
O. c. couesi	98774	MVZ		13.666670	-89.533330	7ElSalv
O. c. couesi	98762	MVZ				3ElSalv
O. c. couesi	98763	MVZ				4ElSalv
O. c. couesi	98764	MVZ				5ElSalv
O. c. couesi	98765	MVZ				6ElSalv
O. c.	11899/118956	CMNH	Guatemala	15.403739	-89.139150	1Gua
O. sp.	760	USAC		17.225000	-89.613300	2Gua
O. sp.	976	USAC		16.915000	-89.817500	4Gua
O. c. couesi	565124	NMNH (SI)		15.116208	-89.559053	7Gua
O. c. couesi	565129	NMNH (SI)		14.189217	-90.695856	8Gua
O. c. couesi	FJ360633	KUM		15.260000	-90.220000	9Gua
O. c.	FJ971266	ROM		16.990761	-89.690078	10Gua
O. sp.	975	USAC		17.693900	-89.533300	3Gua
O. sp.	978	USAC				6Gua
O. c.	FJ971267	ROM		17.309981	-89.621039	11Gua
O. c.	FJ971268	ROM				12Gua
O. c.	11714/118669	CMNH	Honduras	14.535556	-88.707222	1Hon
O. c. couesi	565477	NMNH (SI)		14.775169	-88.773611	4Hon
O. c. couesi	565478	NMNH (SI)		14.420167	-88.605389	5Hon
O. c.	TK136361/104417	TTU6		14.412683	-86.627956	13Hon

O. c.	TK136999/104088	TTU11		14.610244	-86.303389	14Hon
O. c.	13306/112927	CMNH		15.010278	-85.852500	2Hon
O. c.	13311/112928	CMNH				3Hon
O. c. couesi	182	UAMI		15.510819	-87.948792	6Hon
O. c. couesi	183	UAMI				7Hon
O. c. couesi	184	UAMI				8Hon
O. c.	TK101818/84475	TTU3		15.740479	-87.457278	15Hon
O. c.	EU074666	TTU				16Hon
O. c.	EU074667	TTU				17Hon
O. c.	TK102090/84747	TTU10		14.808898	-85.842817	9Hon
O. c.	TK102091/84748	TTU1				10Hon
O. c.	DQ185383	TTU				11Hon
O. c.	DQ185384	TTU				12Hon
O. c. couesi	30753	CNMA-IBUNAM	Campeche/ México	18.618508	-92.284408	1CamMx
O. c. couesi	31708	CNMA-IBUNAM		20.506945	-90.384160	4CamMx
O. c. couesi	37343	CNMA-IBUNAM		18.800000	-89.367000	5CamMx
O. c. teapensis	EU074657	ASNHC		18.093972	-91.042547	6CamMx
O. c. couesi	30751	CNMA-IBUNAM		19.040000	-91.090000	2CamMx
O. c. couesi	30752	CNMA-IBUNAM				3CamMx
O. c. regillus	141802	MVZ	Chiapas/ México	16.907222	-92.126389	1ChiMx
O. c.	223	ECO-SC-M		16.761000	-91.128000	2ChiMx
O. c.	231	ECO-SC-M		16.701000	-94.169000	3ChiMx
O. c.	975	ECO-SC-M		15.946000	-93.825000	4ChiMx
O. c.	1319	ECO-SC-M		16.191111	-91.308056	5ChiMx
O. c.	TK150243/104676	TTU8		15.419683	-93.071961	6ChiMx
O. c. teapensis	EU074660	ASK		17.423225	-91.981956	7ChiMX
O. c.	EU074650	TTU		17.430878	-92.005475	10ChiMx
O. c.	EU074648	TTU		15.419683	-93.071961	8ChiMx
O. c.	EU074649	TTU				9ChiMx
O. c.	2036	ASK-ROM	Colima/ México	19.250014	-103.516650	3ColMx
O. c.	2103	ASK-ROM		18.912867	-103.872092	4ColMx
O. c.	1916	ASK-ROM		19.386669	-103.571528	1ColMx
O. c.	EU074652	ASK-ROM				2ColMX
O. c.	2123	ASK-ROM		18.929483	-104.065172	5ColMx
O. c.	2124	ASK-ROM				6ColMx
O. c.	2141	ASK-ROM		19.048119	-104.327775	7ColMx

O. c.	2164	ASK-ROM				8ColMx
O. c.	2165	ASK-ROM				9ColMx
O. c.	EU074645	TTU		19.100389	-104.308367	10ColMx
O. c.	EU074646	TTU				11ColMx
O. c.	GQ178245	TTU				12ColMx
O. c.	GQ178246	TTU				13ColMx
O. c. spp. mexicanus	3268	MZFC-M	Guerrero/ México	17.420000	-100.204000	3GueMx
O. c. spp. mexicanus	3274	MZFC-M		17.414000	-100.209000	4GueMx
O. c. spp. mexicanus	6725	MZFC-M		16.796000	-98.712000	5GueMx
O. c. spp. mexicanus	6726	MZFC-M		16.759000	-98.685000	6GueMx
O. c. spp. mexicanus	6730	MZFC-M		16.790000	-98.672000	7GueMx
O. c. spp. mexicanus	3262	MZFC-M		17.342000	-100.252000	1GueMx
O. c. spp. mexicanus	3263	MZFC-M				2GueMx
O. c. subsp. c.	13850	UAMI	Hidalgo/ México	21.178889	-98.895833	3HidMx
O. c. subsp. c.	13853	UAMI		20.964722	-98.705833	4HidMx
O. c. subsp. c.	13855	UAMI		21.200556	-99.006111	5HidMx
O. c. subsp. albiventer	12344	UAMI	Jalisco/ México	19.768056	-104.362500	3JalMx
O. c.	EU074653	TTU		19.491812	-105.044286	4JalMx
O. c. spp. mexicanus	6111	MZFC-M		19.300000	-104.790000	1JalMx
O. c. spp. mexicanus	6127	MZFC-M				2JalMx
O. c. subsp. regillus	999	UAMI	Michoacán/ México	20.118056	-102.691667	1MichMx
O. c.	EU074654	TTU		19.627989	-100.703878	11MichMx
O. c.	1749	ASK-ROM		17.980394	-102.349856	2MichMx
O. c.	1750	ASK-ROM				3MichMx
O. c.	1752	ASK-ROM				4MichMx
O. c.	1755	ASK-ROM				5MichMx
O. c.	1756	ASK-ROM				6MichMx
O. c.	1757	ASK-ROM				7MichMx
O. c.	1758	ASK-ROM				8MichMx
O. c.	1760	ASK-ROM				9MichMx
O. c.	1765	ASK-ROM				10MichMx
O. c.	EU074651	ASNHC	Nayarit/México	21.535000	-105.249861	1NayMx
O. c. subsp. aztecus	38101	CNMA-IBUNAM	Oaxaca/ México	18.030556	-96.699720	1OaxMx
O. c. subsp. aztecus	38103	CNMA-IBUNAM		17.634722	-96.898610	2OaxMx
O. c.	FJ97124	BYU		16.982775	-95.067558	5OaxMx
O. c.	DQ185385	TTU		16.774786	-96.600792	3OaxMx

O. c.	DQ185386	TTU				4OaxMx
O. c. subsp. aztecus	4909	UAMI	Puebla/ México	18.184167	-98.384167	1PueMx
O. c. subsp. aztecus	5276	UAMI		18.333333	-97.283333	2PueMx
O. c. subsp. aztecus	8102	UAMI		18.706111	-98.487222	3PueMx
O. c. subsp. aztecus	8103	UAMI		18.835556	-98.404167	4PueMx
O. c. subsp. c.	9503	UAMI		18.636944	-98.645833	5PueMx
O. c.	7173	ASNHC	Quintana Roo/ México	18.420097	-88.786481	1QRooMx
O. c.	29961	ASNHC		18.348100	-88.941400	4QRooMx
O. c.	29962	ASNHC		20.552250	-89.116542	5QRooMx
O. c.	7187	ASNHC		18.233328	-89.016694	2QRooMx
O. c.	7188	ASNHC				3QRooMx
O. c.	EU074658	ASNHC		20.431511	-86.908500	1CozuMx
O. c. cozumelae	2hMCZMEL	IE				2CozuMx
O. c. cozumelae	2mMCZMEL	IE				3CozuMx
O. c. cozumelae	3hMCZMEL	IE				4CozuMx
O. c. cozumelae	3mMCZMEL	IE				5CozuMx
O. c. cozumelae	1mPCZMEL	IE				6CozuMx
O. c. cozumelae	2mPCZMEL	IE				7CozuMx
O. c. cozumelae	3mPCZMEL	IE				8CozuMx
O. c. cozumelae	1mCTCZML	IE				9CozuMX
O. c. cozumelae	2mCTCZML	IE				10CozuMx
O. c. cozumelae	1hRCZMEL	IE				11CozuMx
O. c. cozumelae	2hRCZMEL	IE				12CozuMx
O. c. cozumelae	3hRCZMEL	IE				13CozuMX
O. c. cozumelae	3mRCZMEL	IE				14CozuMx
O. c. cozumelae	4mRCZMEL	IE				15CozuMx
O. c. cozumelae	66hCZMEL	IE				16CozuMx
O. c. cozumelae	66mCZMEL	IE				17CozuMx
O. c. cozumelae	70mCZMEL	IE				18CozuMx
O. c.	3089	CNMA-IBUNAM	San Luis Potosí/ México	22.341300	-99.038608	1SLPMx
O. c.	16243	UAMI		21.384333	-98.990386	2SLPMx
O. c.	22031	CNMA-IBUNAM		22.437800	-99.305600	4SLPMx
O. c.	FJ971271	CMC	Tabasco/ México	17.742300	-91.711983	11TabMx
O. c.	FJ971272	BYU				12TabMx
O. c.	2514	ASK-ROM		17.961756	-92.987833	1TabMx
O. c.	2516	ASK-ROM				2TabMx

O. c.	2518	ASK-ROM				3TabMx
O. c.	2527	ASK-ROM				4TabMx
O. c.	EU074656	ASK-ROM				5TabMx
O. c.	135	ASK-ROM		17.766667	-91.283333	6TabMx
O. c.	136	ASK-ROM				7TabMx
O. c.	141	ASK-ROM				8TabMx
O. c.	143	ASK-ROM				9TabMx
O. c.	153	ASK-ROM				10TabMx
O. c.	TK27059/44927	TTU	Tamaulipas/ México	24.861350	-98.181108	8TmlpMx
O. c. aquaticus	92286	MVZ		23.148333	-99.009722	9TmlpMx
O. c.	3385/2197	ASNHC-ASK		25.842017	-97.497692	5TmlpMx
O. c.	3383/2199	ASNHC-ASK				6TmlpMx
O. c.	3381/2229	ASNHC-ASK				7TmlpMx
O. c.	3375/2190	ASNHC-ASK		22.247506	-97.848122	1TmlpMx
O. c.	3377/2192	ASNHC-ASK				2TmlpMx
O. c.	EU074659	ASK				3TmlpMx
O. c.	3380/2195	ASNHC-ASK				4TmlpMx
O. c. goldmani	EU074661	TTU	Veracrúz/ México	18.035603	-94.196031	8VerMx
O. c. subsp. c.	13858	UAMI		19.100556	-97.030833	9VerMx
O. c. subsp. c.	13861	UAMI		18.893333	-97.006944	10VerMx
O. c. subsp. c.	13869	UAMI		19.921111	-97.274722	11VerMx
O. c.	M-7796	LSUMZ		17.795539	-95.114192	1VerMx
O. c.	M-7797	LSUMZ				2VerMx
O. c. spp. c.	6734	MZFC-M		18.600000	-95.076000	6VerMx
O. c. spp. c.	6737	MZFC-M				7VerMx
O. sp.	M-7654	LSUMZ		19.637653	-96.398125	3VerMx
O. sp.	M-7655	LSUMZ				4VerMx
O. sp.	M-7656	LSUMZ				5VerMx
O. c. goldmani	FJ971269	BYU		18.589697	-95.100925	12VerMx
O. c. goldmani	FJ971270	BYU				13VerMx
O. c. goldmani	FJ971273	CMC				14VerMx
O. c.	6273	ASNHC	Yucatán/ México	19.821586	-89.337986	1YucMx
O. c.	7194	ASNHC		21.590000	-87.986192	2YucMx
O. c.	7195	ASNHC				3YucMx
O. c.	Oc1b	IE		21.576389	-88.075278	4YucMx
O. c.	Oc2	IE				5YucMx

O. c.	Oc3	IE				6YucMx
O. c.	Oc4	IE				7YucMx
O. c.	Oc13	IE				8YucMx
O. c.	Oc15	IE				9YucMx
O. c.	Oc1	IE		21.132222	-90.008333	10YucMx
O. c.	Oc02	IE				11YucMx
O. c.	Oc24	IE				12YucMx
O. c.	Oc28	IE				13YucMx
O. c.	Oc29	IE				14YucMx
O. c.	Oc31	IE				15YucMx
O. c. richmondi	337782	NMNH (SI)	Nicaragua	12.171800	-84.319000	1Nic
O. c. couesi	555693	NMNH (SI)		13.817400	-86.035900	2Nic
O. c.	TK119170/100561	TTU4		13.675500	-84.991833	3Nic
O. c.	FJ971264	ROM		11.928006	-85.959861	9Nic
O. c.	FJ971265	ROM				10Nic
O. c.	TK119172/100575	TTU12		12.166667	-83.916667	4Nic
O. c.	TK119180/100586	TTU16				5Nic
O. c.	TK119186/100602	TTU18				6Nic
O. c.	EU074663	TTU				7Nic
O. c.	EU074664	TTU				8Nic
O. c.	EU074668	MSB	Panamá	7.240000	-80.630000	1Pan
O. c.	EU074669	MSB				2Pan
O. c.	2764	ASNHC	Estados Unidos de América	26.071614	-97.476303	1TxUs
O. c.	EU074665	ACUNHC		26.160439	-98.373403	4TxUs
O. c.	DQ370034	TTU		25.990292	-97.500489	2TxUs
O. c.	EU074662	TTU				3TxUs

La información de la tabla incluye el nombre de identificación de la muestra, donde el nombre de la colección biológica a la que pertenece la muestra son: ACUNHC, Abilene Christian University Natural History Collections; ASNHC, Angelo State Natural History Collection; BYU, Brigham Young University; CMC, Colección de Mamíferos del CEAMISH; Universidad Autónoma del Estado de Morelos; CMNH, Carnegie Museum of Natural History; CNMA-UNAM, Colección Nacional de Mamíferos/UNAM; ECO/SC-M, Colegio de la Frontera Sur/San Cristobal de las Casas; FMNH, The Field Museum of Natural History; IE, Instituto de Ecología/UNAM; KUM, Kansas University Museum; LSUMZ, Louisiana State University Museum of Natural Science; MVZ, Museum of Vertebrate Zoology-University of Berkeley; MZFC-UNAM, Museo de Zooología de la Facultad de Ciencias/UNAM; NMNH(SI), National Museum of Natural History/Smithsonian Institute; RMT, Robert M. Timm/vouchers at University of Kansas Museum of Natural History KUM; ROM, Royal Ontario Museum; TTU, Texas Tech University Mammal Collection; UAMI, Universidad Autónoma de México/Iztapalapa; USAC, Universidad de San Carlos/Guatemala. Respecto al nombre de la especie O. c. es la abreviación de *Oryzomys couesi*.

Tabla 2. *Características de los primers diseñados*

Nombre	Dirección	Secuencia 5' a 3'	Tamaño pb	GC	% contenido de GC	Tm ˚C
1Ory_CytB_47F	F	ACTCATTCATTGACCTGCCAACCC	24	12	50.0	59.4
1Ory_CytB_219R	R	TACGTCTCGGCAGATGTGAGTTACTG	26	13	50.0	59.5
2Ory_CytB_159F	F	ACACTACACATCAGATACAACAACAGC	27	11	40.7	56.8
2Ory_CytB_270R	R	GAATATTGATGCGCCGTTAGCGTG	24	12	50.0	58.5
3Ory_CytB_230F	F	GACTTATYCGATATGCCCACGCTA	24	12	50.0	58.0
3Ory_CytB_347R	R	CCGATGTTTCAGGTTTCGTTGAGT	24	11	45.8	57.3
4Ory_CytB_338F	F	TCACGTCGGACGAGGAATGTACTA	24	12	50.0	58.7
4Ory_CytB_461R	R	TTGTCCTCATGGAAGTACGTAGCC	24	12	50.0	58.1
5Ory_CytB_401F	F	CATGAGGACAAATATCATTCTGAGGAGC	28	12	57.0	42.9
5Ory_CytB_542R	R	AAGGCGAAGAATCGGGTTAGTGTG	24	12	58.9	50.0
6Ory_CytB_507F	F	AGTAGACAAAGCCACACTAACCCG	24	12	58.5	50.0
6Ory_CytB_646R	R	CTGAGTTTGAGTTTAGTCCTGAGGG	25	12	56.6	48.0
7Ory_CytB_622F	F	CCCTCAGGACTAAACTCAAAC	21	10	52.5	47.6
7Ory_CytB_752R	R	CCGAGAACATCTGGGAAA	18	9	51.4	50.0
8Ory_CytB_738F	F	CCCAGATGTTCTCGGA	16	9	50.2	56.3
8Ory_CytB_852R	R	GATTGAGCGTAGAATAGCGT	20	9	51.9	45.0

Donde: F, forward o en dirección adelante; pb, pares de bases; R, reverse o hacia atrás y Tm, tiempo de fusión;

Tabla 3. *Valores de diversidad genética y pruebas de neutralidad para los grupos filogenéticos de O. couesi*

Grupo	N	Hp	h (±DS)	Π (±DS)	S	*k*	Tajima's D	Fu's F	R2	SSD	P (SSD Sim ≥ SSD Obs)
Golfo	63	52	0.990 (0.006)	0.01443 (0.00124)	107	10.447	**-1.87054***	**-3.19425***	**0.0416*****	0.00155	0.9387
América Central	29	26	0.993 (0.011)	0.01411 (0.00169)	76	10.224	-1.79788	**-2.91538***	**0.0498*****	0.00130	0.9446
Pacífico	56	48	0.994 (0.005)	0.01993 (0.00134)	95	14.366	-1.06860	**-2.98314***	0.0678	0.00413	0.8504
Península	35	18	0.859 (0.055)	0.00534 (0.00120)	46	3.887	**-2.35966****	**-3.41231****	**0.0487*****	0.00446	0.8282
Península Basal	12	9	0.939 (0.058)	0.00563 (0.00133)	16	4.106	-0.97152	-1.22080	0.1163	0.00708	0.7784
América del Sur	4	4	1	0.01565 (000380)	20	11.333					
Costa Rica	2	2	1	0.00410 (0.500)	3	3					

Los códigos corresponden a: *N*, número de muestras; *Hp*, número de haplotipos; *h* diversidad haplotípica y π nucleotídica; *S*, número de sitios polimórficos; *k*, media de diferencias pareadas; *r*, índice de Harpending; R_2 índice de Ramos-Onsins y Rozas. Los valores de significancia de las pruebas de neutralidad son: *P<0.05, ** P<0.01, ***P<0.001. Valores bajos de P ($SSD_{Sim} \geq SSD_{Obs}$) indicarían el rechazo del modelo de expansión.

Tabla 4. Divergencia nucleotídica neta (Da ± desviación estándar; sobre la diagonal) y distancia p-no corregida (Dx ± desviación estándar; bajo la diagonal) entre los grupos de *O. couesi*

		Da						
		Golfo	AC	Pacífico	Península	PB	AS	CR
Dx	Golfo		0.00470 (0.00136)	0.01576 (0.00164)	0.00537 (0.00119)	0.00576 (0.00141)	0.04927 (0.00702)	0.09926 (0.01402)
	AC	0.01897 (0.00137)		0.01928 (0.00222)	0.00353 (0.00152)	0.00352 (0.00175)	0.04823 (0.00871)	0.09959 (0.01847)
	Pacífico	0.03294 (0.00139)	0.03631 (0.00206)		0.01917 (0.00241)	0.01774 (0.00249)	**0.05414 (0.00780)**	0.09338 (0.01345)
	Península	0.01526 (0.00111)	0.01326 (0.00152)	0.03181 (0.00220)		0.00230 (0.00132)	0.05233 (0.01371)	0.10131 (0.03129)
	PB	0.01579 (0.00141)	0.01399 (0.00178)	0.03052 (0.00231)	0.00779 (0.00131)		0.05148 (0.01403)	0.09650 (0.02949)
	AS	0.06431 (0.00665)	0.06311 (0.00846)	**0.07193 (0.00745)**	**0.06282 (0.01345)**	0.06282 (0.01354)		**0.10910 (0.04728)**
	CR	0.10853 (0.01398)	0.10870 (0.01844)	0.10540 (0.01340)	0.10603 (0.03127)	0.10131 (0.02947)	**0.11898 (0.04724)**	

Los números resaltados y sombreados indican los valores más altos y más bajos respectivamente.

Tabla 5. Resultados de estructuración genética de *Oryzomys couesi* a partir de cinco hipótesis de agrupamiento

%de la variación	Taxonómica Goldman (1918)	Taxonómica Hanson (2009)	Filogenética	Filogeográfica	Ecológica
Entre grupos	**38.01**	**47.73**	**50.26**	**72.11**	6.46
Entre poblaciones dentro de los grupos	35.95	30.75	30.75	18.99	**65.09**
Dentro de las poblaciones	26.04	21.51	18.99	8.9	28.44
Índices de fijación					
Φ_{CT}	0.38014	0.47734	0.50257	0.72109	0.06461
Φ_{SC}	0.5799	0.58843	0.61814	0.68101	0.69591
Φ_{ST}	**0.73959**	**0.78489**	**0.81005**	**0.91103**	**0.71556**

Donde: los números resaltados en negritas son el mayor de cada agrupación, los números con fondo sombreado son los menores para cada agrupación, los números rojos son el dato mayor del conseguido de todas las agrupaciones y en azul el dato menor obtenido de todas las agrupaciones. Todos los índices tienen valores de significancia $p<0.001$.

Tabla 6. Resultados de estructuración genética de *Ototylomys phyllotis* a partir de cuatro hipótesis de agrupamiento

% de la variación	Taxonómica Lawlor	Filogenética	Filogeográfica	Ecológica
Entre grupos	35.14	**75.3**	**77.11**	51.2
Entre poblaciones dentro de los grupos	**55.19**	13.86	9.29	**37.48**
Dentro de las poblaciones	9.67	10.85	13.6	11.32
Índices de Fijación				
Φ_{CT}	0.35143	0.75297	**0.7711**	0.51196
Φ_{SC}	0.85092	0.56096	0.4059	0.76801
Φ_{ST}	**0.90331**	**0.89154**	0.864	**0.88678**

Donde: los números resaltados en negritas son el mayor de cada agrupación, los números con fondo sombreado son los menores para cada agrupación, los números rojos son el dato mayor del conseguido de todas las agrupaciones y en azul el dato menor obtenido de todas las agrupaciones. Todos los índices tienen valores de significancia $p<0.001$.

Tabla 7. Variables ecológicas relevantes para cada agrupación de *Ototylomys phyllotis*

AGRUPACIONES			
Taxonómica	Filogenética	Filogeográfica	Ecológica
BIO 13	BIO 19	BIO 19	BIO 19
BIO 16	BIO 4	BIO 14	BIO 16
BIO 15	BIO 16	BIO 8	BIO 12
BIO 4	BIO 13	BIO 17	BIO 13
BIO 12	BIO 12	BIO 12	BIO 17

Nota: la selección de las variables se hizo de acuerdo a los valores de Lambda de Wilks, valores de F y significancia mayor al 98%.

Tabla 8. Variables ecológicas relevantes para cada agrupación de *Oryzomys couesi*

AGRUPACIONES				
Taxonómica Goldman	Taxonómica Hanson	Filogenética	Filogeográfica	Ecológica
BIO 15	BIO 15	BIO 15	BIO 19	BIO 4
BIO 19	BIO 2	BIO 19	BIO 17	BIO 12
BIO 4	BIO 19	BIO 3	BIO 14	Altitud snm
BIO 3	BIO 17	BIO 17	BIO 12	BIO 16
BIO 7	BIO 14	BIO 4	BIO 7	BIO 13

Nota: la selección de las variables se hizo de acuerdo a los valores de Lambda de Wilks, valores de F y significancia mayor al 99%.

Tabla 9. Estadísticos de resumen de la estructura genética, diversidad y divergencia de *Oryzomys couesi* y *Ototylomys phyllotis* de acuerdo a la hipótesis de barreras geológicas principales de América Central

Estadístico	Descripción	*Oryzomys couesi*	*Ototylomys phyllotis*
% variación AMOVA	Entre grupos	25.43	**68.38**
	Entre poblaciones dentro de los grupos	**40.98**	22.55
	Dentro de las poblaciones	33.6	9.07
Índices de fijación AMOVA	Φ_{CT}	0.25425	0.68375
	Φ_{SC}	0.54949	0.71311
	Φ_{ST}	**0.66403**	**0.90927**
Divergencias Da/Dx±DS	Península vs Chiapas-Guatemala	0.02281±0.02013/ 0.06897±0.01949	0.04898±0.00341/ 0.06223±0.00333
	Península vs AC	0.00147±0.00127/ 0.01675±0.00126	**0.05326±0.00413/ 0.07536±0.00362**
	Chiapas-Guatemala vs AC	0.00043±0.00372/ 0.02069±0.00391	0.04668±0.00528/ 0.06668±0.00506
	AC vs Panameño	**0.02829±0.01381/ 0.05503±0.01082**	0.02616±0.00854/ 0.04054±0.00851
Diversidad haplotípica h	Península	0.953±0.018	0.977±0.007
	Chiapas-Guatemala	1.000±0.126	0.926±0.026
	AC	0.995±0.007	0.991±0.013
	Panameño	1.000±0.126	N
Diversidad nucleotídica π	Península	0.01006±0.00096	0.01525±0.00170
	Chiapas-Guatemala	0.02005±0.00521	0.01125±0.00127
	AC	0.02048±0.00172	**0.02876±0.00273**
	Panameño	**0.03300±0.01064**	N

Donde: los valores más altos están en letras negritas y los valores más bajos están sombreados; N, significa que no se calculó.

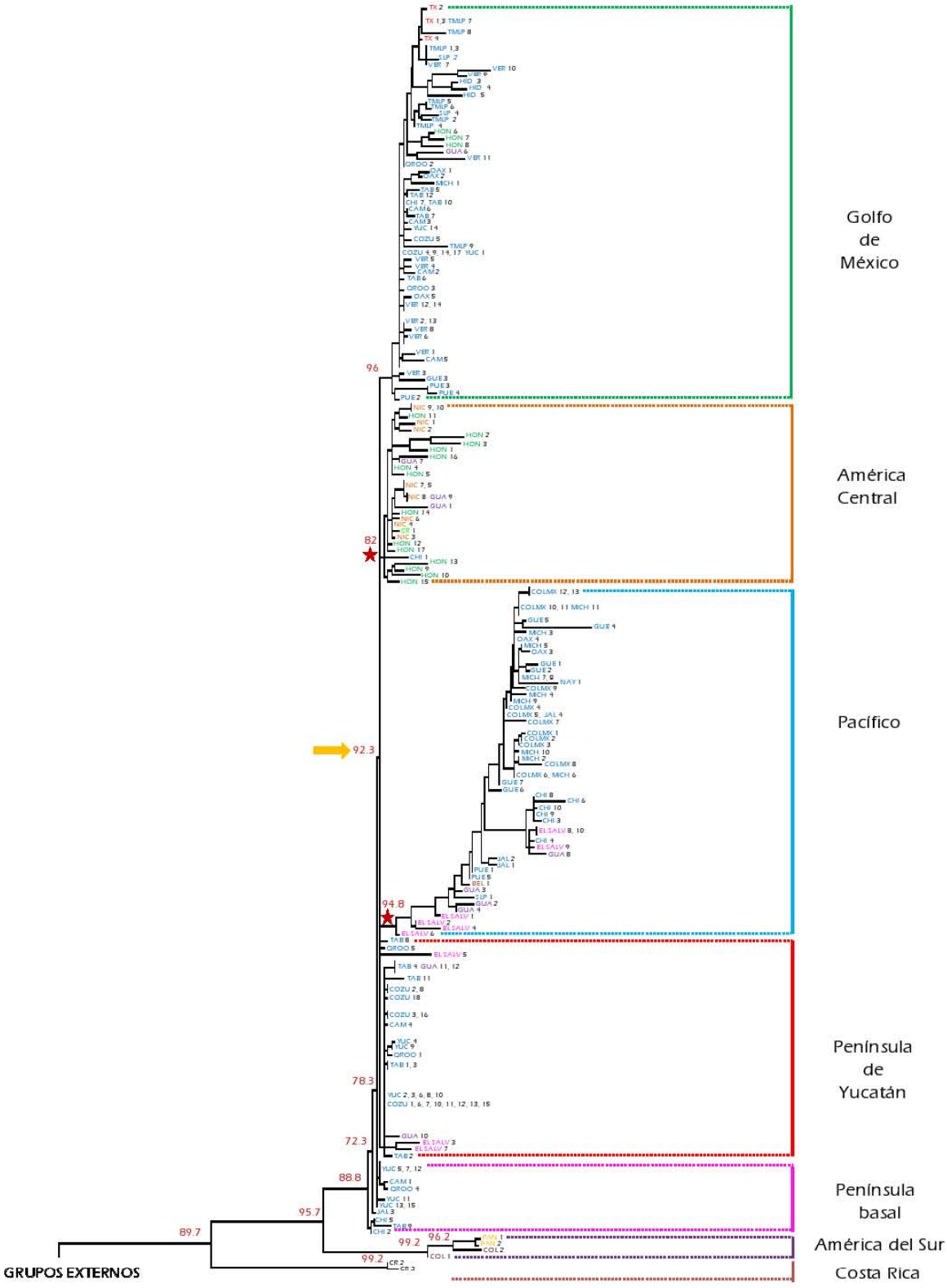


Figura 1. Árbol filogenético de máxima verosimilitud de *O. couesi* con los grupos filogenéticos. Los números rojos corresponden a los valores de aLRT. La punta de las ramas tiene el nombre de la muestra, tabla 1. La distribución geográfica de los grupos se pueden observar en el Anexo 3.

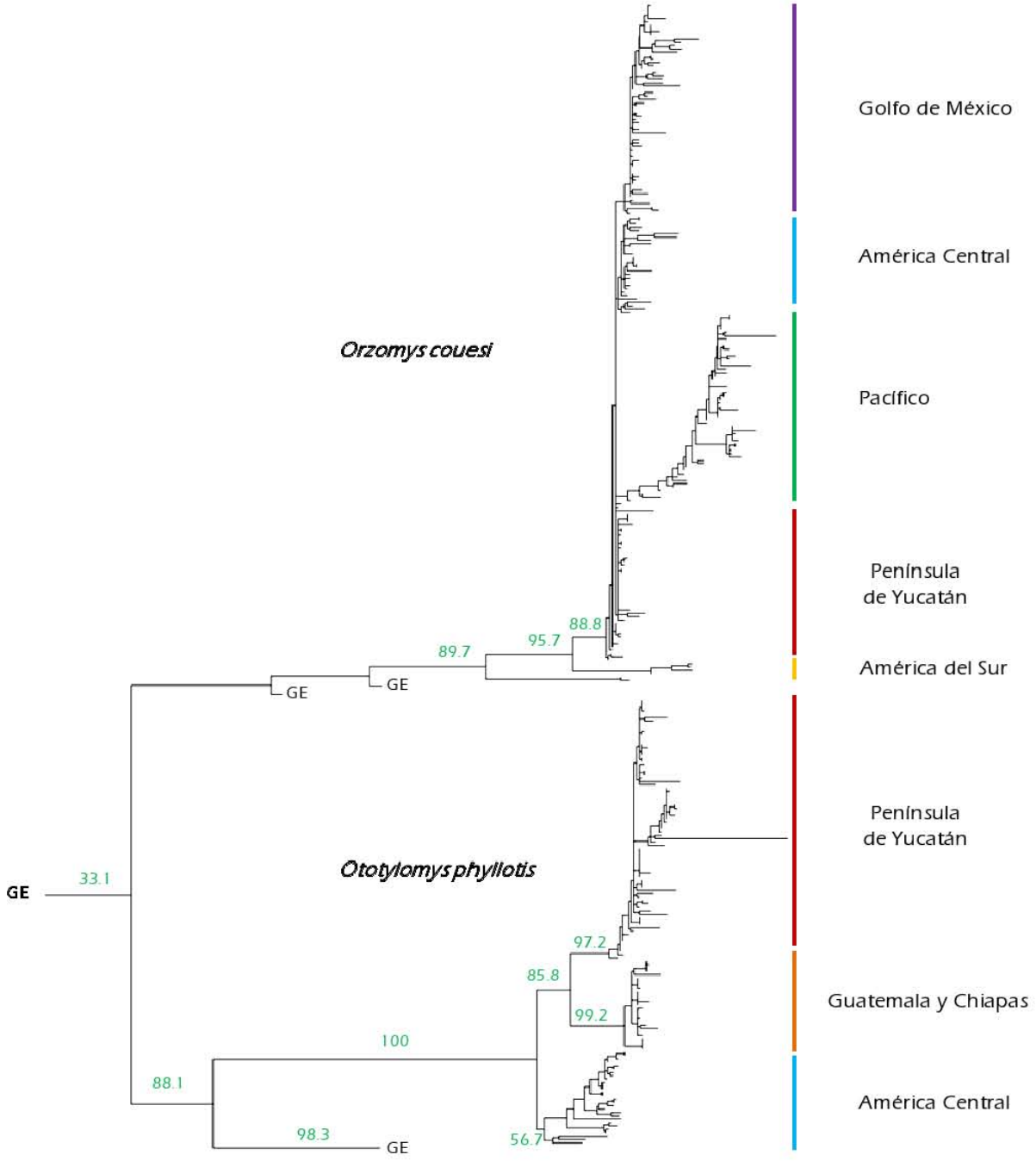


Figura 2. Topología del árbol de máxima verosimilitud de 710 pares de bases de citocromo b de *O. couesi* y *O. phyllotis*. En el árbol, GE indica el lugar donde están posicionados los grupos externos. A la derecha se indican los grupos filogenéticos. Los valores en verde indican el valor de aLRT.

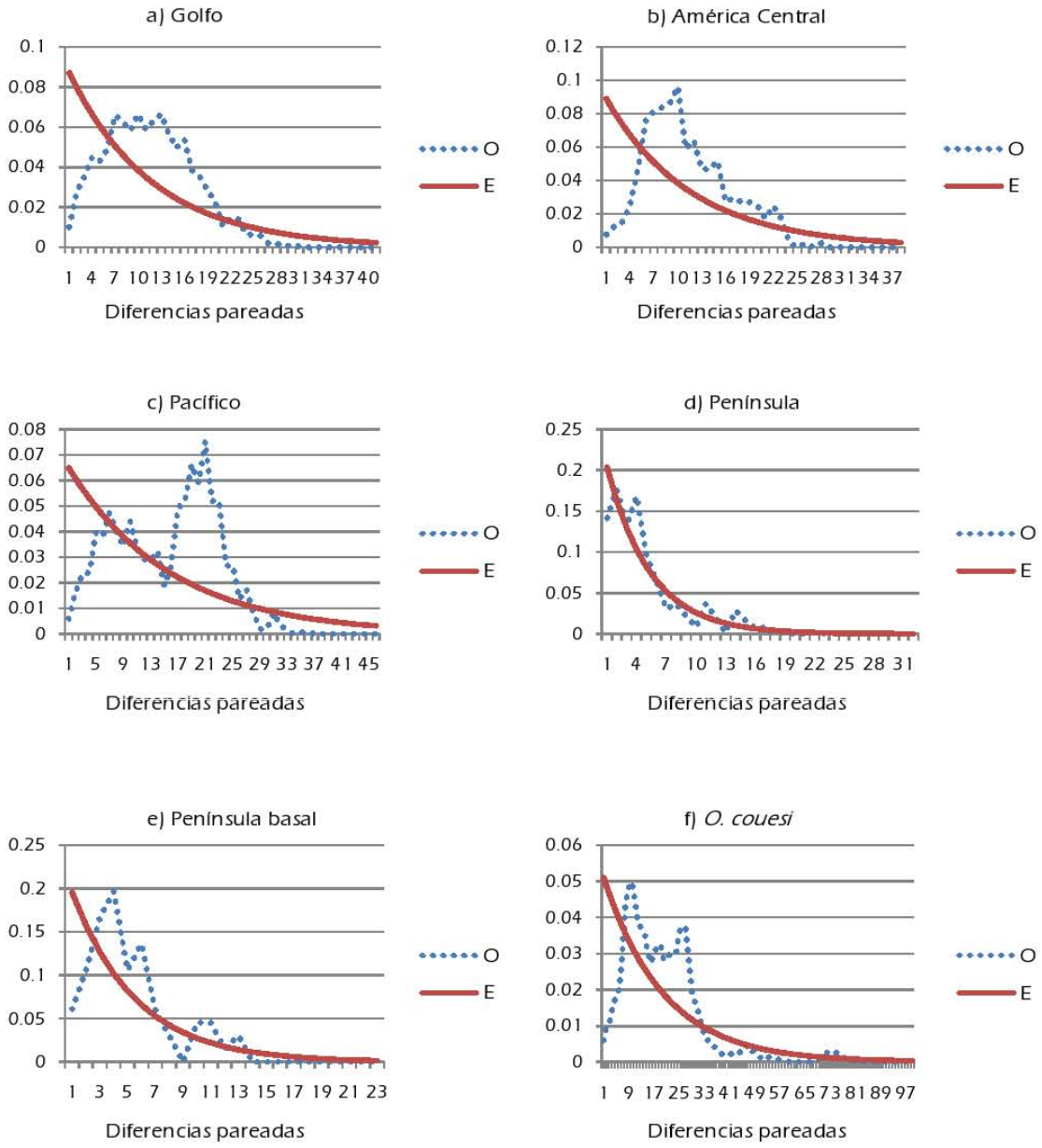


Figura 3. Gráficas del análisis de mismatch para los grupos filogenéticos de *O. couesi* donde se representan las frecuencias observadas (O) y las frecuencias esperadas (E) de acuerdo a un modelo de población de tamaño constante. Los valores para los grupos filogenéticos corresponden a las gráficas *a-e*, mientras que el análisis para la especie completa se observa en la *f*.

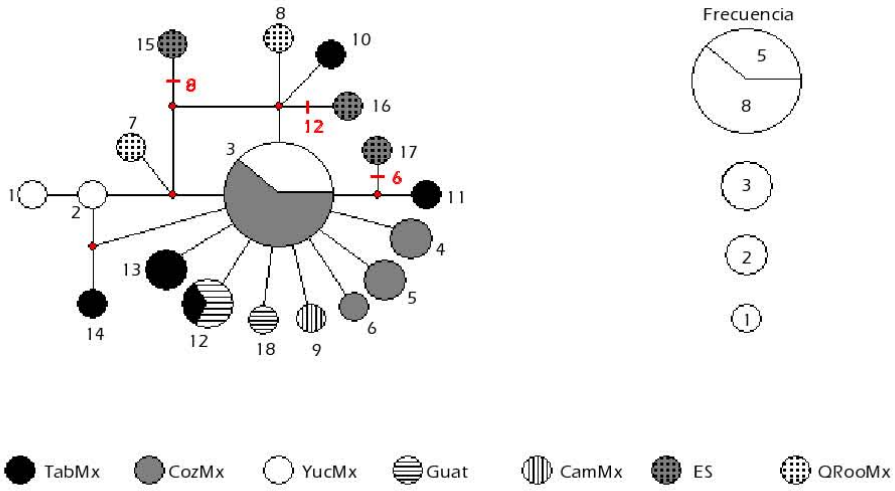


Figura 4. Red de haplotipos del grupo filogenético Península (PY) de *O. couesi*. Los números en rojo corresponden al número de pasos mutacionales. Las muestras a las que corresponden los haplotipos están en el Anexo 1. Las localidades están organizadas por país, ES corresponde a El Salvador y Guat a Guatemala. En el caso de que las localidades de México se agregó la terminación (Mx) y son: TabMx, Tabasco; CozMx, Cozumel; YucMx, Yucatán; CamMx, Campeche; y QRooMx, QuintanaRoo.

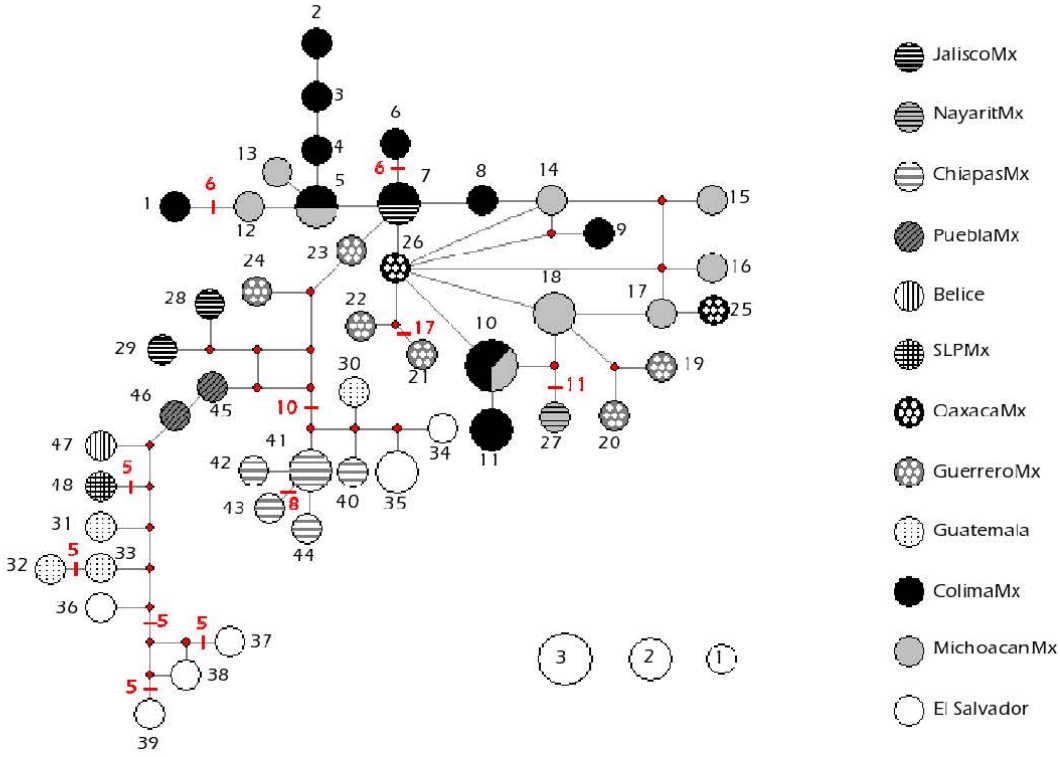


Figura 5. Red de haplotipos del grupo filogenético Pacífico (PA) de *O. couesi*. Los números en rojo corresponden al número de pasos mutacionales. Las localidades están organizadas por país y en el caso de México se agregó la terminación Mx a cada estado. La abreviación SLP corresponde al estado San Luis Potosí. Las muestras a las que pertenecen los haplotipos están en el Anexo 1.

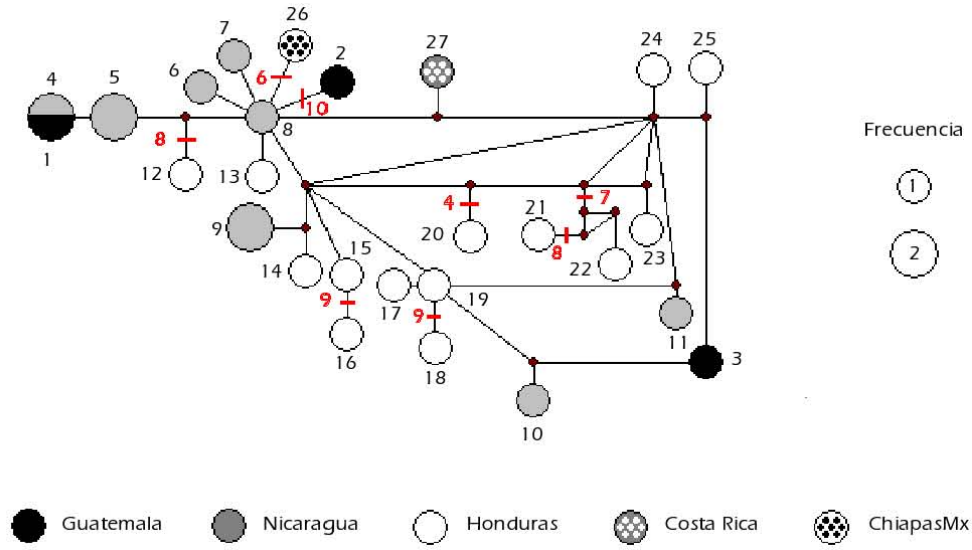


Figura 6. Red de haplotipos del grupo filogenético América Central (AC) de *O. couesi*. Los números en rojo corresponden al número de pasos mutacionales. Las localidades están organizadas por país. En el caso de México se agregó la terminación Mx. Las muestras a las que corresponden los haplotipos están en el Anexo 1.

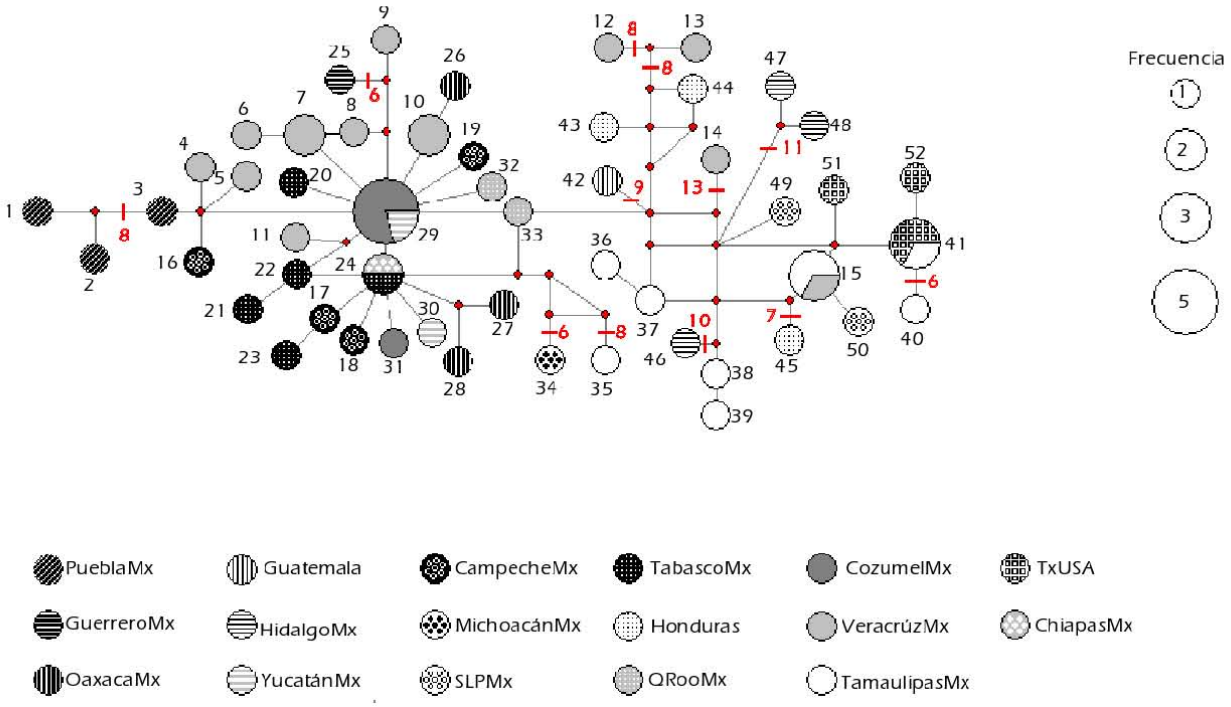


Figura 7. Red de haplotipos del grupo filogenético Golfo (G) de *O. couesi*. Los números en rojo corresponden al número de pasos mutacionales. Las muestras a las que corresponden los haplotipos están en el Anexo 1. Las localidades están organizadas por país. En el caso de Estados Unidos de Norteamérica y México se agregaron las terminaciones USA y Mx respectivamente a cada Estado. Las abreviaciones son: QRoo, QuintanaRoo y SLP, San Luis Potosí.

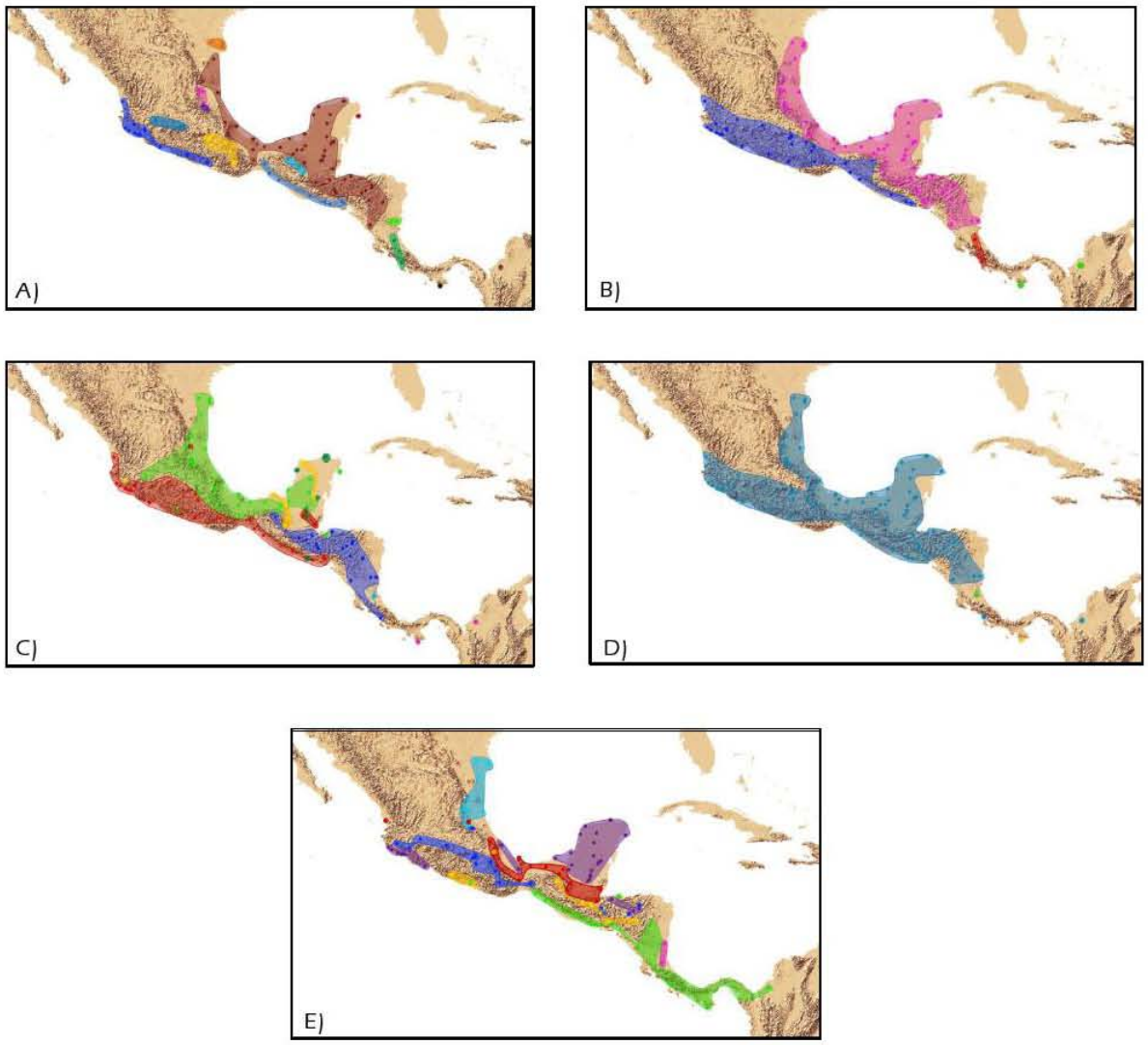


Figura 8. Agrupaciones de las muestras de *Oryzomys couesi* de acuerdo con diferentes criterios. El detalle de los grupos está en el anexo 2. Las figuras corresponden a las siguientes agrupaciones: A) Taxonómica de Goldman (1918), B) Taxonómica de Hanson (2010), C) Filogenéticos, D) Filogeográficos (SAMOVA) y, E) Ecológicos

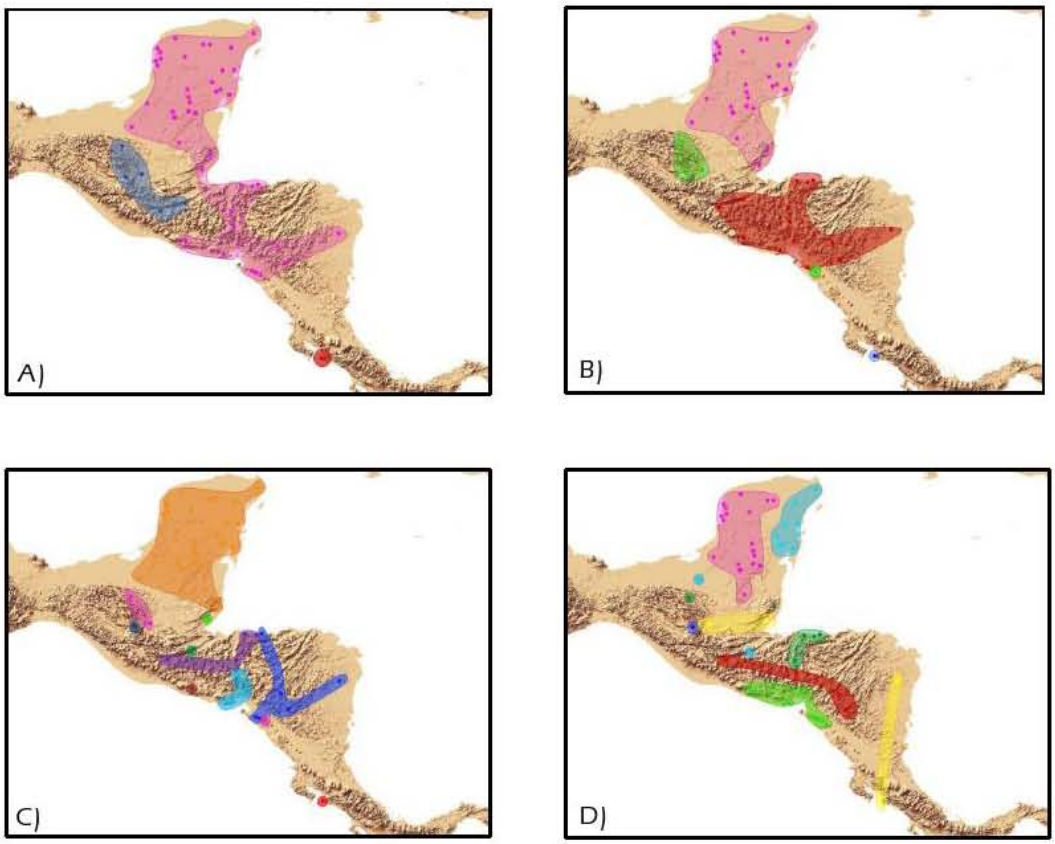


Figura 9. Agrupaciones de las muestras de *Ototylomys phyllotis* de acuerdo con diferentes criterios. El detalle de los grupos está en el anexo 3. Las letras de la figura corresponden a las agrupaciones siguientes: A) Taxonómica de Lawlor (1989), B) Filogenética, C) Filogeográfica y, D) Ecológica

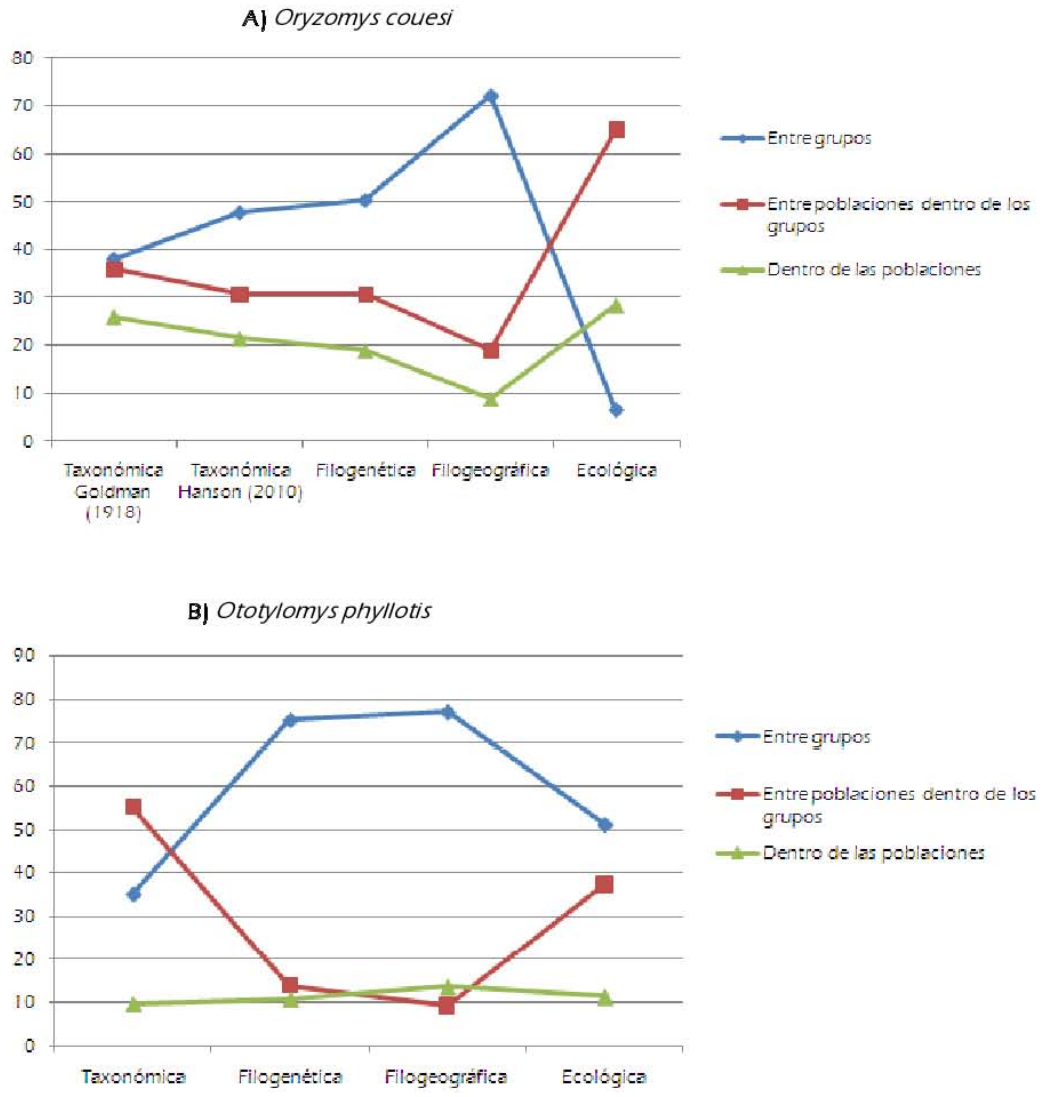


Figura 10. Gráficas del porcentaje de variación calculado para cada agrupación utilizando Análisis de Varianza Molecular (AMOVA)

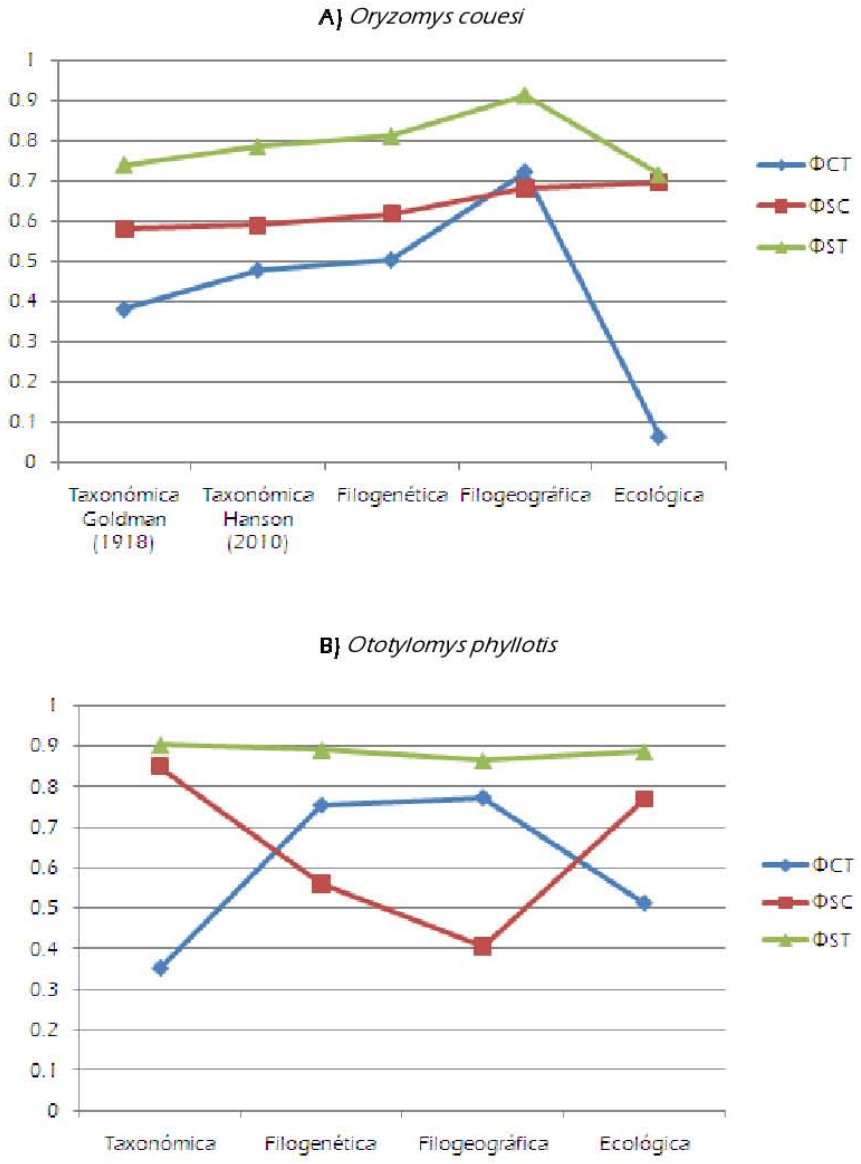


Figura 11. Gráficas del Índice de fijación calculado para cada agrupación utilizando Análisis de Varianza Molecular (AMOVA)

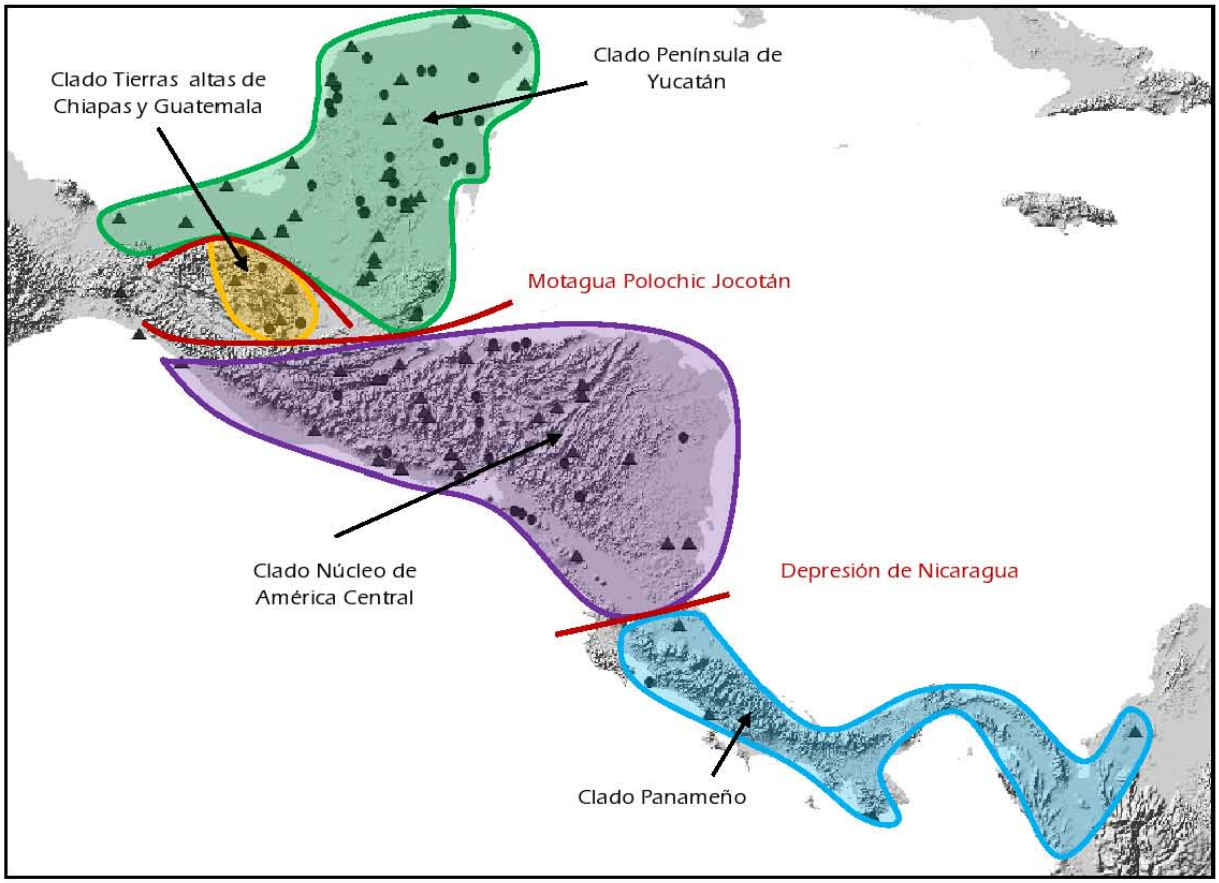


Figura 12. Principales clados de América Central derivados de la historia geológica de acuerdo con la revisión de Gutiérrez-García y Vázquez-Domínguez (2013). Las líneas en rojo representan las barreras geológicas principales. En el mapa se observan las localidades de las muestras usadas para el estudio comparado, con un triángulo las de *O. couesi* y con un círculo las de *O. phyllotis*

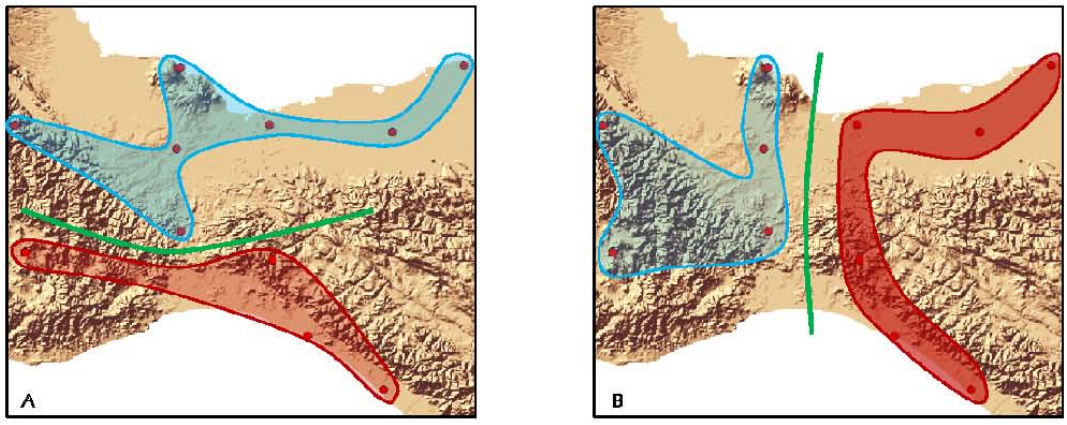


Figura 13. Hipótesis sobre el efecto del istmo de Tehuantepec en las poblaciones de *O. couesi*. El cuadro A muestra la hipótesis norte-sur, el cuadro B muestra la hipótesis este-oeste.

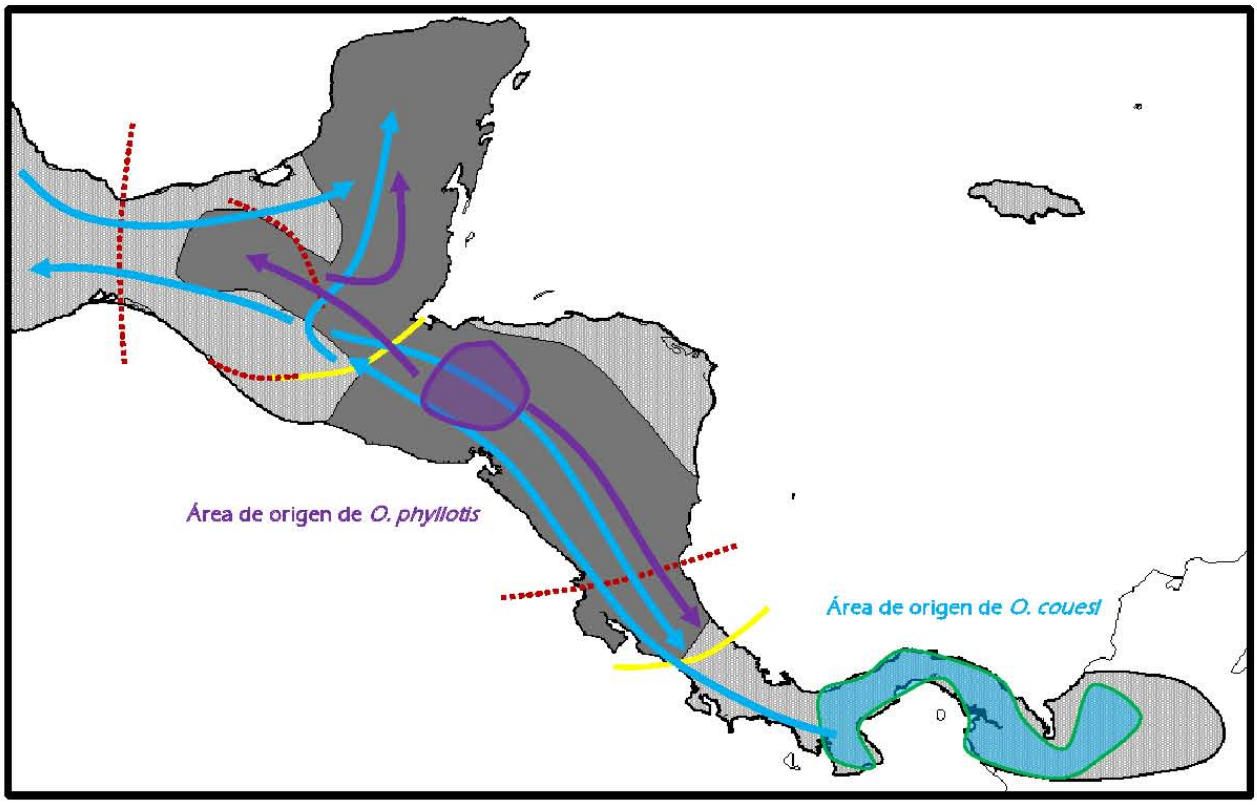
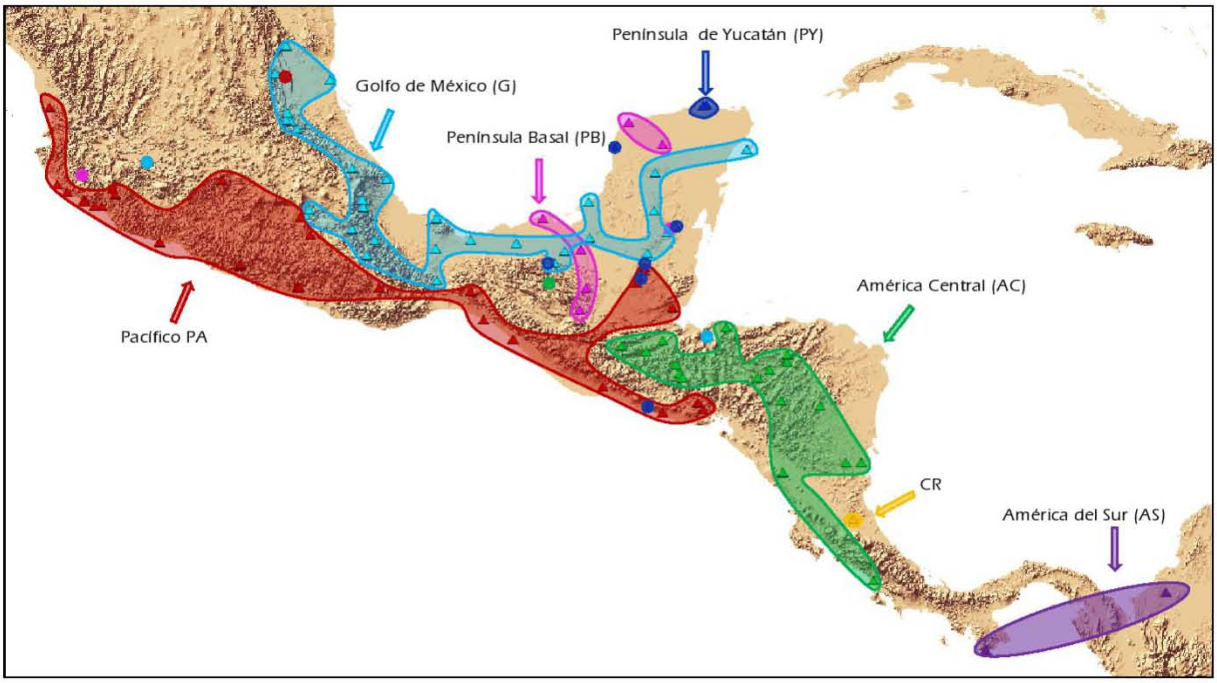


Figura 14. Esquema de resumen de los patrones concordantes y discordantes del origen y migración de *O. couesi* (azul) y *O. phyllotis* (morado). La línea amarilla corresponde a las barreras geográficas concordantes, mientras que la línea punteada roja señala las barreras no concordantes.



Anexo 1. Grupos filogenéticos de *O. couesi*, donde se muestra el grupo altamente divergente de Costa Rica (color amarillo).

ANEXO 2. Tabla de agrupaciones mayores de *O. couesi*

		AGRUPACIONES				
ID muestra	ID de la red de haplotipos	A	B	C	D	E
1Bel	47PA	12	4	1	3	1
1Col	1AS	12	2	6	3	2
2Col	2AS	12	2	6	3	2
1CoRi	27AC	10	1	2	3	2
2CoRi	3CR	10	1	7	1	5
3CoRi	4CR	10	1	7	1	5
1ElSalv	36PA	8	4	1	3	2
2ElSalv	38PA	8	4	1	3	7
3ElSalv	15PY	8	4	4	3	7
4ElSalv	39PA	8	4	1	3	7
5ElSalv	16PY	8	4	1	3	7
6ElSalv	37PA	8	4	1	3	7
7ElSalv	17PY	8	3	4	3	2
8ElSalv	35PA	8	3	1	3	2
9ElSalv	34PA	8	3	1	3	2
10ElSalv	35PA	8	3	1	3	2
1Gua	2AC	12	4	2	3	1
2Gua	32PA	12	4	1	3	6
3Gua	31PA	12	4	1	3	6
4Gua	33PA	12	4	1	3	6
6Gua	42G	12	4	3	3	6
7Gua	3AC	12	4	2	3	7
8Gua	30PA	8	3	1	3	2
9Gua	1AC	12	4	2	3	7
10Gua	18PY	12	4	4	3	6
11Gua	12PY	12	4	4	3	6
12Gua	12PY	12	4	4	3	6
1Hon	17AC	12	4	2	3	7
2Hon	21AC	12	4	2	3	3
3Hon	22AC	12	4	2	3	3
4Hon	19AC	12	4	2	3	3
5Hon	18AC	12	4	2	3	3
6Hon	44G	12	4	3	3	6
7Hon	43G	12	4	3	3	6
8Hon	45G	12	4	3	3	6
9Hon	20AC	12	4	2	3	6
10Hon	16AC	12	4	2	3	6
11Hon	14AC	12	4	2	3	6
12Hon	15AC	12	4	2	3	6
13Hon	12AC	12	4	2	3	3
14Hon	13AC	12	4	2	3	6
15Hon	23AC	12	4	2	3	2

16Hon	25AC	12	4	2	3	2
17Hon	24AC	12	4	2	3	2
1CamMx	5PB	12	4	5	3	6
2CamMx	16G	12	4	3	3	6
3CamMx	18G	12	4	3	3	6
4CamMx	9PY	12	4	4	3	6
5CamMx	19G	12	4	3	3	6
6CamMx	17G	12	4	3	3	6
1ChiMx	26AC	7	3	2	3	7
2ChiMx	6PB	12	4	5	3	1
3ChiMx	42PA	8	3	1	3	3
4ChiMx	40PA	8	3	1	3	2
5ChiMx	7PB	7	4	5	3	1
6ChiMx	43PA	8	3	1	3	2
7ChiMX	24G	12	4	3	3	1
8ChiMx	41PA	8	3	1	3	2
9ChiMx	41PA	8	3	1	3	2
10ChiMx	44PA	12	4	1	3	1
1ColMx	2PA	9	3	1	3	3
2ColMX	3PA	9	3	1	3	3
3ColMx	4PA	9	3	1	3	6
4ColMx	8PA	9	3	1	3	6
5ColMx	7PA	9	3	1	3	6
6ColMx	5PA	9	3	1	3	6
7ColMx	6PA	9	3	1	3	6
8ColMx	1PA	9	3	1	3	6
9ColMx	9PA	9	3	1	3	6
10ColMx	10PA	9	3	1	3	6
11ColMx	10PA	9	3	1	3	6
12ColMx	11PA	9	3	1	3	6
13ColMx	11PA	9	3	1	3	6
1GueMx	20PA	9	3	1	3	7
2GueMx	19PA	9	3	1	3	7
3GueMx	25G	9	3	3	3	7
4GueMx	21PA	9	3	1	3	7
5GueMx	22PA	9	3	1	3	2
6GueMx	24PA	9	3	1	3	7
7GueMx	23PA	9	3	1	3	7
3HidMx	48G	4	4	3	3	4
4HidMx	47G	4	4	3	3	3
5HidMx	46G	4	4	3	3	4
1JalMx	29PA	9	3	1	3	6
2JalMx	28PA	9	3	1	3	6
3JalMx	8PB	9	3	5	3	3
4JalMx	7PA	9	3	1	3	6

1MichMx	34G	6	3	3	3	3
2MichMx	12PA	9	3	1	3	6
3MichMx	16PA	9	3	1	3	6
4MichMx	15PA	9	3	1	3	6
5MichMx	17PA	9	3	1	3	6
6MichMx	5PA	9	3	1	3	6
7MichMx	18PA	9	3	1	3	6
8MichMx	18PA	9	3	1	3	6
9MichMx	14PA	9	3	1	3	6
10MichMx	13PA	9	3	1	3	6
11MichMx	10PA	6	3	1	3	3
1NayMx	27PA	9	3	1	3	1
1OaxMx	28G	3	3	3	3	1
2OaxMx	27G	3	3	3	3	3
3OaxMx	25PA	3	3	1	3	3
4OaxMx	26PA	3	3	1	3	3
5OaxMx	26G	12	3	3	3	1
1PueMx	45PA	3	3	1	3	3
2PueMx	3G	3	3	3	3	3
3PueMx	1G	3	3	3	3	3
4PueMx	2G	3	3	3	3	3
5PueMx	46PA	3	3	1	3	3
1QRooMx	7PY	12	4	4	3	6
2QRooMx	33G	12	4	3	3	6
3QRooMx	32G	12	4	3	3	6
4QRooMx	9PB	12	4	5	3	6
5QRooMx	8PY	12	4	5	3	6
1CozuMx	3PY	13	4	4	3	6
2CozuMx	5PY	13	4	4	3	6
3CozuMx	4PY	13	4	4	3	6
4CozuMx	29G	13	4	3	3	6
5CozuMx	31G	13	4	3	3	6
6CozuMx	3PY	13	4	4	3	6
7CozuMx	3PY	13	4	4	3	6
8CozuMx	5PY	13	4	4	3	6
9CozuMX	29G	13	4	3	3	6
10CozuMx	3PY	13	4	4	3	6
11CozuMx	3PY	13	4	4	3	6
12CozuMx	3PY	13	4	4	3	6
13CozuMX	3PY	13	4	4	3	6
14CozuMx	29G	13	4	3	3	6
15CozuMx	3PY	13	4	4	3	6
16CozuMx	4PY	13	4	4	3	6
17CozuMx	29G	13	4	3	3	6
18CozuMx	6PY	13	4	4	3	6

1SLPMx	48PA	5	4	1	3	4
2SLPMx	50G	5	4	3	3	1
4SLPMx	49G	5	4	3	3	4
1TabMx	13PY	12	4	4	3	1
2TabMx	14PY	12	4	4	3	1
3TabMx	13PY	12	4	4	3	1
4TabMx	12PY	12	4	4	3	1
5TabMx	21G	12	4	3	3	1
6TabMx	20G	12	4	3	3	6
7TabMx	23G	12	4	3	3	6
8TabMx	10PY	12	4	4	3	6
9TabMx	10PB	12	4	5	3	6
10TabMx	24G	12	4	3	3	6
11TabMx	11PY	12	4	4	3	1
12TabMx	22G	12	4	3	3	1
1TmlpMx	15G	12	4	3	3	4
2TmlpMx	36G	12	4	3	3	4
3TmlpMx	15G	12	4	3	3	4
4TmlpMx	37G	12	4	3	3	4
5TmlpMx	38G	2	4	3	3	4
6TmlpMx	39G	2	4	3	3	4
7TmlpMx	41G	2	4	3	3	4
8TmlpMx	40G	12	4	3	3	4
9TmlpMx	35G	12	4	3	3	4
1VerMx	11G	12	4	3	3	6
2VerMx	7G	12	4	3	3	6
3VerMx	9G	12	4	3	3	6
4VerMx	5G	12	4	3	3	6
5VerMx	4G	12	4	3	3	6
6VerMx	8G	12	4	3	3	1
7VerMx	15G	12	4	3	3	1
8VerMx	6G	12	4	3	3	1
9VerMx	13G	12	4	3	3	7
10VerMx	12G	12	4	3	3	1
11VerMx	14G	12	4	3	3	1
12VerMx	10G	12	4	3	3	1
13VerMx	7G	12	4	3	3	1
14VerMx	10G	12	4	3	3	1
1YucMx	29G	12	4	3	3	6
2YucMx	3PY	12	4	4	3	6
3YucMx	3PY	12	4	4	3	6
4YucMx	1PY	12	4	4	3	6
5YucMx	11PB	12	4	5	3	6
6YucMx	3PY	12	4	4	3	6
7YucMx	11PB	12	4	5	3	6

8YucMx	3PY	12	4	4	3	6
9YucMx	2PY	12	4	4	3	6
10YucMx	3PY	12	4	4	3	6
11YucMx	12PB	12	4	5	3	6
12YucMx	11PB	12	4	5	3	6
13YucMx	13PB	12	4	5	3	6
14YucMx	30G	12	4	3	3	6
15YucMx	13PB	12	4	5	3	6
1Nic	10AC	11	4	2	3	2
2Nic	11AC	12	4	2	3	7
3Nic	7AC	12	4	2	3	2
4Nic	8AC	11	4	2	3	5
5Nic	5AC	11	4	2	3	5
6Nic	6AC	11	4	2	3	5
7Nic	5AC	11	4	2	3	5
8Nic	4AC	11	4	2	3	5
9Nic	9AC	12	4	2	3	2
10Nic	9AC	12	4	2	3	2
1Pan	5	1	2	6	2	2
2Pan	6	1	2	6	2	2
1TxUs	41G	2	4	3	3	4
2TxUs	52G	2	4	3	3	4
3TxUs	41G	2	4	3	3	4
4TxUs	51G	2	4	3	3	4

En la tabla, el ID de los haplotipos corresponde a la red de haplotipos del grupo filogenético en que se encuentran: AC América Central, AS América del Sur, CR Costa Rica, G Golfo, PA Pacífico, PB Península Basal y PY Península de Yucatán. Las agrupaciones se refieren a: A) Taxonómica de Goldman (1918), B) Taxonómica de Hanson (2010), C) Filogenética, D) SAMOVA y, E) Ecológica.

Anexo 3. Tabla de agrupaciones mayores de *O. phyllotis*

ID muestra*	ID de la red de haplotipos*	AGRUPACIONES A	B	C	D
Op1832CR	1	1	4	1	1
Op64128SMS	22	3	1	6	4
Op64129SMS	23	3	1	6	4
Op565166LPH	20	3	1	6	2
Op565167VCH	21	3	1	6	4
Op64121LLS	24	3	1	2	4
Op565162ZG	25	2	1	4	6
Op1341SMG	2	2	1	9	2
Op1343SMG	3	2	1	9	2
Op64119LLS	4	3	1	2	4
Op101715AH	5	3	1	9	3
Op101714AH	6	3	1	9	3
Op179814AH	7	3	1	9	3
Op136064CYH	8	3	1	9	3
Op93601SNN	10	3	1	5	2
Op113512SNN	9	3	1	5	2
Op113534SNN	10	3	1	5	2
Op72923SNN	12	3	1	5	2
Op113520SCN	14	3	1	5	4
Op113598SCN	12	3	1	5	4
Op113603SCN	11	3	1	5	4
Op113568BVN	13	3	1	5	4
Op121413NSN	18	3	1	5	2
Op121419NSN	17	3	1	5	2
Op121426ANN	15	3	1	5	1
Op121434ANN	16	3	1	5	1
Op136829CH	19	3	1	5	3
Op136881CH	19	3	1	5	3
Op781CCHM	27	2	2	8	1
Op787CCHM	26	2	2	8	1
Op789CCHM	27	2	2	8	1
Op791CCHM	27	2	2	8	1
Op801CCHM	27	2	2	8	1
Op223319YAG	30	2	2	7	5
Op223320YAG	30	2	2	7	5
Op223321YAG	30	2	2	7	5
Op223322YAG	30	2	2	7	5
Op784CCHM	28	2	2	8	1
Op796CCHM	29	2	2	8	1
Op799CCHM	29	2	2	8	1
Op800CCHM	29	2	2	8	1

Op779CCHM	36	2	2	8	1
Op780CCHM	32	2	2	8	1
Op782CCHM	33	2	2	8	1
Op786CCHM	34	2	2	8	1
Op792CCHM	35	2	2	8	1
Op793CCHM	34	2	2	8	1
Op794CCHM	34	2	2	8	1
Op795CCHM	34	2	2	8	1
Op93683SJN	31	3	2	8	4
Op467OCHM	39	2	2	8	3
Op778CCHM	38	2	2	8	1
Op785CCHM	38	2	2	8	1
Op802CCHM	38	2	2	8	1
Op803CCHM	37	2	2	8	1
Op565152EPG	40	3	3	10	7
OpC2BEQRM	41	3	3	10	7
Op32806TCM	42	3	3	10	7
OpJ7CKCM	43	3	3	10	7
Op4TGQRM	45	3	3	10	7
Op55TGQRM	45	3	3	10	7
OpC7MLQRM	44	3	3	10	7
Op7274UYM	42	3	3	10	7
Op33076TM	46	3	3	10	6
Op33077TM	47	3	3	10	6
Op12ESQRM	49	3	3	10	6
Op20ESQRM	48	3	3	10	6
Op91ENQRM	49	3	3	10	6
Op8787HACM	50	3	3	10	7
Op8797LPCM	51	3	3	10	7
Op9CXQRM	52	3	3	10	6
Op12CXQRM	52	3	3	10	6
Op15CXQRM	52	3	3	10	6
Op510LCQRM	53	3	3	10	6
Op8783TACM	56	3	3	10	7
Op8789TACM	57	3	3	10	7
Op14ESQRM	55	3	3	10	6
Op67ESQRM	55	3	3	10	6
Op68ENQRM	55	3	3	10	6
Op69ENQRM	55	3	3	10	6
Op75ENQRM	55	3	3	10	6
OpSK4LQRM	55	3	3	10	6
OpSK6LQRM	55	3	3	10	6
Op10CXQRM	54	3	3	10	6
Op50ACQRM	55	3	3	10	6
Op8801LPCM	58	3	3	10	7

Op8865HACM	59	3	3	10	7
Op7238TCM	60	3	3	10	7
Op9789ERCM	66	3	3	10	7
Op7236TCM	68	3	3	10	7
OpJ6CKCM	61	3	3	10	7
Op21MQRM	63	3	3	10	6
Op22MQRM	71	3	3	10	6
Op23MQRM	65	3	3	10	6
Op26MQRM	71	3	3	10	6
Op27MQRM	71	3	3	10	6
Op28MQRM	65	3	3	10	6
Op29MQRM	70	3	3	10	6
Op31MQRM	64	3	3	10	6
Op32MQRM	62	3	3	10	6
Op33MQRM	65	3	3	10	6
Op34MQRM	65	3	3	10	6
Op36MQRM	63	3	3	10	6
Op39ACQRM	69	3	3	10	6
Op42ACQRM	69	3	3	10	6
Op54ACQRM	69	3	3	10	6
Op9788NBQRM	67	3	3	10	6
Op18967LTYM	72	3	3	10	7
OpJ2CIYM	73	3	3	10	7
Op7242EGCM	77	3	3	10	7
Op7243TCM	76	3	3	10	7
Op28ESQRM	74	3	3	10	6
Op7273UYM	77	3	3	10	7
Op30784CIYM	75	3	3	10	7
Op128048TB	78	3	3	3	1
Op583075SCB	79	3	3	10	3
Op128043TB	80	3	3	3	1
Op128045TB	81	3	3	3	1
Op565482EPG	82	3	3	10	7
Op3TGQRM	83	3	3	10	7
Op58TGQRM	83	3	3	10	7
OpC1BEQRM	84	3	3	10	7
OpJ4CKCM	85	3	3	10	7
Op56TGQRM	87	3	3	10	7
Op57TGQRM	88	3	3	10	7
OpC3MLQRM	87	3	3	10	7
OpC4MLQRM	87	3	3	10	7
OpC5MLQRM	90	3	3	10	7
OpC6MLQRM	87	3	3	10	7
OpC8MLQRM	89	3	3	10	7
OpC9MLQRM	86	3	3	10	7

Op7244XQRM	87	3	3	10	7

*Los nombres de identificación y de haplotipos de las muestras corresponden a los utilizados por Gutiérrez-García y Vázquez-Domínguez (2012). Loa grupos son: A) Taxonómica de Lawlor (1982), B) Filogenética, C) SAMOVA y, D) Ecológica

Discusión y conclusiones

El objetivo de la mayoría de los estudios filogeográficos comparados es el análisis del efecto de los eventos geológicos y climáticos históricos en conjuntos de individuos, es decir, a nivel de comunidades. La incongruencia de patrones fiogeográficos de múltiples taxones codistribuidos puede deberse, entre otros, a la particularidad topográfica de la región, susceptibilidad diferencial de las especies a periodos glaciares y/o a efectos estocásticos, características de historia de vida, etc. (Michaux *et al.*, 2005). Cuando las hipótesis biogeográficas no explican la distribución de la variación genética y/o los patrones filogeográficos conocidos son incongruentes, es importante determinar si se debe a la respuesta al ambiente y ecología de las especies (Sullivan *et al.,* 2000), si a que están recientemente asociadas y/o debido a las tasas de evolución de los marcadores moleculares utilizados en el análisis (Turner *et al.*, 1996). En el caso de *O. coue*si y *O. phyllotis* fue posible evaluar comparativamente el efecto de la ecología de cada especie en la estructuración de la variación genética, corroborando que ésta influye en la forma de respuesta a la historia geológica y climática de la región.

El patrón de estructuración genética de las comunidades puede coincidir con lo que se conoce como "ecología comparada" de las especies de estudio, de tal forma que la ecología especie-específica puede ayudar a comprender los patrones actuales de diversidad genética y (Stuart-Fox *et al.*, 2001). Al estudiar dos especies distintas en una misma región se pueden detectar eventos de hibridización e introgresión reciente, contactos secundarios y diferencias demográficas en cuanto a tamaño poblacional histórico, e incluso inferir aspectos de historia de vida como la biología reproductiva de la especie y su dinámica durante eventos climáticos del pasado (Alvarado *et al.*, 2005). Es decir, al momento de generar conclusiones sobre la distribución de la estructura genética de las especies se debe considerar la respuesta particular de cada especie a eventos vicariantes o de dispersión. En el caso de los roedores de América Central de este estudio, a partir de la información de un marcador molecular mitocondrial, fue posible analizar la respuesta de cada especie a los cambios climáticos. Sin embargo, todavía es necesario estudiar con precisión los patrones de radiación de sus poblaciones y las estrategias de respuesta al aislamiento de cada una, lo cual de acuerdo con Pastorini *et al.* (2003), es importante para tener una mejor comprensión de la dinámica entre las especies y sus posibles respuestas a los cambios de la región.

La verificación de que un patrón filogeográfico congruente entre taxones es resultado de una historia evolutiva común no es fácil, dado que incluso especies hermanas difieren en sus características

ecológicas o de historia de vida (Churikov y Gharrett, 2002), por lo que hay que enfatizar en la generación de nuevos métodos o combinaciones de metodologías que permitan comparar especies y localidades de manera cuantitativa. Las características demográficas y ecológicas especie-específicas que generen diferentes patrones de respuesta al mismo evento histórico resultan en la pérdida de patrones filogeográficos entre especies (Goldstien *et al.*, 2006) por lo que la información generada a partir de la filogeografía intraespecífica que resulta en la presencia o ausencia de patrones biogeográficos es fundamental.

Estudiar la estructuración genética de especies de América Central es importante, pues permite descubrir diferencias genéticas significativas entre especies morfológicamente similares y poblaciones geográficamente cercanas, además de detectar linajes crípticos que en conjunto pueden sugerir una reorganización taxonómica a diferentes niveles. América Central presenta poblaciones y especies distintivas, así como linajes alopátricos que podrían ser considerados como unidades evolutivas significativas (ESU) (Fraser y Bernatchez, 2001) y/o unidades taxonómicas operacionales (OTU). Además, los caracteres biológicos intrínsecos son importantes para detectar cuales eventos geológicos están relacionados con una región como la incluida en este estudio (Zeh *et al.*, 2003).

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