2015-04-01

Evolution and Biogeography of Mesoamerican Small Mammals: With Focus on the Genus Handleyomys and Related Taxa

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A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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March 2015

ABSTRACT

Evolution and Biogeography of Mesoamerican Small Mammals: Focus on the Genus *Handleyomys* and Related Taxa

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Mesoamerica is considered a biodiversity hot spot with levels of endemism and species diversity likely underestimated. For mammals, the patterns of diversification of Mesoamerican taxa still are controversial. Reasons for this include the region's complex geologic history, and the relatively recent timing of such geological events. Previous studies, however, support the view that substantial migration between North (NA) and South America (SA) occurred prior or/and during the Great American Biotic Interchange (GABI) ~3.5 Ma. This was followed by repeated periods of isolation during Pleistocene climatic oscillations, which produced most of the diversification in the region. From a North American origin, the subfamily Sigmodontinae migrated to SA, where most of its present day diversity exists. The taxonomic history of this subfamily, and of Oryzomynii, its largest tribe, has been exceptionally complex. Recently, extensive studies have helped to clarify genealogical relationships among major clades, but have left the evolutionary histories of several groups unresolved. Such is the case for the genus *Handleyomys* that includes nine species; seven of which are endemic to Mesoamerica; and of its phylogenetic position among closely related genera *Euryoryzomys*, *Hylaeamys*, *Oecomys*, *Nephelomys* and *Transandinomys*.

The results supported the monophyly of *Handleyomys*, and four clades with inter-generic levels of divergence within the genus, three of these clades restricted to Mesoamerica (the *alfaroi*, *chapmani* and *melanotis* species groups). Furthermore, the estimated time for the split of the Mesoamerican *Handleyomys* is on average, 2.0 Myr older than the proposed migrations to NA during the GABI. In addition, the position of *Handleyomys* as the sister clade to *Euryoryzomys*, *Hylaeamys*, *Oecomys*, *Nephelomys* and *Transandinomys* was well supported, as it was a biogeographic hypotheses that depicted a polyphyletic origin for these genera and *Handleyomys* 5.5-6.0 Ma. The integrative approach implemented in this dissertation allowed the development of more biologically realistic hypothesis than has previously been conducted in Mesoamerica, where half of the endemic mammals are listed under the IUCN Red list; and where mammals with small ranges, which are the most vulnerable to extinction, are found largely outside reserves. The continued decline of the ecosystems health in this region calls for a more precise account of its biodiversity for its proper conservation; and for rigorous biogeographic studies for its management, since the region also serves as a biological corridor for intercontinental connectivity.

Keywords: Mesoamerica, rodents, biogeography, molecular phylogenetics, ecological niche models, Pliocene-Pleistocene

ACKNOWLEDGEMENTS

I want to acknowledge my advisor, Duke S. Rogers, for his teachings, continuous support and thorough guidance. I am fortunate to have had this opportunity to learn from him. I am very grateful for the expert advice and encouragement that I have received from my advisory committee members, Byron J. Adams, Jerald B. Johnson, Leigh A. Johnson and Eric Rickart; and for the remarkable contribution of major collaborators; Mark D. Engstrom and Francisco X. González-Cózatl.

I acknowledge Bruce L. Patterson (The Field Museum), Consuelo Lorenzo-Monterrubio (El Colegio de la Frontera Sur), Elizabeth Arellano-Arenas (Centro de Investigación en Biodiversidad y Conservación, UAEM), Livia León-Paniagua (Museo de Zoología, UNAM), Mark. D. Engstrom (Royal Ontario Museum), Robb T. Brumfield (Louisiana State University Museum of Natural Science), Robert J. Baker (Texas Tech University) and Suzanne Peurach (National Museum of Natural History) for providing indispensable tissue samples.

I am indebt to the Mexican National Council of Science and Technology, the principal sponsor for pursuing a Ph.D. abroad. And I want to acknowledge the BYU's College of Life Sciences, Department of Biology and Monte L. Bean Museum, for additional funding and for research and teaching assistantships. Also, the Dissertation Improvement Grant from the National Science Foundation, the American Society of Mammalogists Grants-in-Aid of Research and the Theodore Roosevelt grant from the American Museum of Natural History, were valuable financial sources for my Ph.D. research.

I thank the BYU's College of Life Sciences, Department of Biology and Monte L. Bean Museum administrative team, for the continuous assistance and sincere care. I also thank my

BYU colleagues, for their helpful advice and genuine manners, which made of my time at BYU a pleasant growing experience.

Finally, I would like to extend my appreciation to Parker Frech and family, for their generosity, constant encouragement, and for welcoming me into their family. And to express my deepest gratitude to all of my kin and to my friends, for their absolute love and care, which provides me with an unparalleled emotional comfort that certainly eased the hardest times.

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INTRODUCTION

Mexico and Central America hold approximately 10% and 7% of the World's biological diversity, respectively, and from that, more than 35% is endemic to these areas, that roughly account for 2.5% of the World. This elevated biodiversity is hypothesized to be a result of a complex geological history since the Miocene, mainly associated with the reconnection of North and South American continents at an area of intersection of five tectonic plates (Coates & Obando 1996; Woodburne 2010). As a consequence, the at least 35 physiographic provinces in the region are not only of varied age but also of diverse provenance (Cervantes-Zamora *et al.* 1990; Marshall 2007). Furthermore, the intermediate continental location of Mexico and Central America strengthens the ecological and evolutionary implications of their natural environments. Typically, two main Eco regions in these countries are given global priority for conservation (Mittermeier *et al.* 2004). The Madrean pine-oak woodlands, biogeographically characterized as the transition between the Nearctic and Neotropical regions, and the Mesoamerica hotspot, where tropical ecosystems have predominance (Morrone 2014a).

Mammal groups often exhibit some of the most intricate evolutionary relationships in Mesoamerica; for instance, a 60% species turnover is implied in the fossil record of the Pleistocene (Arroyo-Cabrales *et al.* 2010). What obscures the evolutionary origin of the more than 250 extant endemic species (Jenkins & Giri 2008), from which close to 32% have constrained distributions in mountain environments (>1000 m) (Ceballos *et al.* 2010; Reid 2009). This pattern is particularly noticeable in rodents (Amori *et al.* 2013), that accounts for 82% of mammalian endemism in Mexico (Ceballos 2007), and ~70 of Central America. In addition, the large amounts of cryptic diversity being exposed with the use of molecular data suggest that the majority of the undescribed species in the region are likely to be endemic (Pimm *et al.* 2014;

Scheffers *et al.* 2012). Similarly, the phylogenetic information exposed with these methods has shed light into the biogeographic history. Pliocene-Pleistocene biogeographic hypothesis for most Mesoamerican mammals propose that the Pliocene mammalian assemblages in the region were essentially Nearctic (Woodburne *et al.* 2006). South America taxa appear in the fossil record of Mesoamerica by the beginning of the Pleistocene ~2.58 Ma, indicative of an over the Panamanian Land Bridge migration (Montellano-Ballesteros & Jimenez-Hidalgo 2006). Throughout the Pleistocene, South American lineages were largely localized in the eastern mountains of the Mesoamerica hotspot, while the majority of the Nearctic lineages subsisted in the Trans-Mexican Volcanic Belt and the mountain ranges of western Mesoamerica, corresponding to the Madrean pine-oak woodlands (Ferrusquía-Villafranca *et al.* 2010; Morrone 2014b). These biogeographic hypotheses are largely based on the assumption of niche conservatism (Peterson 2011; Wiens & Graham 2005). However, cases in which the amount of niche divergence between sister species mammals has been evaluated, suggest that substantial niche differentiation has accompanied the allopatric speciation of small mammals in the region (Martínez-Gordillo *et al.* 2010).

The genus *Handleyomys* represents an ideal system to test biogeographic hypotheses and niche conservatism in Mexico and Central America. It includes 9 species, 7 of which are endemic to Mesoamerica, whereas its proposed closest relatives have predominantly South American distributions (*Euryoryzomys*, *Hylaeamys*, *Nephelomys*, *Transandinomys* and *Oecomys*) (Weksler *et al.* 2006). Within *Handleyomys*, *H. alfaroi* occupies a relatively wide geographic distribution in evergreen and mountain forests ecosystems (500 – 1400 m) along Mesoamerica and the eastern slopes of the Ecuadorian Central Andes. *H. rostratus* and *H. melanotis* are typically found at lower elevations (< 800 m; rarely ~1000 m). *H. rostratus* favors deciduous and

evergreen tropical forests in eastern Mexico and northern Central America (north of the Nicaraguan Depression), whereas *H. melanotis* is endemic to subtropical and mixed forests in western Mexico (Musser & Carleton 2005; Reid 2009). In contrast, *H. fuscatus, H. intectus*, *H. chapmani*, *H. guerrerensis*, *H. rhadbops* and *H. saturatior* are limited to high elevation montane forests (>1200 m). The first two species are endemic to the Cordillera Occidental and Cordillera Central of Colombia, respectively, and the last four are restricted to Mexico or northern Central America. Nevertheless, the taxonomic history of these species has been problematic, and the number of recognized species has ranged from 7 (Goldman 1918) to 14 (Allen 1891, 1913; Allen & Chapman 1897; Goldman 1915; Merriam 1901; Voss *et al.* 2002), with another eight forms proposed as subspecies (Musser & Carleton 2005). In addition, their evolutionary relationships remain unclear, as it is the phylogenetic position of this genus with respect to *Euryoryzomys*, *Hylaeamys*, *Nephelomys*, *Transandinomys* and *Oecomys* (Parada *et al.* 2013; Pine *et al.* 2012; Salazar-Bravo *et al.* 2013; Weksler *et al.* 2006).

In this context, the dissertation was structured to comprise four chapters. For chapter 1, a comprehensive review of the literature on the biogeography of Central American mammals was compiled in a book chapter coauthored with Duke S. Rogers. Chapter 2 focused on the phylogeography of the Mexican endemic *Handleyomys chapmani* using mitochondrial (*Cytb*) and nuclear (*Fgb*-I7) DNA sequences, presented as a peer-reviewed article in the Journal of Mammalogy coauthored with Francisco X. González-Cózatl and Duke S. Rogers. In chapter 3, sequence data from four mitochondrial and eight nuclear loci was generated to develop a time calibrated phylogenetic hypothesis for *Handleyomys* and to evaluate its evolutionary relationships with respect to *Euryoryzomys*, *Hylaeamys*, *Nephelomys*, *Oecomys,* and *Transandinomys*. In addition, species limits among species-level clades in the genus

Handleyomys were demarcated with an integrative approach under the unified species concept, and niche conservatism hypothesis between these clades were objectively tested and quantified. This manuscript is intended for submission to the journal of Molecular Phylogenetic and Evolution. Finally, chapter 4 was built on chapter 3 results to explore the intra and inter-generic biogeographic patterns of the aforementioned taxa in a hypothesis-testing framework. This last chapter was designed for submission to the Journal of Biogeography.

The inclusive research approach of this dissertation, allows for development and testing of more biologically realistic hypothesis than has generally been done in Mesoamerica, and will have an impact in other groups with similar histories and distributions. The continued decline of the ecosystems health in this region calls for a more precise account of its biodiversity for its proper conservation; and it is particularly necessary in this region where the distributions of 90% of the endemic mammals have been reduced significantly (DeClerck *et al.* 2010; Fuller, Trevon *et al.* 2006), and where mammals with small ranges, which are the most vulnerable to extinction, are found largely outside reserves (Ceballos 2007; Jenkins & Giri 2008; Visconti *et al.* 2011). Furthermore, nearly half of these endemics are listed under one of the three IUCN (International Union for the Conservation of Nature) Red List categories. Similarly, more rigorous biogeographic studies in this region are critical for its management, as Mesoamerica continues to show some of highest deforestation rates in the world (FAOSTAT 2013), what will further degrade the roughly 17% of the original vegetation that remains intact, and because the region also serves as a biological corridor for intercontinental connectivity.

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CHAPTER 1

Biogeography of Central American Mammals: Patterns And Processes

Ana Laura Almendra and Duke S. Rogers

[Manuscript published in Historical Biogeography of Neotropical Mammals (B. D. Patterson a. L. P. Costa, eds.). University of Chicago Press, Chicago, Illinois. 2012. Pp. 203-229.]

Abstract

Compared with its total land area, Central America contains a disproportionately large amount of biodiversity owing to its complex topography and position between the Nearctic and Neotropical realms. The geologic history of the region is correspondingly complex and our understanding is far from complete. Likewise, both biogeographic and phylogeographic patterns for mammals are not well articulated, although some conclusions based on analysis of molecular data are emerging. For example, the actual biodiversity for mammals, particularly for rodents, likely is much higher than currently documented. The historical events and geographic features that have shaped Central America seem to have affected mammals and other groups in the similar fashion. These include dispersal events both prior and subsequent to the permanent land bridge between North and South America, the northern Andes orogeny, in situ divergence both between and within the northern and southern Central American mountain ranges as well as between Atlantic and Pacific lowlands separated by these highland areas, and the barriers represented by three areas (Isthmus of Tehuantepec, Nicaraguan Depression and Central Panama) that were submerged for various times in the past. In general, mid-and high-elevation faunas are relatively diverse and contain higher levels of endemism than do lowland areas, although radiations have occurred among both lowland and montane taxa. Rodents exhibit more genetic structure than do bats, ungulates and primates over comparable geographic sampling. In many cases, estimated levels of molecular divergence correspond to events that occurred in the early Pleistocene or late Pliocene. Unfortunately, continued and rapid change in land–use practices throughout Central America may preclude a complete and accurate reconstruction of the historical biogeography of the region.

No phenomenon in the whole realm of nature forced itself earlier upon the notice of man than certain facts of geographic distribution. – C. H. Merriam (1892:3)

Introduction

Central America extends more than 1500 km from southeastern Mexico to eastern Panama and encompasses habitats ranging from low elevation savannah, semiarid scrub and humid tropical forests to montane habitats that exceed 4000 m in elevation (Savage 1982) and with no fewer than 15 recognized physiographic provinces (Marshall 2007). This region represents a bridge, both literally and figuratively, between the Nearctic and Neotropical biogeographic realms (Halffter 1987; Marshall and Liebherr 2000; Morrone 2006; Webb 2006). As a result of its location, complex physiography and climatic fluctuations, Central America is one of the most biologically diverse regions in the world (Marshall and Liebherr 2000; Patterson 2001; Webb 2006). The region supports more than 6% of the World's mammal diversity (Ceballos 2007; Ceballos et al. 2002; Reid 2009) superimposed on only 0.4% of the Earth's total land surface (Marshall 2007). Furthermore, more than 100 mammal species (approximately 30% of the total) are endemic to Central America (Jenkins and Giri 2008; Reid 2009). It has long been known that taxa with South American origins decrease northwards, with the reverse being true for North American species (Merriam 1892). Despite earlier contributions to our understanding of Central American biogeography by Rosen (1978), Halffter (1987), and Savage (1982), and by Marshall et al. (1982) and Webb (1991, 2006) for mammals in particular, many details regarding the historical processes involved in shaping mammalian distributions and diversification are lacking. Most molecular studies of mammals within the region have focused on estimating genealogical relationships rather than explicitly examining biogeographic hypotheses and thus largely precluding rigorous evaluation of alternate scenarios leading to diversification. However,

molecular phylogeographic studies generally agree with patterns recovered for other organisms (discussed below). Certainly, molecular studies of mammals demonstrate that the biodiversity within Central America is underestimated. This finding is in agreement with Carleton et al. (2002) who predicted that the mammalian diversity in the Central American highlands likely is 30 – 40% greater than currently understood. Undoubtedly, levels of endemism also are greater than currently is appreciated, as are amounts of evolutionary history change (i.e., branch lengths in a phylogenetic tree) expected from biodiversity estimates alone (Sechrest et al. 2002).

Below we summarize the geologic history and major events leading to faunal diversification in Central America based primarily on fossil and distributional data for mammals. We then describe phylogeographic patterns of mammalian taxa, restricting our discussion to studies relying on sequence data and incorporating results from other animal and plant groups as appropriate. Finally, we summarize these findings and comment on new directions and methodologies that may be applied to the new and still developing field of molecular biogeography.

Geologic Processes and the Great American Biotic Interchange

The paleogeology of Central America is complex and our understanding is far from complete. In the north, it involved subduction of the Cocos plate beneath the western margin of the Caribbean plate together with the Motagua-Polochic fault system that transects central Guatemala roughly east to west and demarcates the boundary between the North American (Maya Block) and Caribbean plates (Chortis Block; Marshall 2007). Tectonic activity has resulted in the formation of the Central Massif which includes the Sierra Madre de Chiapas and the Maya highlands (Briggs 1994; Halffter 1987; Marshall 2007). However, there is disagreement over whether these events took place during the Paleocene, 65-55 Ma (Raven and

Axelord 1974; Moran-Zenteno 1994) or the Miocene, 5.7-2.2 Ma (Escalante et al. 2007). Southern Central America consists of the Chorotega Block (Costa Rica and western Panama) and the Chocó Block (eastern Panama), which are situated within an area of tectonic interactions among the Caribbean, Cocos, Nazca and South American plates (Marshall 2007). In this region lies the Cordillera de Talamanca, an area of uplift that stretches across Costa Rica and Panama. This area may have begun its deformation in the late Cretaceous (65 Ma) and continued through most of the Cenozoic as part of the volcanic arc which extended along the Pacific coast from Guatemala to Panama (Coates and Obando 1996). However, major uplifts occurred as a result of the underthrusting of the Cocos Ridge and subduction of the Nazca Plates (Silver et al. 1990), events that occurred during the end of the Miocene and beginning of the Pliocene, 5.7-3 Ma (Abratis and Wörner 2001; Coates and Obando 1996; Gräfe et al. 2002).

The terrestrial and freshwater biotas of South America were isolated from those of North America throughout the Cretaceous until the Pliocene. This ended with the formation of the Panamanian land bridge in the middle Pliocene 3.5-3.1 Ma at the junction of the Pacific and Caribbean plates (Coates and Obando 1996; Coates et al. 2004). These geological events represented an intercontinental corridor for terrestrial dispersal (Briggs 1994; Vermeij 1991; Wallace 1876; Webb 2006) which initiated the episode known as the Great American Biotic Interchange, or GABI (Stehli and Webb 1985). Correspondingly, the final closure of the isthmus had oceanographic and climatic effects and coincided with the beginning of the major northern hemisphere glaciations (Cronin and Dowsett 1996). Miller et al. (1987) estimated a 4°C decline on global temperatures from the mid-Pliocene to the Pleistocene. This resulted in an increase in cool to cold climate plant pollens found in Central America and northern South American palynofloras (Graham 1999). Analysis of pollen and spore flora from central Panama ~ 3 Ma

document the presence of grass species indicative of tropical dry forest or savannah habitats (Graham 1991) and fossil leaves and paleosols from the mid-Miocene in Central Panama indicate a cooler and drier climate than currently exists in the region (Retallack and Kirby 2007). According to Graham (1989), upland habitats in the isthmian region were in place approximately 2.5 Ma. It is therefore likely that considerable topographic and corresponding habitat diversity existed during, or even prior to the formation of a permanent land bridge connecting the two continents and may have facilitated dispersal by both tropical savannah and tropical forest-adapted taxa.

The process of connecting North and South America is hypothesized to have begun in the early Oligocene (30 Ma), with volcanic islands along the present position of Central America. Thus, there could have been a connection between western Panama and North American as early as 10-12 Ma (Raven and Axelord 1974). Whether this corridor was a discontinuous, island arc (Coates and Obando 1996) through present-day lower Central America, or a peninsula connecting North America with portions of present-day western Panama (Whitmore and Stewart 1965) is contentious. However, recent studies support a peninsular configuration extending to central Panama. Kirby and MacFadden (2005) compared mammalian tooth size (as a surrogate for body mass) between counterparts of six fossil species in Panama and Texas. They argued that if central Panama was at one time an island, fossil mammals should exhibit the "island rule" (Van Valen 1973), wherein mammals > 1 kg in body mass become smaller, whereas those < 1 kg evolve a larger size (Damuth 1993; Lomolino 1985). Results of their study showed no difference in tooth size between taxa found in Texas and Panama, failing to support the archipelago model. Kirby et al. (2008) presented lithostratigraphic, biostratigraphic and chemostratgraphic evidence for a Central American Peninsula that existed as early as 19 Ma. Whichever model is correct, it

must account for dispersal of taxa prior to the closure of the Panamanian portal. This is because fossil evidence documents movement of mammals as long ago as the middle Miocene (ca. 9 Ma). For example, *Thinobadistes,* an extinct mylodontid sloth, and *Pliometanastes,* a megalonychid sloth, arrived in North America about 9 Ma from South America. Conversely, *Cyonasua*, a large procyonid from North America, appears in the fauna of northwestern Argentina in the middle to late Miocene (9-7 Ma) (Marshall 1988; Webb 1991, 2006). Based on fossil evidence, some investigators hypothesize that members of the rodent family Cricetidae entered South American prior to the formation of the Panamanian bridge (Marshall 1979; Woodburne and Swisher 1995). Others suggest that cricetids likely diversified in southern Central America and that once the Panamanian land bridge was completed in the Pliocene (3-2.7 Ma), one or several lineages of cricetids were among the first mammalian groups to enter South America (Pardiñas et al. 2002; Steppan et al. 2004). Recently, Verzi and Montalvo (2008) described late Miocene fossils belonging to the rodent subfamily Sigmodontinae and the carnivore family Mustelidae from the Cerro Azul Formation in Caleufú, Argentina (5.8-5.7 Ma). However, Prevosti and Pardiñas (2009) argued that the late Miocene age of this site is not well established and provided evidence that the purported carnivore is in fact a didelphimorphian marsupial.

After the formation of the Panamanian land bridge, representatives of the family Tayassuidae migrated into South America and from the Pliocene until the middle Pleistocene (4-1.5 Ma; see Gibbard et al. 2010), a large group of taxa dispersed to South America including members of the Camelidae, Canidae, Cervidae, Equidae, Felidae, Gomphotheriidae (mastodonts), Heteromyidae, Tapiridae, and Ursidae (Marshall et al. 1982; Webb 2006), whereas Pascual (2006) suggested that representatives of the family Heteromyidae entered South America

during the Holocene. Pleistocene glaciations are assumed to have modified the biotic patterns (Betancourt et al. 1990; Horn 1990), and during the Holocene, four additional families of mammals (Geomyidae, Leporidae, Sciuridae, and Soricidae) are hypothesized to have entered South America (Marshall et al. 1982).

During the late Pliocene and Pleistocene (4.7-1.8 Ma), the South American "legions" (sensu Marshall et al. 1982) including members of the families Dasypodidae, Erethizontidae, Glyptodontidae, and Hydrochoeridae migrated to North America, followed by the families Didelphidae and Megatheriidae in the middle to late Pleistocene (1.8-0.3 Ma). During the Holocene, the families Atelidae, Bradypodidae, Callitrichidae, Cebidae, Choleopodidae, Dasyproctidae, Echimyidae, and Myrmecophagidae are also hypothesized to have migrated from South America northward (Marshall et al. 1982), although molecular evidence suggests earlier entries (see below). However, relatively few families became established north of Central America. Lone members of the families Dasypodidae and Erethizontidae and two species each in the families Atelidae and Didelphidae occur west and north of the Isthmus of Tehuantepec in Mexico. Moreover, three other South American families (Megatheriidae, Glyptodontidae and Toxodontidae) dispersed into Central America but became extinct during the Pleistocene (Arroyo-Cabrales et al. 2009; Webb 2006).

It is evident that the process of inter-continental colonization is more complex than explained by the GABI alone. Many mammals diversified in northern South America as a result of the Andean orogeny, with east and west lineages (cis- and trans-disjunctions, respectively, following Haffer 1967) that began ~12 Ma (Albert et al. 2006; Patterson et al. this volume), but this cordillera had reached only half its modern elevation by 10 Ma (Gregory-Wodzicki 2000). Therefore, considerable habitat diversity must have existed in northwestern South America since

the late Miocene. Ford (2006) proposed three post-land bridge dispersal events from northwestern Colombia into Central America for primates. Similarly, Santos et al. (2009) demonstrated repeated colonization of Central America prior to the formation of the Panamanian isthmus by amphibian lineages whose origins are from the Amazonian and Chocó region of South America. This biogeographic hypothesis of recurrent colonization coincides with geological periods of isolation and potential connections between North America and Central America both before and after the formation of the Panamanian land bridge (Coates and Obando 1996). These events are assumed to have led to rapidly changing distributions of the new immigrants and accompanied by subsequent diversification of the lineages that colonized Central America (Marshall and Liebherr 2000; Webb 2006). Partly as a result of dispersal and subsequent in situ diversification, one-third of the mammalian fauna in Central America is comprised of endemic species. Accordingly, the region is viewed as a hotspot of species richness and endemism (Ceballos and Ehrlich 2006; Jenkins and Giri 2008; Mittermeier et al. 2004; Myers et al. 2000; Sechrest et al. 2002).

Mexico and Central America

The Isthmus of Tehuantepec represents a biogeographic demarcation between Central America and areas to the north and west in Mexico. This pattern is well supported for a variety of taxa (see Weir et al. 2008 for a recent summary). A series of papers have examined relationships among montane taxa distributed across the isthmus; these have typically concluded that climatic changes (Toledo 1982), coupled perhaps with the most recent marine incursion (Beard et al. 1982; Maldonado-Koerdell 1964), are the vicariant events likely responsible for divergences.

To date, all examples of molecular-based, species-level divergence among mammals associated with the Isthmus of Tehuantepec involve montane rodent taxa that originated in North America. Sullivan et al. (1997) determined that samples of *Peromyscus aztecus* south and east of the Isthmus of Tehuantepec represented a distinct species-level clade from samples of *P. aztecus* from Mexican Sierra Madre Oriental and Oaxacan Highlands west of the isthmus. Sullivan et al. (2000), Hardy et al. (ms submitted), and Arellano et al. (2005) likewise recovered what they considered species-level divergence among allopatric samples of *Reithrodontomys sumichrasti* and *R. microdon*, respectively, distributed on either side of the isthmus. Ordóñez-Garza et al. (2010) hypothesized that the Isthmus of Tehuantepec formed a vicariant barrier separating two clades within the *Peromyscus mexicanus* species group and both Rogers et al. (2007) and León-Paniagua et al. (2007) uncovered comparable evidence for species of *Habromys*. Edwards and Bradley (2002) also found what they considered a species-level split for allopatric populations of *Neotoma* on either side of the isthmus based on cytochrome *b* (this relationship was not recovered with a nuclear marker in a follow-up study by Longhofer and Bradley 2006). Sullivan et al. (2000) determined that the deepest node separating two codistributed taxa (*P. aztecus* and *R. sumichrasti*) from their congeners corresponded geographically to Isthmus of Tehuantepec. According to Sullivan et al. (2000), the amount of sequence divergence was consistent with isolation by an early Pleistocene, trans-isthmus marine barrier (Barber and Klicka 2010). Estimates for the timing of separation between samples of *R. sumichrasti* on either side of the isthmus (3.4-1.8 Ma) are consistent with this hypothesis (Hardy et al. submitted). Thus far, the only exception to this general pattern is evidenced by the genus *Glaucomys*. In this instance, samples spanning the Isthmus of Tehuantepec do not approach species-level divergence based on cytochrome *b* (Kerhoulas and Arbogast 2010) or the mitochondrial control region (Ceballos et al.

2010), prompting Kerhoulas and Arbogast (2010) to propose that occupation of the Sierra Madre de Chiapas by flying squirrels was a relatively recent event.

This scenario also is consistent with the phylogeographic structure recovered for toads (Mulcahy et al. 2006), suggesting that the isthmus may represent a barrier to taxa that occur in both lowland and montane habitats. However, based on molecular data, Rogers and González (2010) recovered a clade within the Desmarest's spiny pocket mouse known from two disjunct localities in Oaxaca and Chiapas, Mexico. Both sites are within a transition zone between Cloud Forest and Tropical Evergreen Forest (Leopold, 1950) and span the Isthmus of Tehuantepec with only minimal cytochrome *b* divergence, suggesting that this habitat type may have been continuous across the Isthmus in the recent past. Molecular analyses of rodent species whose origins likely are southern Central America indicate that the Isthmus of Tehuantepec does not represent a barrier. Although sampling was limited, both *Oligoryzomys fulvescens* sensu stricto (Rogers et al. 2009) and *Oryzomys couesi* (Hanson et al. 2010) occur in lowland habitats throughout Central America and northward along the eastern and western coasts of Mexico. Rogers et al. (2009) hypothesized a relatively recent northward dispersal of *Oligoryzomys fulvescens* from Central America to northern Mexico. Lack of genetic divergence across the isthmus for both *Oligoryzomys fulvescens* and *Oryzomys couesi* is best explained by dispersal once the isthmian marine barrier no longer existed (Beard et al. 1982).

Northwestern South America and Central America

Portions of Central America and the Pacific coastal region of South America extending to southern Ecuador were first recognized by Hershkovitz (1958) as a separate zoogeographic region. A series of molecular phylogenetic studies have recovered what are considered co-distributed species-level clades linking Central America with western (trans-Andean) South

America. Examples include the marsupial *Marmosa isthmica* (Gutiérrez et al. 2010; Rossi et al. 2010), the primate species *Alouatta palliata* (Cortés-Ortiz et al. 2003), and *Ateles geoffroyi* (Collins and Dubach 2000a; 2000b), the bats *Artibeus jamaicensis* (Larsen et al. 2007), *Carollia brevicauda,* and *C. castanea* (Hoffman and Baker 2003), *Dermanura rava* (Solari et al. 2009), *Glossophaga soricina* (Hoffman and Baker 2001), *Uroderma bilobatum* (Hoffman et al. 2003), *Vampyressa thyone* (Hoofer and Baker 2006), and the rodents *Orthogeomys dariensis* (Sudman and Hafner 1992) and *Reithrodontomys mexicanus* (Arellano et al. 2005).

Other studies have recovered a sister-group relationship between species distributed in western Colombia and Ecuador and those found in Central America. Examples include *Artibeus fraterculus* and *A. hirsutus* (Larsen et al. 2007; Redondo et al. 2008), *Dermanura rosenbergi* and *D. watsoni* (Solari et al. 2009), as well as the *Heteromys anomalus* and *H. desmarestianus* species groups (Rogers and González 2010). This pattern is replicated in other groups such as amphibians (Vallinoto et al. 2010), birds (Cracraft and Prum 1988; Marks et al. 2002; Ribas et al. 2005), and snakes (Zamudio and Green 1997) and likely represents relatively more ancient vicariant events that were contemporaneous with the Andean orogeny (Velazco and Patterson 2008). Hanson and Bradley (2008) examined phylogenetic relationships among samples of *Melanomys caliginosus* from Costa Rica, Ecuador, Nicaragua, Panama, and Venezuela. They recovered an Ecuadorian clade as basal to all other samples, including two *Sigmodontomys* (one sample each from Panama and Ecuador) which formed a sister group to samples of *M. caliginosus* from Panama and Venezuela. If species identifications are correct, then this genealogical pattern renders *Melanomys* paraphyletic and is suggestive of a cis- and trans-Andean split followed by differentiation among samples west of the Andes in Central America

and northern South America. However, limited geographic sampling and lack of estimates for the timing of these events preclude additional interpretation.

Amazon and Central America

Several alternative explanations have been described to account for the pattern of genetic diversity documented among taxa distributed in Amazonia and Central America. The first involves a western Amazon diversification followed by entry into Central America. This pattern has been recovered for *Marmosa* (Gutiérrez et al. 2010), *Micoureus* (Patton and Costa 2003), *Oligoryzomys* (Miranda et al. 2008; Rogers et al. 2009), *Philander* (Patton and da Silva 1997), and the primate taxa *Ateles* (Collins and Dubach 2000a, 2001), *Alouatta* (Cortéz-Ortiz et al. 2003), and *Saimiri* (Lavergne et al. 2010). Precursors of the primate lineages *Alouatta, Ateles*, and *Saimiri* are thought to have migrated from South America shortly after the completion of the Panamanian land bridge (Collins and Dubach 2000a, 2000b; Ford 2006; Lavergne et al. 2010). These genera are unrelated to fossil primates found in the Greater Antilles, which comprise a monophyletic group whose closest mainland relative is the South American genus *Callicebus* (Horovitz and MacPhee 1999; MacPhee and Horovitz 2004). The molecular phylogeny for the genus *Didelphis* by Patton and Costa (2003) can best be explained by two dispersal events into Central America–the first by a precursor to the modern *D. virginiana*, followed by the more recent entry of *D. marsupialis*. Based on more limited sampling, the phylogeographic pattern recovered for *Carollia perspicillata* indicates a South American origin and relatively recent range expansion into Central America (Hoffman and Baker 2003). Baker et al. (1994) showed that *Chiroderma salvini* is sister to the remaining species in the genus, and hypothesized that this could be explained by its isolation in Central America from the common ancestor to the remaining *Chiroderma* species. This latter pattern is similar to that identified by Hanson and

Bradley (2008) for *Melanomys caliginosus,* in which Central American samples from Nicaragua and Costa Rica formed a sister group with those from Panama and Venezuela. Based on the phylogenetic reconstruction of relationships among members of the genus *Myotis*, Stadelmann et al. (2007) proposed a complex scenario that involved "early *Myotis* lineages" colonizing South America ca. 10-7 Ma and subsequently dispersing northward across the Isthmus of Panama.

The phylogenetic relationships among samples of the widely distributed *Desmodus rotundus* recovered by Martins et al. (2009) indicate that Central American populations are not most closely related to vampire bats from northern South America or even the remainder of Amazonia. Instead, Central American samples form a sister group with vampire bats from the Brazilian Pantanal, prompting these authors to hypothesize gene flow along the Andes cordillera. This particular pattern has not been replicated in other taxa. Other molecular data for mammalian taxa support the notion that movement from South America northward occurred primarily after the formation of the Panamanian land bridge. The relatively recent and rapid northward expansion out of South America is a pattern shared by parrots (Eberhard and Bermingham 2004), freshwater fish (Bermingham and Martin 1998; Perdices et al. 2002; Reeves and Birmingham 2006) and caimans (Venegas-Anaya et al. 2008).

Relatively deep molecular divergences have been recovered among species with origins in North or Central America that subsequently entered South America. Arellano et al. (2005) examined phylogeographic relationships among samples of *Reithrodontomys mexicanus* sensu stricto and recovered the sample from Colombia as basal relative to individuals from Central and Middle America. Gongora et al. (2006) recovered two clades of South American peccaries which currently are allocated to separate genera. Perini et al. (2010) proposed that the initial diversification of South American canids (4 Ma) predates the Panamanian land-bridge and

proposed that these two, closely related lineages entered South America. In turn, these lineages gave rise to an extant fauna that includes five endemic genera of canids. Rogers and González (2010) confirmed monophyly of the *Heteromys anomalus* species group, a basal clade within the genus that is distributed primarily in northern South America. Sequence divergence between the *H. anomalus* group and other basal clades in the genus support a single, Pliocene entry into South America and thus may predate closure of the Panamanian portal, as suggested by Engel et al. (1998) for sigmodontine rodents. Regardless of the absolute timing of these events, it is clear that entry into South America by many mammalian groups occurred earlier than was assumed previously.

Divergence within Central America

Some species endemic to Central America exhibit considerable geographic structure in DNA sequence data. The phylogeographic patterns recovered indicate in situ isolation of lowland taxa by the Sierra Madre de Chiapas and Central Massif and the Cordillera de Talamanca (fig. 1.1), separation of montane taxa that occur in both of these upland areas (as well as isolation within the complex Cordillera de Talamanca), and separation of lowland taxa by a marine incursion in southern Nicaragua. For example, Cortés-Ortiz et al. (2003) determined that occupation of Mesoamerica by *Alouatta* coincides with formation of the Panamanian land bridge and the 3 Ma split between the two Mesoamerica *Alouatta* clades. Lower sea-levels ~ 2.5 Ma (Haq et al. 1987) likely facilitated range expansions of these taxa. Baumgarten and Williamson (2007) contend that ancestral populations of *Alouatta* were isolated by a cooling period that separated the Yucatan Peninsula (*A. pigra*) from lowlands to the south (*A. palliata*); this vicariant event was driven by the Sierra Madre de Chiapas and the Maya highlands (fig. 1.1) and also may be responsible for isolating one of two clades recovered among samples of *Marmosa mexicana*

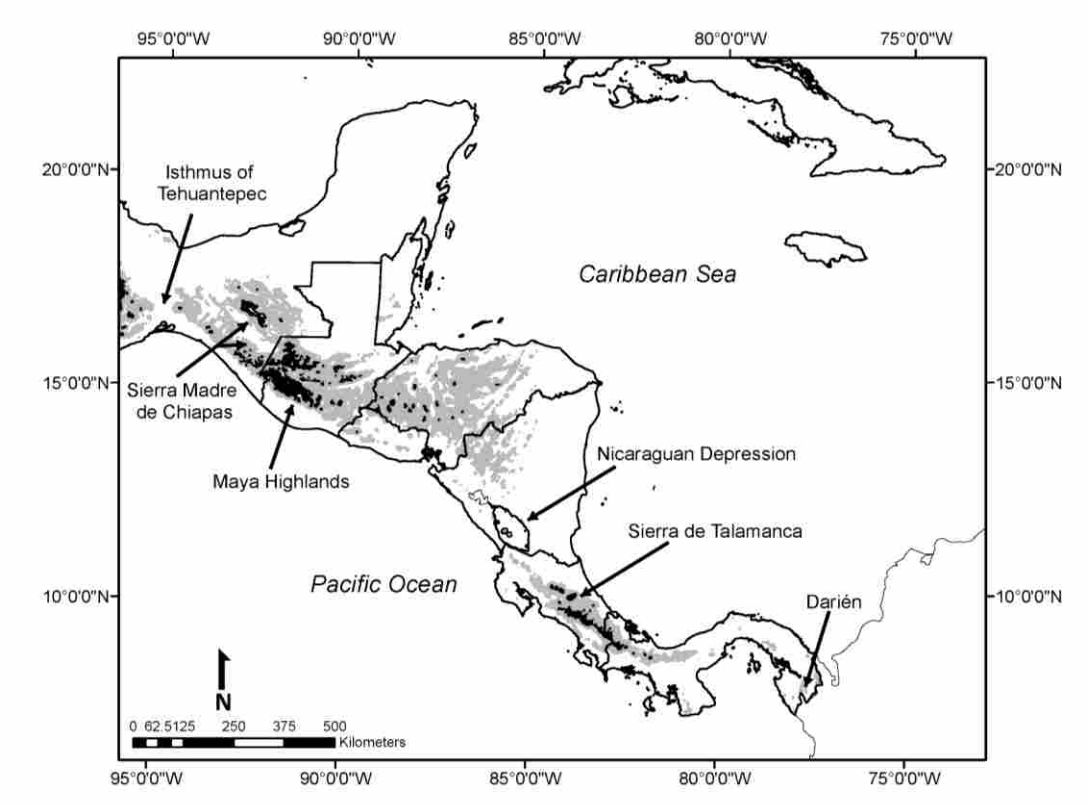

Figure 1. 1. Map of Central America illustrating geologic features discussed in text. Elevation ranges are white (<800 m), gray (800–2000 m), and black (>2000 m). Stippled areas indicate lakes Managua and Nicaragua.

by Gutiérrez et al. (2010). In addition, Baumgarten and Williamson (2007) argue that sea level increases ~ 2 Ma (Bermingham and Martin 1998; Perdices et al. 2002) may have reinforced separation of *A. pigra* and *A. palliata* (Ford 2006). Rogers and Vance (2005) documented a deep split among samples of *Liomys salvini* from the dry forests along the Pacific versant of Chiapas, Mexico, compared to samples from similar habitats in Honduras and Costa Rica. They also confirmed the sister group relationship between *L. salvini* and *L. adspersus*, the latter species known only from savanna habitat in central Panama. The relatively deep phylogenetic split between *L. salvini* and *L. adspersus* is consistent with proposition that wet and dry forest habitats have existed in southern Central America since the late Miocene or early Pliocene (Crawford et al. 2007).

Differentiation among rodent taxa in Central America has been extensive. Hardy et al. (submitted) recovered two well differentiated clades of *R. sumichrasti* corresponding to the Central Massif and the Talamancan range in Costa Rica and Panama. Rogers et al. (2009) determined that Central American populations of *Oligoryzomys fulvescens* (sensu stricto) and the Costa Rican and southern Nicaraguan endemic *Oligoryzomys vegetus* were sister taxa. Cladogeneis in these groups may have resulted from a marine gap (Nicaraguan Depression) during the Miocene and most of the Pliocene (Coates and Obando 1996; Iturralde-Vincent 2006). The Nicaraguan Depression currently serves as a physiographic break for a variety of vertebrate, insect, and plant taxa (Castoe et al. 2009 and references therein).

Rogers and González (2010) evaluated phylogenetic relationships within the broadly distributed lowland rodent species *Heteromys desmarestianus*, which they recovered as paraphyletic. They documented three possible species within this taxon; one from the Atlantic drainage of Costa Rica, another from the Pacific slopes of Costa Rica and Panama and a third from the Darién region of eastern Panama (fig. 1.1). Each of these candidate species corresponded to a different physiographic province as delimited by Marshall (2007). Well supported genetic subdivisions also were recovered within *H. desmarestianus* sensu stricto, including separation of northern Central American samples from those in Costa Rica (Rogers and González 2010), a pattern reminiscent of that described by Hoffman and Baker (2003) for *Carollia sowelli*.

Although limited in geographic sampling, other strictly molecular phylogenetic studies have uncovered a series of species-level rodent taxa in lower Central America. Arellano et al. (2005) recognized *Reithrodontomys cherrii* (formerly a subspecies of *R. mexicanus*) as a deeply divergent clade from the Cordillera de Talamanca, Costa Rica, with affinities to the *R.*

tenuirostris species group. The species-level status of *R. cherrii* was confirmed by Gardner and Carleton (2009), based on detailed examination of morphological evidence. Miller and Engstrom (2008) identified two undescribed species of *Reithrodontomys,* one from the Cerro de la Carpintera and another from Volcán Poas in Costa Rica. Rogers and Gonzalez (2010) confirmed the species status of *Heteromys nubicolens*, a species known only from the Cordillera de Tilarán and Cordillera de Guanacaste, Costa Rica and whose sister taxon, *H. oresterus*, occurs to the south in the Cordillera de Talamanca (Anderson and Timm 2006). At least for rodents, it appears that vicariant events driven by climatic oscillations during the Pleistocene (or earlier) were sufficient to drive speciation. Panama's Darién region (fig. 1.1) likely was isolated from South America until ~ 13-7 Ma (Coates et al. 2004) and from Central America until the formation of the Panamanian land bridge. As such, the Darién is regarded as a separate physiographic province by Marshall (2007). A series of species-level splits have been identified within several rodent taxa distributed in eastern Panama compared with populations in western Panama and Costa Rica. These include *Melanomys caliginosus* (Hanson and Bradley 2008) and *Heteromys desmarestianus* and *H. australis* (Rogers and González 2010).

A pattern similar to that found for *Ateles pigra* and *A. palliata* also was recovered for mammal species whose distributions are not restricted to Central America. Bradley et al. (2008) determined that *Sigmodon toltecus* (generally distributed north of the Central American Highland Massif) and *S. hirsutus* (southern Central America and northern South America) were sister taxa. Hanson et al. (2010) evaluated genealogical relationships among samples of *Oryzomys couesi* from Mexico and Central America. Four species-level clades were identified; one each from the Atlantic and Pacific versants in northern Central America, a third from the Atlantic coast of Costa Rica, and a fourth from the Pacific coast of central Panama. Hoffman et al. (2003)

documented isolation between two chromosomal races of *Uroderma bilobatum* from Central American: one generally distributed on the Pacific slopes of southern Mexico, Guatemala and El Salvador and the other found throughout the rest of Central America as well as western Colombia and Ecuador. Hoffman et al. (2003) estimated that their isolation occurred in the Pleistocene (0.9 – 0.2 Ma). This distribution pattern is identical to the sister-group relationship for *Carollia sowelli* and *C. subrufa* identified by Hoffman and Baker (2003), although *C. sowelli* is not known occur in South America. In addition, Hoffman and Baker (2003) identified a well supported phylogenetic split between populations of *C. sowelli* from northern Central America compared to those sampled from Costa Rica and Panama. Taken together, these genetic data indicate that multiple species-level clades exist within taxa that would not have been recovered based solely on morphological data.

Summary and Prospectus

The impacts of GABI are relatively well understood for mammals compared to other groups (Marshall et al. 1982; Vrba 1992), due in major part to their relatively abundant fossil record. Unfortunately, molecular studies of mammals designed specifically to decipher phylogeographical patterns within Central America are limited compared to other vertebrates (Patten and Smith-Patten 2008). The primary focus for most of these molecular studies has been phylogenetic reconstruction. As a result, detailed geographic sampling and estimates of divergence times among clades often are lacking. Despite these drawbacks, molecular studies have been useful in identifying some biogeographic (or phylogeographic) patterns within Central American mammals. In general, non-volant small mammals exhibit greater genetic diversity over comparable geographic areas than do bats, primates, or artiodactyls. This pattern of relatively low levels of intraspecific divergence among larger mammals and bats is comparable

to that of birds (Ditchfield and Burns 1998) and is not attributable to differences in rate of mitochondrial DNA evolution among mammalian groups (Ditchfield 2000). Rather, differences in vagility apparently explain this marked pattern among taxa. In general, levels of molecular differentiation between closely related taxa are consistent with biogeographic boundaries delimited by a variety of non-molecular methods. These barriers include the Sierra Madre de Chiapas and Maya highlands from the Talamancan Range and the Nicaraguan Depression (Halffter 1987; Luna-Vega et al. 2001; Patten and Smith–Patten 2008; Rosen 1978) as well as eastern Panama (Coates 1997) and the western Andes Cordillera (Patten and Smith–Patten 2008). For non-mammals, species-level biodiversity seems associated with tectonic and climatic events that predate the Pleistocene or even the Pliocene (Castoe et al. 2009). Whether or not this pattern holds for mammals should be tested rigorously, but preliminary results support species-level diversification occurring during the Pleistocene or late Pliocene, contra Savage (2002). Overall, results from a handful of molecular studies of Central American mammals have documented extensive *in situ* diversification that is driven by vicariant events. These findings are concordant with similar investigations for a variety of vertebrate (García-Moreno et al. 2006 and references therein) and plant taxa (Novick et al. 2003) and underscores the need for detailed sampling throughout Central America to fully appreciate its mammalian biodiversity.

The majority of molecular studies for mammals have used one or several mitochondrial markers. Although mitochondrial sequences offer advantages such as relatively rapid coalescence times and generally lack the problem of reticulation, these phylogenetic estimates represent gene trees rather than species trees, which can potentially be problematic (Degnan and Rosenberg 2006; Pamillo and Nei 1988). A subset of studies have used both mitochondrial and nuclear gene segments (Collins and Dubach 2000a, 2001; Cortés-Ortiz et al. 2003; Gongora et al.

2006; Hanson et al. 2010; Martins et al. 2009; Miller and Engstrom 2008; Redondo et al. 2008; Rogers and González 2010; Stadelmann et al. 2007; Velazco and Patterson 2008) to estimate phylogenetic relationships. However, the nuclear sequences employed typically yielded fewer phylogenetically informative characters (and less resolved genealogies) than gene trees obtained from mitochondrial sequence data; when concatenated with mitochondrial sequences in a combined evidence approach, the resulting trees tended to reflect clades based on mitochondrial sequences alone (Rogers and González 2010; Stadelmann et al. 2007).

Central America is experiencing some of the highest deforestation rates in the world. Recent studies estimate that only 20% of the original forested vegetation remains intact (Mittermeier et al. 2004). Moreover, only 12.6% of the land area is afforded some level of environmental protection, and only 3% is under protection that prevents alteration of native vegetation (Jenkins and Giri 2008). Unfortunately, the locations and sizes of these reserves apparently were selected without first obtaining data for species richness, biodiversity, distribution or dispersal requirements of the mammals that were the focus of the conservation effort. As a result, mammals with small ranges (and most vulnerable to extinction) are found largely outside reserves. Given that species ranges within Central America generally are not well known and often are fragmented, incorporating inferential methods that provide predictive information of geographic distribution such as ecological niche modeling (Graham et al. 2004; Peterson et al. 1999) are essential and can be especially useful in prioritizing conservation areas (Esselman and Allan 2010). Paleoclimate and future climate change models can be combined with molecular phylogeographic studies (Solomon et al. 2008) and coalescent simulations (Carstens and Richards 2007) to infer speciation events and address conservation issues. Incorporation of highly variable nuclear markers (Carstens and Knowles 2006; Shaffer and

Thomson 2007; Thomson et al. 2010) would result in more accurate estimates of a group's evolutionary history. This is particularly true for phylogeographic studies due to the relatively shallow genealogical patterns typically recovered. Likewise, recent developments in estimating species trees (Degnan and Rosenberg 2009; Heled and Drummond 2010; Liu et al. 2009), even with substantial incongruence among individual gene trees (Knowles 2009) and incomplete lineage sorting (Carstens and Knowles 2007b; McCormack et al. 2009), promise to revolutionize our understanding of biological processes that have shaped evolutionary history (Knowles 2009) of mammals in the region. Sequence data from multiple, unlinked loci also should be employed to estimate divergence times more precisely using Bayesian MCMC or maximum likelihood analyses of molecular sequences (Carstens and Knowles 2007a; Drummond and Rambaut 2007; Lemmon and Lemmon 2008; Pyron 2010) and other statistical phylogeographic approaches. Relatively few molecular studies of Central American mammals were designed to test a priori biogeographic or phylogeographic hypotheses (e.g., Sullivan et al. 2000). Fortunately, new approaches in molecular phylogenetics (Johnson and Crandall 2009; Riddle et al. 2008) together with recent advances in methods and modeling techniques (Carstens et al. 2009; Richards et al. 2007) have enabled investigators to develop biologically realistic phylogeographic hypotheses, even in the absence of a well corroborated fossil record for the group under study. Finally, detailed phylogeographic studies should incorporate well justified sampling designs together with an emphasis on analysis of ecological components (Buckley 2009). Studies such as those conducted by Robertson and Zamudio (2009) and Robertson et al. (2009) should serve as templates for examining mammalian systems in this incredibly biodiverse region.

Acknowledgements

We thank the organizers of IMC-10, especially Bruce D. Patterson and Leonora Pires Costa, for the opportunity to present this summary. Nicole Lewis-Rogers and three anonymous reviewers read this paper and provided useful comments.

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CHAPTER 2

Molecular Phylogenetics of the *Handleyomys chapmani* complex in Mesoamerica

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[Manuscript published in Journal of Mamamlogy Vol. 95, Pp. 26-40]

Abstract

Handleyomys chapmani (Chapman's Handley's mouse) is a Mexican endemic rodent inhabiting humid montane forest of the Sierra Madre Oriental (SMO), the Oaxacan Highlands (OH), and the Sierra Madre del Sur (SMS). The systematic status of populations currently classified as *H. chapmani* has been problematic and to date evolutionary relationships among populations remain unresolved. In this study we use sequences from the mitochondrial cytochrome-*b* gene (*Cytb*; 1,143 base pairs) and intron 7 of the beta fibrinogen gene (*Fgb-I7*; 621 bp) to reconstruct a phylogeny, estimate divergence times and assess patterns of sequence variation over geography among samples of *H. chapmani.* This species was recovered as 2 monophyletic clades corresponding to the SMO-OH and SMS mountain ranges. Moreover, *H. saturatior*, the purported sister taxon to *H. chapmani*, was consistently recovered as the sister lineage to the SMO-OH clade, rendering *H. chapmani* paraphyletic. The geographic distribution of the 2 *H. chapmani* clades and of *H. saturatior* strongly correlate with the geographic extent of the SMO-OH, SMS, and the Trans-Isthmian Highlands (TIH; highlands east of the Isthmus of Tehuantepec through Central America) mountain ranges. Divergence times associate their isolation to late Pleistocene climatic changes that likely were reinforced by barriers such as the Isthmus of Tehuantepec, the Tehuacán-Cuicatlán Valley, and the Central Valleys of Oaxaca. The fact that populations of *H. chapmani* represent 2 independent evolutionary lineages results in a substantial reduction in the distributional range for both entities. Therefore, the conservation status of *H. chapmani* should be reevaluated.

Key words: conservation, cytochrome-*b*, *Fgb-I7*, *Handleyomys*, phylogeography, species delimitation, systematics.

Introduction

Recent studies employing molecular data have demonstrated that rodent populations from different mountain ranges in Mexico exhibit considerable levels of genetic differentiation (Arellano et al. 2005; Hardy et al. 2013; Harris et al. 2000; León-Paniagua et al. 2007; Rogers and González 2010; Rogers et al. 2007; Sullivan 1997; Vallejo and González-Cózatl 2012). Within the *Handleyomys alfaroi* group, some forms are confined to medium and high elevation forests in different mountain ranges of Mesoamerica (Musser and Carleton 2005). Taxonomically, this species group had been included in *Oryzomys,* but a systematic evaluation conducted by Weksler et al. (2006) proposed that the genus be restricted to the "*palustris* group" and the remaining 10 clades were elevated to generic rank, including the "*alfaroi* group", which was provisionally assigned to *Handleyomys*. As a result, we refer to members of the *H. alfaroi* group as *Handleyomys* rather than *Oryzomys* throughout this paper.

The *Handleyomys alfaroi* group (Goldman 1918; Hall 1981), as currently defined (Weksler et al. 2006), is a complex of 6 species that includes *H. alfaroi* (Alfaro's Handley's mouse), *H. chapmani* (Chapman's Handley's mouse), *H. melanotis* (Black-eared Handley's mouse), *H. rostratus* (Long-nosed Handley's mouse), *H. rhabdops* (Highland Handley's mouse), and *H. saturatior* (Cloud Forest Handley's mouse). Although previous workers (Goldman 1915, 1918; Hall 1981; Musser and Carleton 1993, 2005) had retained *H. melanotis* and *H. rostratus* in the *melanotis* group, Weksler et al. (2006) included these 2 species in the *H. alfaroi* group. The systematics of this species group has been controversial and, over time, this complex has included from 5 (Goldman 1918) to 12 species (Allen 1891, 1913; Allen and Chapman 1897; Goldman 1915; Merriam 1901). Within this complex, the taxonomy of the Mexican endemic *H. chapmani* also has been problematic. The 1st specimens referable to this taxon were collected

near Jalapa, Veracruz, in the Sierra Madre Oriental (SMO), and were regarded as *H. melanotis* by Allen and Chapman (1897). Later, Thomas (1898) referred to these specimens as *H. chapmani*. In 1901, Merriam recognized *H. chapmani* (sensu Thomas 1898) and described specimens from northern Oaxaca (Oaxacan Highlands; OH) as *H. c. caudatus* and those from Puebla as *H. c. dilutior* (SMO). Goldman (1915) then described specimens from Guerrero and southern Oaxaca in the Sierra Madre Sur (SMS) as *H. guerrerensis*. In his revision of North American rice rats, Goldman (1918) retained *H. guerrerensis* as a full species, but relegated *H. chapmani* and the 2 subspecies contained therein (*caudatus* and *dilutior*) as subspecies of *H. alfaroi*. Specimens collected by Dalquest (1951) from Tamaulipas and San Luis Potosí (SMO) were described as a new subspecies of *H. alfaroi* (*H. a. huastecae*). Interestingly, Goodwin (1969) recognized 2 different forms of *Handleyomys* in the mountains of northeastern Oaxaca (OH); the larger specimens were described as *H. caudatus* whereas the smaller form was viewed as *H. a. chapmani*. Additionally, specimens of *H. guerrerensis* from southern Oaxaca were relegated to a subspecies of *H. alfaroi* (*H. a. guerrerensis*—Goodwin 1969). Engstrom (1984) reported a unique karyotype for *H. caudatus* and recognized it as distinct from *H. alfaroi*. More recently, Musser and Carleton (1993, 2005) considered that all described forms restricted to cloud forests in the SMO, OH, and SMS (*H. a. chapmani, H. a. dilutior, H. a. guerrerensis*, *H. a. huastecae,* and *H. caudatus*) were conspecific and classified under the name *H. chapmani* with *H. saturatior* as the sister group.

Given that *H. chapmani* is distributed allopatrically across a series of mountain ranges in northern Mesoamerica and has a complicated taxonomic history, we used DNA sequence data from the mitochondrial gene cytochrome-*b* (*Cytb*) and the nuclear intron 7 of the beta fibrinogen (*Fgb-I7*) as a first approach to estimate phylogenetic relationships among populations of *H.*

chapmani. Other members of the *H. alfaroi* group for which tissue samples were available (*H. alfaroi*, *H. melanotis*, *H. rostratus*, and *H. saturatior*), also were included to estimate the phylogenetic affinities relative to *H. chapmani*. Specifically, we use our sequence data to test Musser and Carleton's (1993, 2005) proposal that all forms of *Handleyomys* restricted to cloud forest elevations of the SMO, OH, and SMS and currently considered as conspecific forms of *H. chapmani* (*H. a. chapmani, H. a. dilutior, H. a. guerrerensis*, *H. a. huastecae,* and *H. caudatus*) represent a monophyletic assemblage. Also, we test the hypothesis that *H. chapmani* and *H. saturatior* represent sister species (Musser and Carleton 1993, 2005).

Materials and Methods

Specimens examined and genes sequenced.—Specimens used in this study were wild-caught following guidelines approved by the American Society of Mammalogists (Sikes et al. 2011) or obtained via tissue loans and represent localities sampled across the known distribution of *H. chapmani* and species representing other members of the *H. alfaroi* species group (*H. alfaroi*, *H. melanotis*, *H. rostratus,* and *H. saturatior*; Fig. 1). Taxonomy follows Musser and Carleton (2005) with nomenclatural updates from Weksler et al. (2006). A total of 79 individuals were used in this study, of which 72 and 39 individuals were sequenced for *Cytb* and Intron *Fgb-I7*, respectively. In addition, 7 *Cytb* sequences were obtained from GenBank (Appendix I).

DNA extraction, amplification, and sequencing.—Total genomic DNA was extracted from liver tissue frozen or preserved in 95% ethanol either following Fetzner (1999) phenol–chloroform method, or using the QIAGEN DNeasy Tissue Kit (Cat. No. 69504; Qiagen, Valencia, California). Amplification of *Cytb* and *Fgb-I7* was performed via polymerase chain

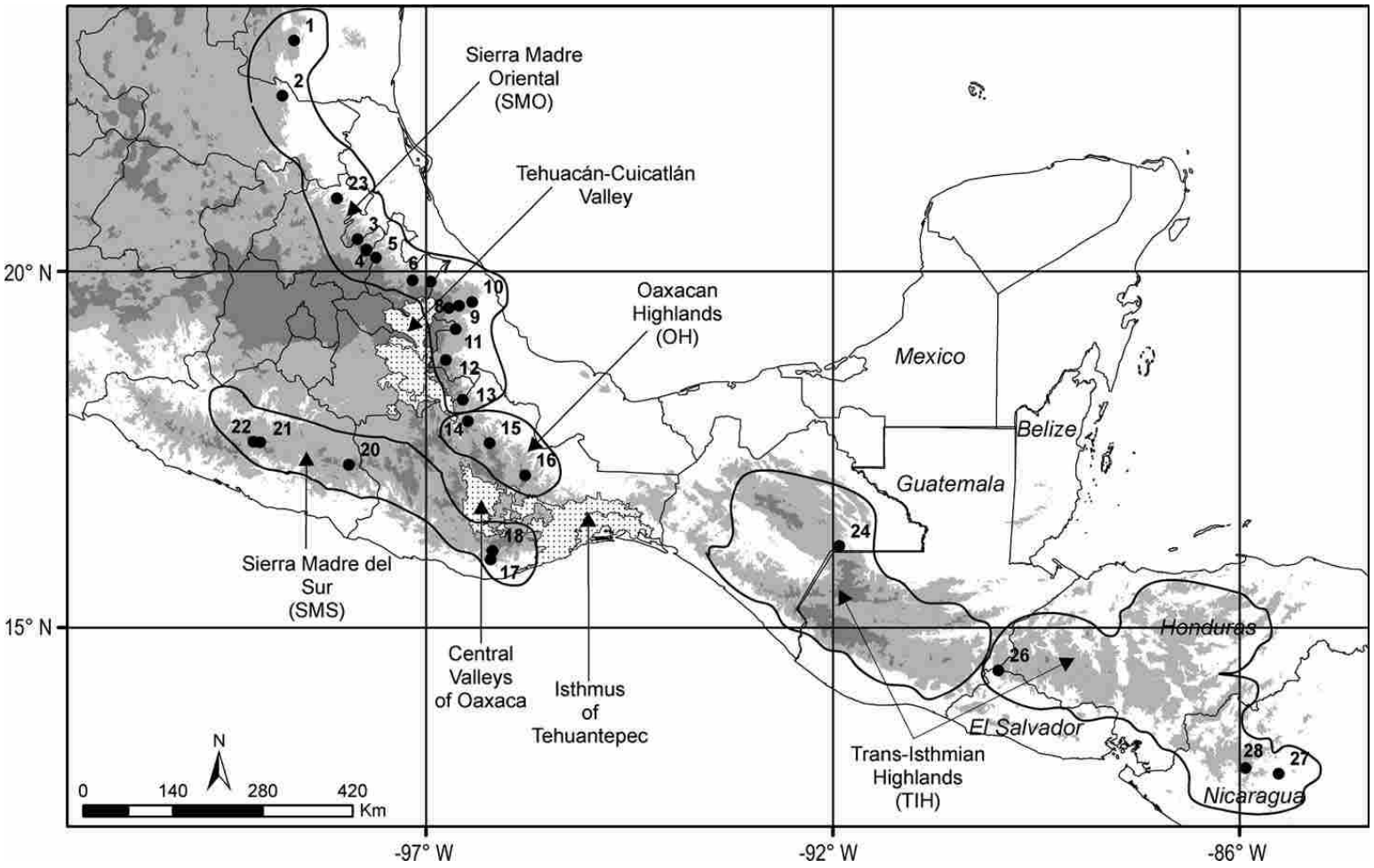

Figure 2. 1. Map of Mexico and northern Central America showing collecting localities (numbered dots) for *Handleyomys chapmani* and *H. saturatior*. Main geological features in the region are delineated in black and hypothesized barriers for dispersion are shown as stippled areas. Numbers correspond to those shown in Figs. 2 and 3 and in Appendix I. An elevation gradient is represented with white <800 m; light gray 800–2,200 m, and dark gray >2,200 m

reaction (PCR) with negative controls used for all amplifications. The complete *Cytb* gene was amplified with the primers MVZ-05 and MVZ-14-M (modified from Smith and Patton 1993 by Arellano et al. 2005) and internal primers MVZ-45, MVZ-16 (Smith and Patton 1993). *Fgb-I7* was amplified with primers B17 and Bfib (Wickliffe et al. 2003). For *Cytb*, the PCR master mix contained 1.0 µl template DNA, 1.0 µl dNTPs (1.25 mM), 0.5 µl of each primer (100 µM), 3.0 µl $MgCl_2$ (25 mM), 11.85 µl distilled H_20, and 0.15 µl Taq polymerase. For *Fgb-I7*, reactions included 3.0 µl template DNA, 1.7 µl dNTPs (1.25 mM), 2.5 µl of each primer (100 µM), 1.7 $MgCl_2$ (25 mM), 0.8 µl GeneAmp 10X PCR buffer, 13.7 µl HPLC-H_2O, and 0.125 µl Platinum Taq polymerase (Promega Corp., Madison, Wisconsin). Thermal profiles for *Cytb* were: 3 min at 94° C, 39 cycles of 1 min at 94° C, 1 min at 50° C, and 1 min at 72° C, and 5 min at 72° C followed by a soak at 4° C. For *Fgb-I7*, a hot start of 15 min at 85°C was used prior to the addition of dNTPs; this was followed by 10 min at 94° C, 32 cycles of 1 min at 94° C, 1 min at 65° C, and 1 min at 72° C, and a soak at 4° C. PCR products were purified either with a Gene-Clean PCR purification kit (Bio 101, La Jolla, California) or by using a Millipore (Billerica, Massachusetts) Multiscreen PCR 96-Well Filtration System (Cat. No. MANU03050). Sequencing reactions of purified PCR products were done with the Perkin–Elmer ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California). Excess dye terminator was removed using a Sephadex 50G solution (3g/50 ml H_2O) or with a Millipore MultiscreenFilter Plate (Cat. No. MAHVN4510). Light and heavy strand sequences were determined with an ABI 3100 automated sequencer (Applied Biosystems) housed in the DNA Sequencing Center at Brigham Young University or by Macrogen Inc., Seoul, Korea (http://www.macrogen.com). Sequences were edited manually using Sequencher version 4.1.1 and 4.1.2 (Gene Codes Corp., Ann Arbor, Michigan).

Phylogenetic analyses of the Cytb data set.—Alignment for *Cytb* was done by translating nucleotide sequences into amino acids with Codon Code Aligner v2.0.6 (Codon Code Corp., Dedham, Massachusetts). Unique haplotypes were identified with TCS v1.21 (Clement et al. 2000) and models of nucleotide substitution and genetic variation parameters that best fit our data were selected using jModelTest v1.1 (Posada 2008 using the Akaike information criteria). The model of evolution selected was TVM+Γ (Posada and Crandall 1998). Base frequencies were A = 0.3332, C = 0.3209, G = 0.0981, and T = 0.2480; transversion rates were (A-C) 0.2990, (A-G) 2.4623, (A-T) 0.3909, (C-G) 0.2127, (C-T) 2.4623, and the gamma shape parameter (Γ) was 0.2310. Maximum Likelihood (ML—Felsenstein 1981) and Bayesian Inference (BI—Yang and Rannala 1997) optimality criteria were used to estimate relationships among taxa using RAxML v7.4.8 (Stamatakis et al. 2006) and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), respectively.

Handleyomys alfaroi was designated as the outgroup for our phylogenetic analyses following its current taxonomic position as the sister lineage to *H. chapmani* and *H. rostratus* (Weksler et al. 2006). We allowed *H. rostratus* and *H. melanotis* (its presumed sister lineage) to be part of the ingroup along with *H. saturatior;* this as an alternative test of monophyly for *H. chapmani* (Nixon and Carpenter 1993).

For BI, 2 analyses with 3 chains were run independently for 10 million Metropolis coupled Markov Chain Monte Carlo (MCMC) generations using the default priors on model parameters starting from a random tree. For all analyses, a tree was sampled every 2,000 generations. Stationary was determined by monitoring the fluctuating value of the likelihood parameters using Tracer v1.4 (Rambaut and Drummond 2007). All the trees prior to stationarity were discarded as "burn in." For ML, a heuristic search starting from a random tree was

conducted with 1,000 replicates using RAxML v7.4.8 (Stamatakis et al. 2006). Kimura 2-parameter (Kimura 1980) genetic distances were calculated to assess within and among species genetic divergence using PAUP v4.0b10 (Swofford 2002) as they are directly comparable to distance values reported in treatments dealing with phylogeny reconstruction or species definitions of mammals (Baker and Bradley 2006; Smith and Patton 1993; Tobe et al. 2010).

Phylogenetic analyses of the Fgb-I7 data set.—Alignment of *Fgb-I7* data was done using the software MUSCLE (Edgar 2004). The model of evolution selected by jModelTest v1.1 as most appropriate for *Fgb-I7,* was HKY (Hasegawa et al. 1985). Base frequencies were A = 0.2949, C = 0.1725, G = 0.2035, and T = 0.3290; Transition/Transversion Ratio (ti/tv) = 1.2408. Phylogeny estimation was done as for *Cytb* data set for ML and BI.

Phylogenetic analyses of the combined data set.—Prior to combining the data partitions, we assessed the level of disagreement between the *Cytb* and *Fgb-I7* data sets with the Incongruence Length Difference Test (ILD—Farris et al. 1995; see also Hipp et al. 2004) using simple taxon addition, Nearest Neighbor Interchange (NNI) branch swapping, and a heuristic search using 1,000 replicates in PAUP v4.0b10 (Swofford 2002). Initially, the test was run by comparing 1,143 base pairs (bp) of *Cytb* with 621 bp of *Fgb-I7*, which resulted in rejecting the null hypothesis of data homogeneity ($P = 0.01$). Then, *Cytb* was reduced to its first 621 bp and to its last 621 bp in order to match the length of *Fgb-I7*. In both cases, these tests failed to reject the null hypothesis of data homogeneity ($P = 0.09$). This inconsistency highlights some of the criticisms of the ILD test (Barker and Lutzoni 2002; Hipp et al. 2004; Yoder et al. 2001). Alternatively, studies have demonstrated that total evidence may provide better resolution than separate analyses that are not fully resolved (Chippindale and Wiens 1994; Jackman et al. 1997), especially when the conflict is small and most regions of the tree are shared between partitions

(Wiens 1998). Therefore, we followed Wiens (1998) methodology for data combinability and analyzed each partition separately to identify parts of the tree where there was incongruence; then combined the data sets and considered the conflicted branches with caution. All major haploclades recovered by *Cytb* were represented in the combined data set. For BI and ML combined analysis, the partition substitution models formerly selected were specified. Combined data analyses were run with the same settings as described for the *Cytb*.

Nodal support.—ML branch support was determined with 1,000 non-parametric bootstrap pseudo-replicates (Felsenstein 1985). Bootstrapping was synchronized with phylogeny reconstruction in RAxML v7.4.8 (Stamatakis et al. 2006). Clades with bootstrap proportions (BP) above 70% were considered relatively well supported (Hillis and Bull 1993). For Bayesian analyses, the posterior probabilities (*pP*) for individual clades were obtained by constructing a majority rule consensus of the trees not discarded as burn-in using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003).

Topology tests.—Statistical support for the tree topologies was estimated as the posterior probability for the subset of possible trees in agreement with the topology we recovered (*pP*—Huelsenbeck and Rannala 2004). Specific ML topology tests were performed with the approximately unbiased (AU) test (Shimodaira 2002) with 10 sets of 10,000 bootstrap replicates. Both tests were performed on the combined data set and run in Consel (Shimodaira and Hasegawa 2001) using site log-likelihoods calculated with PAUP v4.0b10 (Swofford 2002). The model of nucleotide substitution for the AU test was GTR with optimized parameters using RAxML.

Divergence times estimates.—Phylogeny dating was assessed with the coalescent Bayesian approach for multilocus data implemented in BEAST v1.7.4 (Drummond and Rambaut

2007). For each partition, parameters for the model of nucleotide substitution were the same used for the phylogenetic analyses (unlinked substitution models). Two MCMC analyses were run for 10,000,000 generations with trees sampled every 1,000 generations. Stationary, appropriate Effective Sample Size (ESS) and convergence of independent MCMC was visualized with Tracer v1.5. The first 1,000 trees of each run (10% respectively) were discarded as burn in and the remaining trees were then combined to build a maximum credibility tree in TreeAnnotator v1.6. Analyses were done under the assumption of a relaxed molecular clock to account for heterogeneity of substitution rates among lineages (Arbogast et al. 2002). Using a Yule tree prior on the net rate of speciation, rates among lineages were assumed to be uncorrelated, and the rate for each branch was independently drawn from a lognormal distribution (uncorrelated log normal model, UCLN—Drummond et al. 2006). As calibration points we used fossil records for *H. alfaroi* and *H. rostratus* (= *H. melanotis*) from the Rancholabrean 0.3 million years ago (mya—Ferrusquía-Villafranca et al. 2010) and *H. alfaroi* from the late Quaternary (0.5–1.0 mya—Arroyo-Cabrales et al. 2002). This information was incorporated in the *H. rostratus* and *H. alfaroi* nodes to set hard lower bounds of 0.3 mya and 0.5 mya, respectively for the tMRCA (time of Most Recent Common Ancestor).

Delimiting species boundaries.—Although species delimitation is an inherent practice in phylogenetics, until recently, implementation of species boundaries had lacked a theoretical framework on which such limits could be tested explicitly (Rogers and Gonzalez 2010; Sites and Marshall 2003, 2004; Wiens 2007). This is attributable, at least in part, to the natural subjectivity of species concepts and incompatibilities among them (de Queiroz 2007). Nevertheless, this topic is receiving more attention and hypothesis-testing methods for

delimitation of species boundaries have been developed (see Camargo et al. 2012; Fujita et al. 2012; Wiens 2007).

The amount and direction of gene flow was estimated using MIGRATE-N v.3.3 (Beerli and Felsenstein 2001) as the mutation scaled effective migration rate (*M*) to account for the autosomal inheritance of *Fgb-I7*. *M* in turn was multiplied by the estimated effective population size (Theta = Θ) to obtain the effective number of migrants per generation (*Nm*). F_{ST} estimates were used as starting values to run 3 replicate chains with 100,000 genealogies. If migration between lineages was not perceived, the degree of exclusive ancestry was quantified with the Genealogical Sorting Index (GSI—Cummings et al. 2008). GSI ranges from 0 to 1, where values < 1 basically reflect additional coalescent events from the minimum required to unite all members of the group through a most recent common ancestor. Statistical significance for the GSI values (probability of finding that degree of exclusive ancestry in our groupings by chance) is estimated with a permutation test (Cummings et al. 2008). For BI trees (*Cytb*, *Fgb-I7*, and concatenated), GSI values were calculated for the last 100 trees of the MCMC search. For ML trees (*Cytb*, *Fgb-I7*, and concatenated), GSI values were calculated on the best tree found during the heuristic search (with RAxML v7.4.8; see "Materials and Methods"). We also calculated the GSI for the *Cytb* and *Fgb-I7* gene topologies ensemble (GSI_T).

Geographic association of the recovered lineages was assessed with the Nested Clade Phylogeographical Analysis (NCPA—Templeton 1998) and GeoDis (Posada et al. 2000) run in their automated form as implemented in ANeCA (Panchal 2007). Although the NCPA has been criticized, most of these arguments are based on the lack of statistical assessment of uncertainty and related to inconsistencies of the inferences of complex phylogeographic histories, particularly involving high migration rates (Beaumont and Panchal 2008; see Beaumont et al.

2010 for a detailed review). However, the performance of this method has been defended (Templeton 2008, 2010a, 2010b). For the purpose of this paper, migration rates were explicitly estimated previous to this test, and under those circumstances the test provides a concrete way to describe the distribution of genetic variation over geography.

Finally, using the sequence data from both markers, we estimated the posterior probability (BpP) for a model of speciation using those clades that were characterized by a lack of migration and suggested as significantly exclusive based on results of the GSI tests. These analyses were implemented in the software Bayesian Phylogenetics and Phylogeography (BPP v.2.0—Yang and Rannala 2010). The coalescent species delimitation method used in BPP relies on a reversible-jump Markov chain Monte Carlo (rjMCMC) for taking into account uncertainty due to unknown gene trees and ancestral coalescent processes. An equal prior probability was assigned to all species delimitation models (1–4 species, 5 species, and 6 species), and to ensure convergence of the estimates; rjMCMC was run with algorithm 0 and 1 (with fine-tune parameter $\boldsymbol{\varepsilon} = 15$) starting from 3 different trees (fully resolved; 6 species, 5 species, and 1 species). The rjMCMC was run for 500,000 generations with a sampling frequency of 5 after a burn-in period of 10,000. The mean value for the ancestral population size (Θ) was estimated with MIGRATE-N v.3.3 to set a gamma prior (α, β) of $\Theta = (5.0, 100)$ and root age (Tau = τ) $\tau 0 = (2, 1{,}000)$. The Kimura 2-parameter (Kimura 1980) genetic distances for *Cytb* were then used for sequence divergence comparisons within and among the lineages identified.

Results

Phylogenetic analysis of individual genes.—Of the 1,143 *Cytb* nucleotides, 329 were variable. Both ML and BI phylogenetic analyses converged on basically the same tree topology (Fig. 2). Nodal support was high for all species-level taxa and geographically exclusive clades.

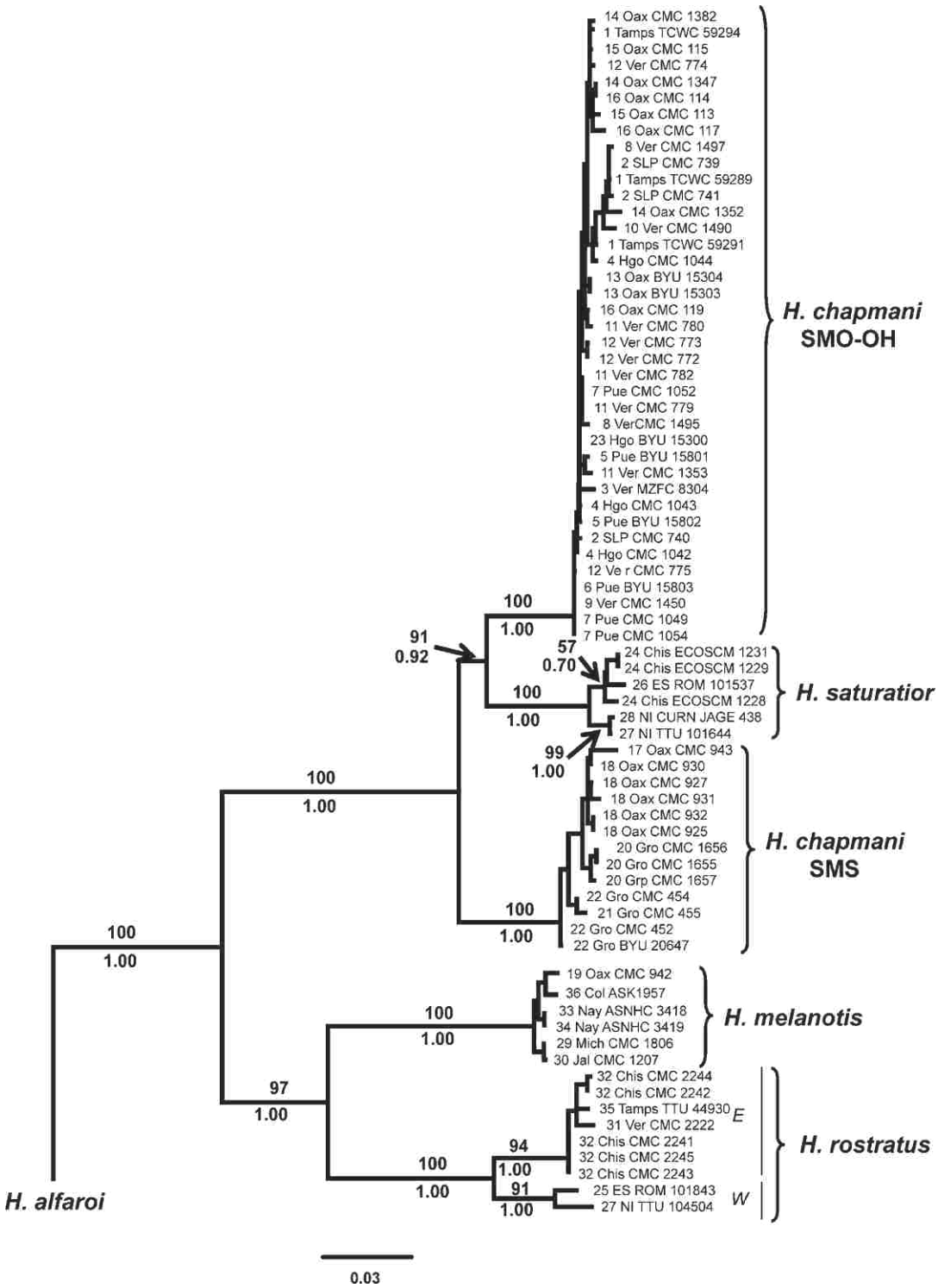

Figure 2. 2. Maximum likelihood (ML) phylogram based on *Cytb* sequence data. ML bootstrap values are shown above nodes and Bayesian inference (BI) posterior probabilities are shown below. Terminal labels indicate locality number; abbreviation for country (ES ¼ El Salvador and NI ¼ Nicaragua) or the Mexican states as listed in Appendix I, museum acronym, and museum voucher number.

A total of 65 haplotypes in the *Cytb* data set were identified, of which 49 represented samples of *H. chapmani* and 16 belonged to other species of *Handleyomys*. With only 3 exceptions, *H. chapmani* haplotypes also were exclusive by locality. These exceptions included 1 haplotype found at localities 11 (CMC 779, CMC 782) and 7 (CMC 1052; Fig. 1), and 1 haplotype present in localities 9 (CMC 1450), 6 (BYU 15803), and 7 (CMC 1049, CMC 1054); all within SMO. Similarly, within the OH a haplotype from locality 16 (CMC 114) was found at locality 14 (CMC 1347). Haplotypes representing *H. alfaroi*, *H. melanotis*, and *H. rostratus* also were exclusive by locality, except for a *H. melanotis* haplotype that was present at locality 33 (ASNHC 3418) and locality 34 (ASNHC 3419). Topologies generated under both ML and BI optimality criteria showed that *H. chapmani* is not a monophyletic group. Samples of this species were recovered in 2 divergent clades (SMO-OH and SMS; Fig. 2) with high nodal support (*pP* = 1.0, BP = 100 for both). Furthermore, *H. saturatior* was placed as the sister group to the *H. chapmani* SMO-OH clade (*pP* = 0.92, BP = 91). *H. melanotis* and *H. rostratus* were recovered as sister taxa (*pP* = 1.00, BP = 100), and samples of each species constituted strongly supported monophyletic assemblages (*pP* = 1.00, BP = 100). *H. rostratus* was recovered in 2 well supported clades (*pP* = 1.0, BP = 91-94) corresponding to samples from east and west of the Isthmus of Tehuantepec (Fig. 2).

The *Fgb-I7* data set consisted of 621 characters, of which 52 were variable. Three indels were inferred for our *Fgb-I7* sequences based on *H. alfaroi* as the outgroup. One was assigned at position 283 (single bp deletion for *H. chapmani* and *H. saturatior*), a 2nd gap was identified at positions 382-383 (an insertion inferred for all ingroup taxa), and a 3rd indel was set at positions 407-417 (a deletion inferred for *H. rostratus*). There were 14 *Fgb-I7* haplotypes identified by TCS (Clement et al. 2000), 8 of which were present only in *H. chapmani*. Of these 8, 3 were

unique haplotypes and 5 were shared but exclusive by regions (SMS, SMO-OH). *H. saturatior* was represented by 2 haplotypes corresponding to different localities, *H. alfaroi* by the same haplotype found at 2 localities, *H. melanotis* by 1 haplotype present in all 5 localities, and *H. rostratus* by 2 haplotypes from a single locality.

Phylogenetic analyses of *Fgb-I7* based on ML and BI optimality criteria estimated genealogies that were highly concordant, albeit less resolved than those recovered with *Cytb* (Fig. 3a). Samples of *H. chapmani* and *H. saturatior* grouped together as a well-supported monophyletic assemblage (*pP* = 1.00, BP = 100), although this clade resulted in an unresolved polytomy. Nonetheless, within this clade samples were arranged following a geographic pattern by mountain range. Samples of *H. chapmani* from the SMS formed 2 clades; 1 comprising CMC 1655 and CMC 1657 from El Tejocote, Guerrero (locality 20; *pP* = 1.00, BP = 89), and the other comprising the remaining samples. Similarly, all *H. saturatior* samples except ECOSCM 1231 (locality 24) also were recovered in a well-supported clade (*pP* = 1.00, BP = 98). *H. melanotis* and *H. rostratus* were recovered as monophyletic clades (*pP* = 1.00, BP = 100, for both clades). However, *H. melanotis* was placed as sister group to the *H. chapmani* and *H. saturatior* clade (*pP* = 0.90, BP = 90).

Phylogenetic analysis of combined data set.—Trees estimated from the combined data set (*Cytb* and *Fgb-I7*) converged on basically the same tree topology for both ML and BI optimality criteria, as recovered by the *Cytb* tree (Fig. 2). Fig. 3b depicts the ML tree (lnL = - 5754.025). *H. chapmani* was recovered as 2 polyphyletic clades (SMO-OH and SMS). Each of the clades recovered was strongly supported by Bayesian *pP* and ML bootstrap values (pP = 1.00, BP = 100). Additionally, the *H. chapmani* SMO-OH clade was placed as the sister group to *H. saturatior* (*pP* = 0.90, BP = 83).

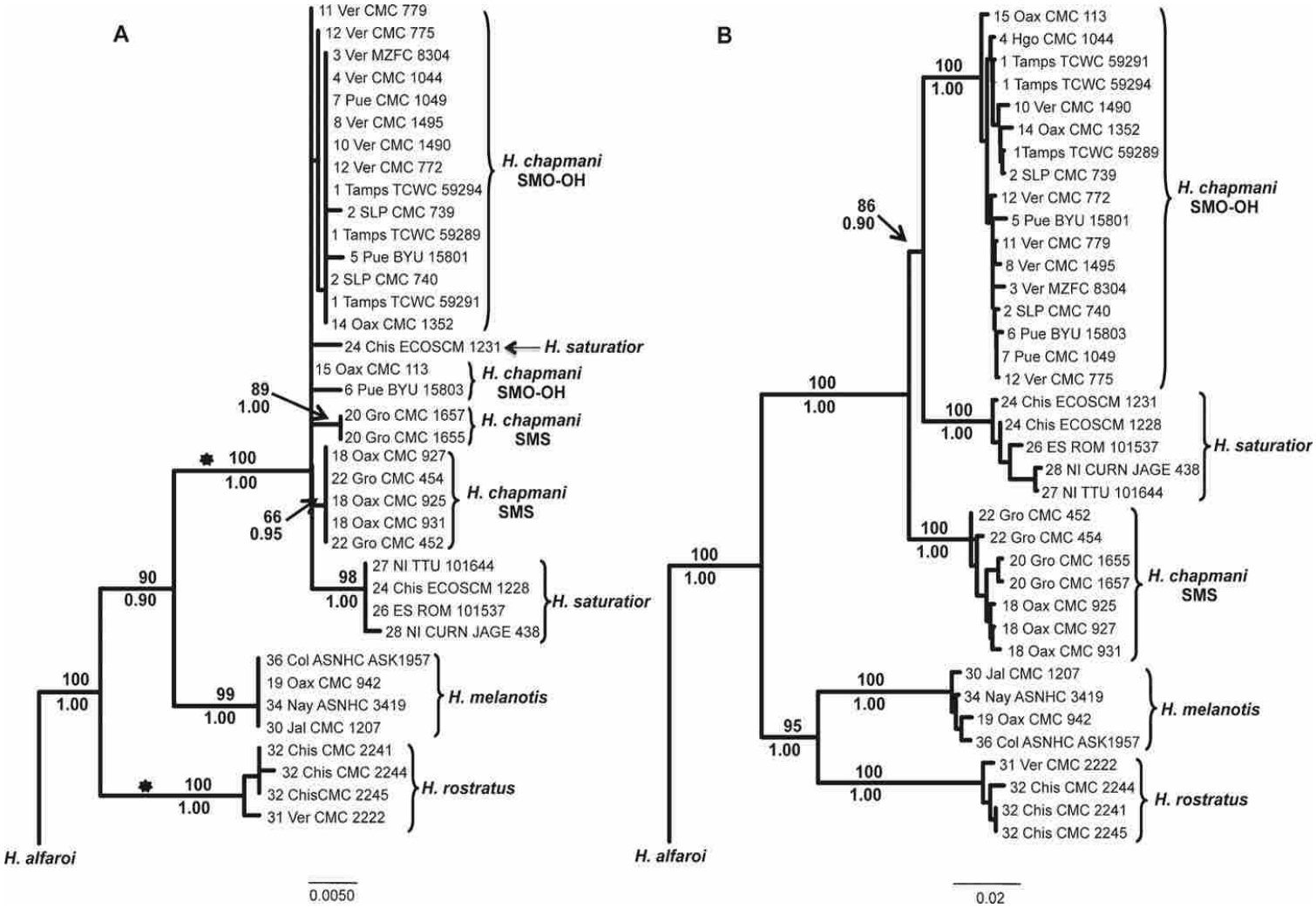

Figure 2. 3. Maximum likelihood (ML) phylograms based on a) *Fgb-I7* sequence data and b) the combined (*Cytb* and *Fgb-I7*) data set. For both trees, ML bootstrap values are shown above nodes and Bayesian inference (BI) posterior probabilities are shown below. Stars represent inferred gaps in the *Fgb-I7* sequence data mapped onto the tree. Terminal labels are as in Fig. 2.

Topology tests.—The *pP* value for a topology that constrained clades representing *H. chapmani* to be monophyletic was *pP* = 0.142, whereas the probability of a topology with *H. chapmani* paraphyletic (as recovered in this study) was *pP* = 0.858. With the AU test, the hypothesis of *H. chapmani* monophyly was rejected (AU = 0.0451; $P < 0.05$). Log-likelihood of the constrained topology (*H. chapmani* monophyletic) was lnL = - 5,762.05701, whereas the unconstrained topology (*H. chapmani* paraphyletic) was lnL = -5,754.025.

Divergence times estimates.—The MCMC combined runs reached ESS above 450 for all parameters. The standard deviation of the uncorrelated lognormal relaxed clock for *Cytb* had a mean of 0.883 and 1.982 for *Fgb-I7*, indicating that both were not behaving in a clock-like manner. The mean substitution rate (per site per million years) was 0.027 for *Cytb*, and 0.009 for *Fgb-I7*. On a time scale, *H. alfaroi* was not used to root the tree because the root is implicit as the most recent common ancestor (MCRA). The root was placed with a mean age of 2.51 mya (highest posterior density interval [95% HPD] = 1.30, 3.76). A mean divergence time of 1.45 mya was estimated for the *H. chapmani* SMS clade (95% HPD = 0.65, 2.40), whereas the *H. chapmani* SMO-OH and *H. saturatior* split was estimated at 1.08 mya (95% HPD = 0.54, 1.86). The divergence time estimate for *H. melanotis* and *H. rostratus* was placed at 1.53 mya (95% HPD = 0.68, 2.50). When it was not constrained as the root, *H. alfaroi* was positioned as sister to the *H. melanotis* and *H. rostratus* clade; the tMRCA for this clade was estimated at 2.07 mya (95% HPD = 1.08, 2.97).

Inferred species boundaries.—There was no evidence of gene flow between *H. chapmani* (SMO-OH) - *H. saturatior Nm* = 0.09 (95% HDP = 0.00 – 0.18), between *H. chapmani* (SMO-OH) - *H. chapmani* (SMS) *Nm* = 0.07 (95% HDP = 0.00 – 0.14), or between *H. chapmani* (SMS)

H. saturatior Nm = 0.09 (95% HDP = 0.00 – 0.18). The opposite migration estimates were equivalent and are not shown.

The GSI values for *H. chapmani* as currently defined (SMO-OH and SMS clades labeled as *H. chapmani*) averaged 0.794 (min = 0.552, max = 0.894; Table 1). When *H. chapmani* was labeled according to the 2 clades recovered in this study, the GSI values were higher for each lineage (SMO-OH averaged 0.889 [min = 0.655, max = 1] and SMS averaged 0.862 [min = 0.604, max = 1]). The GSI values for *H. saturatior* were smaller (min = 0.379, max = 1, average = 0.779) than those calculated for each *H. chapmani* clade. For each basal lineage recovered in our study, *Cytb* and concatenated data topologies consistently recovered values of 1 (achieved monophyly); and weighted GSI analyses (*Cytb* and *Fgb-I7* topologies combined; GSI_T) ranged from 0.664 to 0.827. All GSI statistics had significant *P*-values (< 0.0004). *Fgb-I7* trees yielded lower GSI values for all the groupings (Table 1).

The species delimitation analyses (BPP) strongly supported a model of 5 speciation events (6 species; Fig. 4), corresponding to *H. alfaroi*, *H. melanotis*, *H. rostratus*, *H. chapmani* SMS*, H. saturatior*, and *H. chapmani* SMO-OH (BpP = 0.99620). Under this model, *H. chapmani* SMO-OH, *H. chapmani* SMS, and *H. saturatior* have a BpP = 1.00000; *H. alfaroi* a BpP = 0.99999, and *H. rostratus* and *H. melanotis* a BpP = 0.99666. In contrast, a model of 5 species had a BpP = 0.00374, and a model of < 5 species had a BpP = 0.00005. To examine the effect of excessive a priori subdivision, we further split *H. chapmani* SMO-OH to create a model with 7 species (all of the above species plus *H. chapmani* OH populations as the 7th). This model had a much lower probability BpP = 0.09518 than our 6 species speciation model (BpP = 0.99620).

Table 2. 1. Genealogical sorting index = **GSI** and **BPP** posterior probability (**BpP**) for *H. chapmani* as currently recognized (SMO-OH and SMS clades labeled as *H. chapmani*), and for the SMO-OH and SMS clades labeled as different groups. Values for *H. saturatior* also are shown as a reference for a recognized and diagnosable species in the group. **GSI** values correspond to individual genes topologies ($\mathbf{GSI}_{Cytb}$ and $\mathbf{GSI}_{Fgb\text{-}I7}$), the concatenated data tree ($\mathbf{GSI}_{\text{Concatenated}}$) and for an ensemble from the *Cytb* and *Fgb-I7* topologies ($\mathbf{GSI}_{T}$); and for the trees generated with the two analyses, **ML** and **BI**. All **GSI** values had highly significant *p*-values (< 0.0004).

	ML				**BI**				**BpP**
	$\mathbf{GSI}_{Cytb}$	$\mathbf{GSI}_{Fgb\text{-}I7}$	**GSI** Concatenated	$\mathbf{GSI}_{T}$	$\mathbf{GSI}_{Cytb}$	$\mathbf{GSI}_{Fgb\text{-}I7}$	**GSI** Concatenated	$\mathbf{GSI}_{T}$	
SMO-OH	1.000	0.808	1.000	0.827	1.000	0.655	1.000	0.827	1.000
SMS	1.000	0.604	1.000	0.703	1.000	0.830	1.000	0.762	1.000
H. saturatior	1.000	0.379	1.000	0.664	1.000	0.379	1.000	0.813	1.000
H. chapmani SMO-OH /SMS	0.817	0.552	0.766	0.870	0.817	0.797	0.894	0.845	0.004

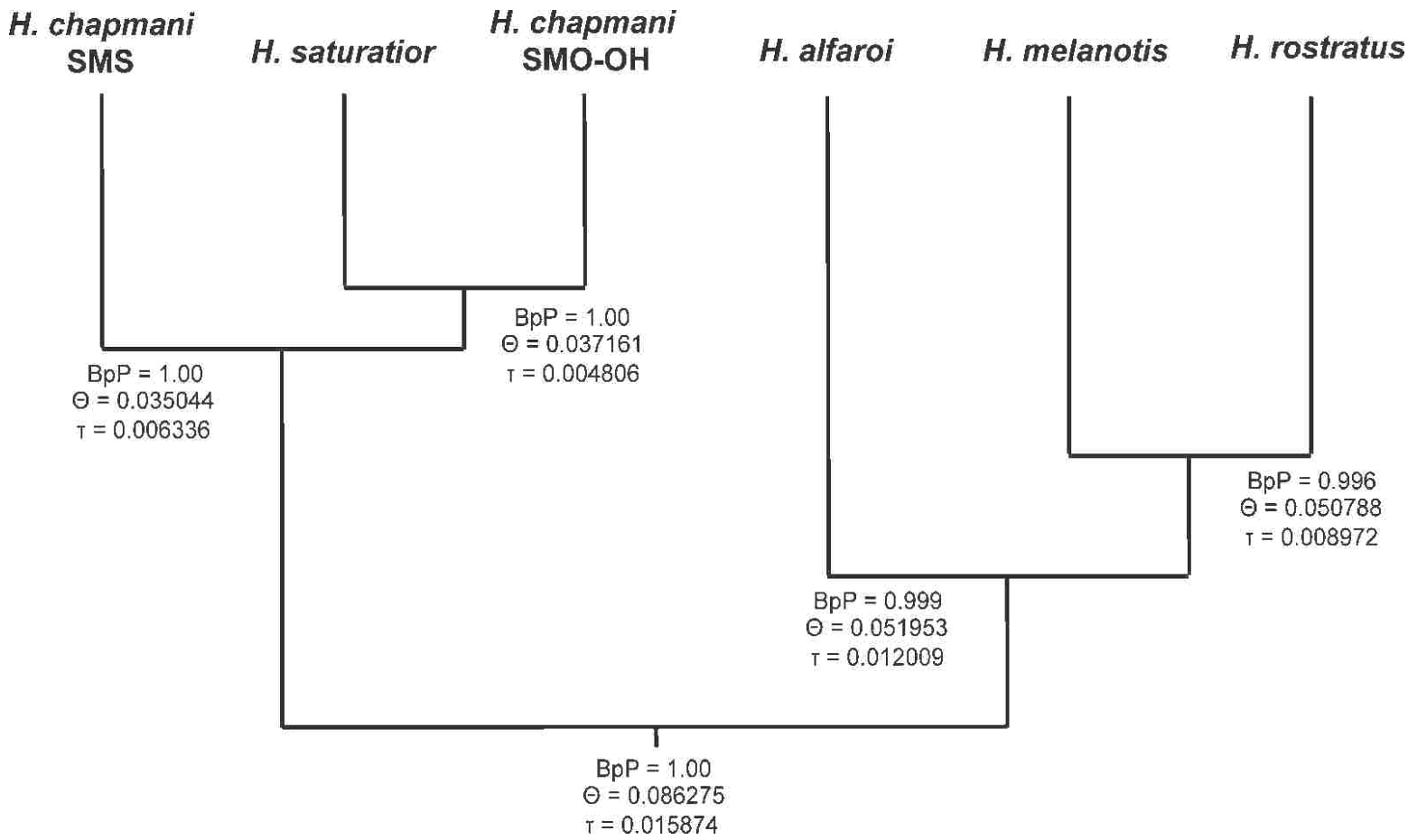

Figure 2. 4 Bayesian species delimitation (6 species) BpP.0.99620. A model with 5 species had a BpP.0.00374, a model with 7 species a BpP.0.09518; and a model of 5 species a BpP.0.00005. Bayesian posterior probability (BpP) for a lineage split and mean posterior estimates for **Θ** and **τ** are shown below nodes.

The *Cytb* haplotype networks we identified corresponded to the clades recovered by ML and BI analyses. Samples of *H. chapmani* were represented in 2 separate networks (SMS and SMO-OH). The NCPA indicated that haplotypes of *H. chapmani* corresponding to the SMS and SMO-OH clades are geographically isolated genetic clusters ($P = 0.0277$ and $P = 0.0411$ respectively). Within SMS (Geodis Dc), the southern Oaxaca and western Guerrero haplotypes are the most geographically restricted ($P = 0.0121$). Within SMO, haplotypes found in San Luis Potosí connected to those in Hidalgo but a significantly large nested clade distance (Dn; $P = 0.0249$). In contrast, haplotypes from northern Oaxaca (OH) were recovered as a separate genetic unit from the rest of *H. chapmani* SMO ($P = 0.0010$; Figs. 1 and 2).

Discussion

Our molecular phylogeny demonstrates that *H. chapmani* is comprised of 2 non-sister lineages that are restricted to different mountain systems (SMO-OH and SMS). Moreover, the SMO-OH clade is the sister group to *H. saturatior*, which occurs to the east of the Isthmus of Tehuantepec in the highlands of Chiapas and Central America (TIH). Together, these 3 lineages constitute a well-supported monophyletic assemblage. By extension, our phylogenetic analyses do not support Musser and Carleton's (1993, 2005) proposal that all forms of *H. chapmani* (*H. a. chapmani, H. a. dilutior, H. a. guerrerensis*, *H. a. huastecae,* and *H. caudatus*) restricted to cloud forest habitat in the SMO, OH, and SMS are conspecific.

The mean *Cytb* genetic distance among clades of *H. chapmani* from the SMO-OH and SMS was 6.5%, whereas values between *H. chapmani* SMO-OH and *H. saturatior* and between *H. chapmani* SMS and *H. saturatior* were 6.0% and 6.9%, respectively. These values are comparable to those among many cryptic species of mammals (Baker and Bradley 2006). Also, the degree of genetic differentiation is in agreement with our divergence time estimates, which

suggests that the 2 lineages of *H. chapmani* (SMO-OH and SMS) and *H. saturatior* are the most recently derived lineages within the *H. alfaroi* group (1.08 - 1.45 mya). According to Arroyo-Cabrales et al. (2002), Ceballos et al. (2010), and Ferrusquía-Villafranca et al. (2010), *H. rostratus* and *H. alfaroi* were well-differentiated forms in the Pleistocene fauna of Mexico. This proposal is consistent with the estimated MRCA for *H. alfaroi – H. rostratus* and *H. melanotis* (2.07 mya), and the relatively large percent *Cytb* sequence divergence between them (13.2%).

Although *Fgb-I7* has been useful in resolving intra-generic relationships in other rodent groups (Hanson and Bradley 2009; Matocq et al. 2007), it typically has a slower substitution rate than *Cytb* in mammals (Wickliffe et al. 2003). We interpret the lack of resolution in the *Fgb-I7* topology as a case of incomplete lineage sorting. This is supported by the lack of evidence for gene flow and the *Fgb-I7* GSI values showing a substantial amount of exclusivity for each *H. chapmani* lineage (0.65 to 0.83) despite the partially resolved phylogeny.

Lack of detectable gene flow between *H. chapmani* clades SMO-OH and SMS (*Nm* = 0.11), and between any of these 2 lineages and *H. saturatior* (average *Nm* = 0.13) also support the notion that these groups represent separate biological species. Similarly, the geographic distributions of these 3 lineages are allopatric as supported by the NCPA analysis. The GSI values for *H. chapmani* clades SMO-OH and SMS showed considerable amounts of exclusive ancestry (mean GSI values of 0.889 and 0.862, respectively) and achieved monophyly (GSI = 1) with *Cytb* and concatenated data topologies. Moreover, the GSI values for the 2 *H. chapmani* clades were consistently larger than for *H. saturatior*, whose average GSI value was 0.779. Accordingly, BPP assigned the highest probability to a speciation model in which *H. chapmani* SMO-OH and *H. chapmani* SMS constitute 2 separate species (BpP = 1.0 for each lineage). This interpretation is also consistent with the phylogenetic species concept (Cracraft 1989).

Therefore, we regard the SMS evolutionary lineage of *H. chapmani* as an unrecognized species based on our molecular genealogy and morphological differences (Goldman 1915, 1918) from the SMO-OH clade.

Goldman (1915) described individuals from Omiltemi, Guerrero (SMS; locality 21; CMC 455) as *H. guerrerensis*. Later, he incorporated individuals from southern Oaxaca (SMS; ~10 km E locality 17; CMC 943) and extended the geographic distribution of this taxon to the "forested Pacific slopes of the Sierra Madre del Sur in Guerrero and Oaxaca" (Goldman 1918:69). In comparison to *H. chapmani* from the SMO-OH, Goldman (1918:70) described skulls representing the SMS form as "smaller and flatter; zygomata tending to curve evenly outward, the sides less nearly parallel; sides of rostrum more tapering anteriorly; ascending branches of premaxillae usually broader posteriorly; maxillary arms of zygoma more slender; incisors smaller." Goodwin (1956) acknowledged the morphological uniqueness of the SMS form described by Goldman (1918) and retained it as species. In a review of the mammals of Oaxaca, Goodwin (1969) included individuals from the SMS localities 17 (CMC 943), 18 (CMC 925, CMC 930, CMC 931, CMC 932, CMC 927) and ~80 km NE (by road) locality 20 (CMC 1655, CMC 1656, CMC 1657) and compared them to *H. chapmani* from the SMO-OH including locality15 (CMC 113, CMC 115), and locality 16 (SMO-OH; CMC 114, CMC 117, CMC 119). Although Goodwin (1969) acknowledged the morphological features underlined by Goldman (1918), he relegated *H. guerrerensis* to a subspecies of *H. alfaroi.*

Because the name *chapmani* first was assigned to voucher specimens of *Handleyomys* from Xalapa, Veracruz (Thomas 1898—but originally described as *H. melanotis* by Allen and Chapman [1897]), and our sampling included 1 specimen from this locality (CMC1450; locality 9) plus 4 more from a nearby location (CMC 1495, CMC 1497; locality 8; CMC 1353, CMC

1490; locality 10), we propose that the SMO-OH clade should retain the name *chapmani*. Determining a valid name for the SMS clade would require sequence data from specimens collected at or near type localities of names currently in synonymy. Goldman (1915) first used the term *guerrerensis* and Goodwin (1956; 1969) preserved the name *guerrerensis*. Therefore, taking into account that our sampling of the SMS included specimens from the type locality of *guerrerensis* (CMC 455; locality 21), we propose that *guerrerensis* is the name with priority and should be applied to the *H. chapmani* SMS clade. *H. saturatior* originally was described as a subspecies of *H. chapmani* (Merriam 1901) and later was retained as a subspecies of *H. alfaroi* (Goldman 1918). Our results support Musser and Carleton's (1993, 2005) recognition of *H. saturatior* as a species-level taxon.

There is general agreement that the highlands of the SMO, OH, SMS, and the different mountain ranges in the TIH represent different biogeographic provinces (Contreras-Medina et al. 2007; Halffter 1987; Liebherr 1994; Marshall and Liebherr 2000; Morrone 2010). Overall, the distributional patterns observed in these studies are supported by a variety of taxa, including plants and various animal groups (Bryson et al. 2011; Contreras-Medina et al. 2007; García-Moreno et al. 2004, 2006; Luna-Vega et al. 2001; Puebla-Olivares et al. 2008). The SMS and SMO provinces are thought to have been separated by intense volcanism in the Miocene (~15 mya) during the formation and migration of the Mexican Transvolcanic Belt, with continuing volcanism until ~3.5 mya (Ferrari et al. 1999). Similarly, the highlands south of the Isthmus of Tehuantepec were repeatedly isolated from mountain ranges to the north and east, with the most recent marine incursion thought to have occurred in the late Pliocene ~3.6 mya (Beard et al. 1982; Coates and Obando 1996; Maldonado-Koerdell 1964). Climatic changes during the

Pleistocene (~2.5 mya) could have reinforced the isolating effects of a low-lying isthmus (Toledo 1982), as supported by our divergence time estimates.

Overall, levels of genetic differentiation within the *H. chapmani* - *H. saturatior* complex are in agreement with 3 main clades that occur in isolated mountain ranges (SMO-OH, SMS, and TIH). Therefore, it is reasonable to assume that these lineages have been subjected to similar historical genetic isolation and diversification as have other montane rodent taxa such as *Peromyscus* (Harris et al. 2000; Sullivan et al. 1997), *Reithrodontomys* (Arellano et al. 2005; Hardy et al. 2013), *Habromys* (León-Paniagua et al. 2007; Rogers et al. 2007), and *Glaucomys* (Kerhoulas and Arbogast 2010), as well as a variety of other vertebrate taxa (see Almendra and Rogers 2012 for a recent summary). However, the degree of divergence of the splits among the 3 main lineages of the *H. chapmani – H. saturatior* complex is not completely consistent with the general patterns observed in other taxonomic groups inhabiting montane systems. Although the lowlands of the Tehuacán-Cuicatlán Valley and the Central Valleys of Oaxaca separate the SMO-OH and SMS, biogeographically, it would be more plausible to expect a closer relationship between the 2 lineages of *H. chapmani* (SMO-OH and SMS). This is because their distributional ranges are closer to each other than either is to *H. saturatior* (TIH).

It has been suggested that the Isthmus of Tehuantepec represents the deepest biogeographic break for closely related taxa of rodents with a geographic distribution along the highlands of México and Central America (Sullivan et al. 2000). Likewise, genetic differentiation recovered herein has been replicated for other rodent clades whose distributions span the Isthmus of Tehuantepec: *Peromyscus* (Sullivan et al. 1997); *Reithrodontomys* (Arellano et al. 2005; Hardy et al. 2013), *Habromys* (León-Paniagua et al. 2007), and *Neotoma* (Edwards and Bradley 2002) as well as other highland taxa (birds—Barber and Klicka 2010; Weir et al.

2008; and reptiles—Castoe et al. 2009). Nevertheless, our data show that within the *H. chapmani* - *H. saturatior* complex, the deepest split corresponds to the Tehuacán-Cuicatlán Valley and the Central Valleys of Oaxaca, rather than the Isthmus of Tehuantepec. The isthmus has played an important role in the evolutionary diversification of *H. chapmani* (SMO-OH) - *H. saturatior*, as noted by Musser and Carleton (1993, 2005).

It is interesting to note that even though the distribution area of the *H. chapmani* SMO-OH clade includes 2 mountain ranges that are split by the Rio Santo Domingo valley in Oaxaca, samples from each mountain system were not separated in our phylogenetic analyses. This pattern is consistent with that of other rodent species that are continuously distributed along highlands of the SMO and OH, but with no apparent genetic differentiation between samples occurring on each mountain system (i.e., the Mexican harvest mouse, *Reithrodontomys mexicanus*—Arellano et al. 2005). There are, however, examples of other groups of rodents in which the Rio Santo Domingo has played an important role in the diversification of populations on either side of this geological barrier (Jico deermouse, *Habromys simulatus* (SMO); Chinanteco deermouse, *H. chinanteco* (OH)—Carleton et al. 2002; Rogers et al. 2007; Nelson's big-toothed deermouse, *Megadontomys nelsoni* (SMO); Oaxacan big-toothed deermouse, *M. cryophilus* (OH)—Vallejo and González-Cózatl 2012). The only evidence of differentiation between samples of *H. chapmani* from SMO and OH was generated by our NCPA analysis which found that haplotypes from northern Oaxaca (OH) constitute an allopatric genetic unit from the rest of *H. chapmani* SMO ($P = 0.0010$).

Mexico is considered a biodiversity hotspot for mammals, both in terms of species richness and endemism (Ceballos 2007; Ceballos and Ehrlich 2006; Ceballos et al. 1998; Giam et al. 2011). Tropical montane cloud forest is the most diverse vegetation type in Mexico, but

comprises only 1% of the land surface of the country (Pedraza and Williams-Linera 2003). Unfortunately, cloud forest habitat has suffered a loss of 41% of its original land area, and of what remains, more than 52% is degraded (Mas et al. 2009; Sánchez Colón et al. 2009). As a result of habitat loss, *H. saturatior* is currently listed as Near Threatened (Reid et al. 2008) and *H. rhabdops* is listed as Vulnerable (Reid and Vázquez 2008). Despite the high rates of cloud forest deforestation, *H. chapmani* is listed as Least Concern mainly because of its relatively large distribution (Castro-Arellano and Vázquez 2008). However, the fact that we recovered 2 evolutionary units within *H. chapmani* results in a substantial reduction in range for both lineages. As a result, the conservation status of *H. chapmani* should be reevaluated.

Acknowledgements

We acknowledge financial support from the Department of Biology, the Office of Research and Creative Activities and the Monte L. Bean Life Science Museum at Brigham Young University (to DSR), and the Consejo Nacional de Ciencia y Tecnología (CONACYT; to ALA). Partial funding for this work was provided by a grant from CONACyT (2002-C01-39711) to F. X. González-Cózatl. Permits for field work in Mexico and were issued by the Secretaria de Medio Ambiente y Recursos Naturales (SEMARNAT) to F. X. González-Cózatl. We thank the following individuals and institutions for providing tissue loans: R. J. Baker, Natural Sciences Research Laboratory, Texas Tech University; F. A. Cervantes, Universidad Nacional Autónoma de México; M. D. Engstrom, Royal Ontario Museum; R. C. Dower, Angelo State University; and C. Lorenzo, Colección Mastozoológica de El Colegio de la Frontera Sur. Data analyses were performed using the Fulton Supercomputer at Brigham Young University. We also thank the following people for their help with specimen collection, laboratory work, or other critical input: E. Arellano, R. Mercado, D.K. Hardy, and J. L. T. Magadán.

Resumen

Handleyomys chapmani (ratón de Handley de Chapman) es un roedor endémico de México con distribución en la Sierra Madre Oriental (SMO), Sierra Norte de Oaxaca (OH) y Sierra Madre del Sur (SMS). El estatus taxonómico de las poblaciones actualmente clasificadas como *H. chapmani* ha sido problemático y hasta la fecha, las relaciones evolutivas entre dichas poblaciones continúan sin resolverse. En este estudio, usamos secuencias del gen mitocondrial citocromo *b* (1143pb) y del intron 7 del gen beta fibrina (621pb) para estimar una filogenia del grupo, tiempos de divergencia y analizar los patrones de variación genética entre poblaciones de *H. chapmani* en un sentido geográfico. *H. chapmani* fue recuperado en 2 clados monofiléticos correspondientes a los sistemas montañosos de la SMO-OH y SMS. Además, *H. saturatior* (ratón de Handley de bosque nublado), reconocido como el grupo hermano de *H. chapmani*, fue consistentemente recuperado como el linaje hermano al clado de las SMO-OH; revelando a *H. chapmani* como un taxón parafilético. La distribución geográfica de los 2 clados en *H. chapmani* y *H. saturatior* muestra una fuerte correlación con la extensión geográfica de la SMO-OH, la SMS y las Tierras Altas Trans-Istmicas (TIH; tierras altas al este del Istmo de Tehuantepec en Chiapas y América Central). Los tiempos de divergencia asocian el aislamiento de éstas entidades con cambios climáticos del Pleistoceno superior, que posiblemente fue reforzado por barreras geográficas como el Istmo de Tehuantepec, el Valle Tehuacán-Cuicatlán y los Valles Centrales de Oaxaca. El hecho de que las poblaciones de *H. chapmani* constituyan 2 entidades evolutivas, tiene como consecuencia la reducción significativa del rango de distribución de estos 2 linajes. Por lo tanto, el estatus de conservación de *H. chapmani* debe ser reevaluado.

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Submitted 10 February 2013. Accepted 6 October 2013.

Associate Editor was Burton K. Lim.

Appendix I

Specimens examined.— For each voucher specimen of *Handleyomys* we list the museum acronym and catalog number as follows: BYU = Monte L. Bean Life Science Museum, Brigham Young University; CMC = Colección de Mamíferos del Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos; CURN = Centro Universitario Regional del Norte de la Universidad Autónoma de Nicaragua; ECOSCM = El Colegio de la Frontera Sur; MZFC = Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México; ROM = Royal Ontario Museum; and TCWC = Texas Cooperative Wildlife Collection, Texas A&M University. For sequences from Genbank we list the accession ID. Specimens are listed by taxon, country, collecting location, locality number, museum voucher number, and specimen field number. Abbreviations *Cytb,* and *Fgb-I7* indicate which gene or gene segment was sequenced for each individual.

Handleyomys alfaroi.—ECUADOR: Esmeralda, Comuna San Francisco de Bogotá (**37**; *Cytb* = EU579488); MEXICO: Veracruz (Ver), Catemaco, 13.0 km NW (by road) Sontecomapan, Estación Los Tuxtlas-IBUNAM, 150 m (**31**; CMC 2246 = DSR 8543 [*Cytb* = KF658401, *Fgb-I7* = KF658443], CMC 2247 = DSR 8544 [*Cytb* = KF658400, *Fgb-I7* = KF658444]); NICARAGUA: Matagalpa, Selva Negra, 250 m (**27**; *Cytb* = EU579489).

Handleyomys chapmani.—MEXICO: Tamaulipas (Tamps), El Cielo, San José, 1329 m (**1**; TCWC 59291 = ICA 36 [*Cytb* = KF658365, *Fgb-I7* = KF658451], TCWC 59294 = ICA 69 [*Cytb* = KF658373, *Fgb-I7* = KF658452], TCWC 59289 = ICA 75 [*Cytb* = KF658375, *Fgb-I7* = KF658450]; San Luis Potosí (SLP), El Naranjo, 3.5 km N 3 km W, Maguey de Oriente (**2**; CMC 739 = FXG 527 [*Cytb* = KF658376, *Fgb-I7* = KF658422], CMC 740 = FXG 528 [*Cytb* = KF658356, *Fgb-I7* = KF658423], CMC 741 = FXG 529 [*Cytb* = KF658377); Veracruz (Ver),

Zacualpan (**3**; MZFC 8304 = HBR 069 [*Cytb* = KF658379, *Fgb-I7* = KF658448]); Hidalgo (Hgo), 26.5 km NE (by road) Metepec, 2210 m (**4**; CMC 1042 = FXG 804 [*Cytb* = KF658348], CMC 1043 = FXG 823 [*Cytb* = KF658353], CMC 1044 = FXG 827 [*Cytb* = KF658361, *Fgb-I7* = KF658431]); Puebla (Pue), Huauchinango, Rancho El Paraíso, 6 km SW Huahuchinango, 2000 m (**5**; BYU 15801 = EAA 643 [*Cytb* = KF658362, *Fgb-I7* = KF658417], BYU 15802 = EAA 644 [*Cytb* = KF658354); Puebla (Pue), La Gloria Falls, Apulco River, 10 km N Zacapoaxtla, 1500 m (**6**; BYU 15803 = EAA 642 [*Cytb* = KF658344, *Fgb-I7* = KF658418]); Puebla (Pue), 4.7 km NE (by road) Teziutlán, 1750 m (**7**; CMC 1049 = FXG834 [*Cytb* = KF658345, *Fgb-I7* = KF658432], CMC 1052 = FXG 837 [*Cytb* = KF658349], CMC 1054 = FXG 839 [*Cytb* = KF658346]); Veracruz (Ver), Xico, Matlalapa, 2070 m (**8**; CMC 1497 = RMV 50 [*Cytb* = KF658378], CMC 1495 = RMV48 [*Cytb* = KF658355, *Fgb-I7* = KF658436]); Veracruz (Ver)**,** Xalapa, El Haya, Old road to Coatepec km 25 (Botanic Garden Francisco Javier Clavijero), 1235 m (**9**; CMC 1450 = RMV 01[*Cytb* = KF658343]; Veracruz (Ver), Acajete, Mesa de la Yerba, 3.4 km intersection to Mazatepec (Xalapa-Perote by road), 2004 m (**10**; CMC 1353 = FXG 873 [*Cytb* = KF658366], CMC 1490 = RMV 84 [*Cytb* = KF658380, *Fgb-I7* = KF658435]); Veracruz (Ver), Huatusco, Las Cañadas, 1340 m (**11**; CMC 779 = FXG 618 [*Cytb* = KF658350, *Fgb-I7* = KF658426], CMC 780 = FXG 619 [*Cytb* = KF658360], CMC 782 = FXG 621 [*Cytb* = KF658351]); Veracruz (Ver), Texhuacán, 1.2 km SE Xochititla, 1670 m (**12**; CMC 772 = FXG 578 [*Cytb* = KF658358, *Fgb-I7* = KF658424], CMC 773 = FXG 579 [*Cytb* = KF658357], CMC 774 = FXG 580 [*Cytb* = KF658372], CMC 775 = FXG 581 [*Cytb* = KF658347, *Fgb-I7* = KF658425]); Oaxaca (Oax), Puerto de la Soledad, 2600 m (**13**; BYU 15303 = EAA 310 [*Cytb* = KF658364], BYU 15304 = EAA 311 [*Cytb* = KF658363]); Oaxaca (Oax), Concepción Pápalo, 14.4 km NE (by road) Santa Flor, 2600 m (**14**; CMC 1382 = FXG 943 [*Cytb*

= KF658370], CMC 1347 = FXG 944 [*Cytb* = KF658367], CMC 1352 = FXG 949 [*Cytb* = KF658381, *Fgb-I7* = KF658434], CMC 1389 = FXG 950 [*Cytb* = KF658369]); Oaxaca (Oax), Ixtlán, 11 km SW (by road) La Esperanza, 2400 m (**15**; CMC 113 = DSR 5800 [*Cytb* = KF658374, *Fgb-I7* = KF658419], CMC 115 = DSR 5827 [*Cytb* = KF658371]); Oaxaca (Oax), Santa María Tlahuitoltepec, Santa María Yacochi, 2400 m (**16**; CMC 114 = DSR 5701 [*Cytb* = KF658368], CMC 117 = DSR 5763 [*Cytb* = KF658382], CMC 119 = DSR 5765 [*Cytb* = KF658359]); Oaxaca (Oax), Candelaria Loxicha, 0.7 km E (by road) La Soledad, 1025 m (**17**; CMC 943 = FXG 682 [*Cytb* = KF658395]); Oaxaca (Oax), Miahuatlán, San Miguel Suchixtepec, Río Molino, 2353 m (**18**; CMC 925 = FXG 691 [*Cytb* = KF658388*, Fgb-I7* = KF658427], CMC 930 = FXG 737 [*Cytb* = KF658389], CMC 931 = FXG 738 [*Cytb* = KF658391, *Fgb-I7* = KF658429], CMC 932 = FXG 739 [*Cytb* = KF658387], CMC 927 = FXG 734 [*Cytb* = KF658390, *Fgb-I7* = KF658428]); Guerrero (Gro), Malinaltepec, 3 km E El Tejocote, 2620 m (**20**; CMC 1656 = FXG 1043 [*Cytb* = KF658392], CMC 1657 = FXG 1044 [*Cytb* = KF658394, *Fgb-I7* = KF658438], CMC 1655 = FXG 1041 [*Cytb* = KF658393, *Fgb-I7* = KF658437]); Guerrero (Gro), Chilpancingo de los Bravos, 6.1 km SW (by road) Omiltemi, 2480 m (**21**; CMC 455 = FXG 412 [*Cytb* = KF658399]); Guerrero (Gro), Leonardo Bravo, 3.4 km (by road) Carrizal, 2480 m (**22**; CMC 452 = FXG 462 [*Cytb* = KF658397, *Fgb-I7* = KF658420], BYU 20647 = FXG 463 [*Cytb* = KF658396], CMC 454 = FXG 464 [*Cytb* = KF658398, *Fgb-I7* = KF658421]); Hidalgo (Hgo), Tlanchinol, 3 km E (by road) Tlanchinol, 1451 m (**23**; BYU 15300 = EAA 272 [*Cytb* = KF658352]).

Handleyomys melanotis.—MEXICO: Oaxaca (Oax), Putla Villa de Guerrero, 5.5 km S (by road) Concepción de Guerrero, 936 m (**19**; CMC 942 = FXG 789 [*Cytb* = KF658412, *Fgb-I7* = KF658430], CMC 939 = FXG 793 [*Cytb* = KF658413]; Nayarit (Nay), Peñita de Jaltemba, 1.8

km N of La Peñita de Jaltemba (ASNHC 3418 = ASK1601 [**33**; *Cytb* = KF658408]); Michoacán (Mich), Coalcomán, 10.9 km NW (by road) Coalcomán (**29**; CMC 1806 = DSR 7715 [*Cytb* = KF658410]); Jalisco (Jal), San Sebastián, 3.4 km W (by road) San Sebastián del Oeste, 1450 m (**30**; CMC 1207 = DSR 7414 [*Cytb* = KF658411, *Fgb-I7* = KF658433]); Nayarit (Nay), 8 KM E of San Blas (**34**; ASNHC 3419 = ASK 1538 [*Cytb* = KF658409, *Fgb-I7* = KF658415]); Colima (Col), Comala, Hacienda San Antonio (**36**; ASNHC = ASK1957 [*Cytb* = KF658414, *Fgb-I7* = KF658416]).

Handleyomys rostratus.—MEXICO: Veracruz (Ver), Catemaco, 13 km NW (by road) Sontecomapan, Estación Los Tuxtlas, IBUNAM, 150 m (**31**; CMC 2222 = DSR 8560 [*Cytb* = KF658407, *Fgb-I7* = KF658439]); Chiapas (Chis), Berriozabal, 12 km N (by road) Berriozabal, 1060 m (**32**; CMC 2241 = DSR 8464 [*Cytb* = KF658403, *Fgb-I7* = KF658440], CMC 2242 = DSR 8465 [*Cytb* = KF658406], CMC 2243 = DSR 8466 [*Cytb* = KF658402], CMC 2244 = DSR 8467 [*Cytb* = KF658405, *Fgb-I7* = KF658441], CMC 2245 = DSR 8468 [*Cytb* = KF658404, *Fgb-I7* = KF658442]); Tamaulipas (Tamps), Rancho Calabazas (near Ciudad Victoria), 3.2 km W Calabazas (**35**; *Cytb* = EU579492). EL SALVADOR: Ahuachapán, Ahuachapán, El Imposible (**25**; *Cytb* = EU579493). NICARAGUA: Matagalpa, Matagalpa, El Tigre (**27**; *Cytb* = EU579491).

Handleyomys saturatior.—MEXICO: Chiapas (Chis), La Trinitaria, Lagos de Montebello (**24**; ECOSCM 1228 [*Cytb* = KF658384, *Fgb-I7* = KF658446], ECOSCM 1229 [*Cytb* = KF658385], ECOSCM 1231 [*Cytb* = KF658383, *Fgb-I7* = KF658447]). NICARAGUA: Matagalpa, Selva Negra-Atajo Trail (**27**; TTU 101644 [*Cytb* = DQ224410, *Fgb-I7* = KF658453]); (**28**; CURN = JAGE 438 [*Cytb* = KF658386, *Fgb-I7* = KF658445]). EL

SALVADOR: Santa Ana, Montecristo National Park (**26**; ROM 101537 [*Cytb* = EU579494, *Fgb-I7* = KF658449]).

CHAPTER 3

Molecular Phylogenetics of the genus *Handleyomys*

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[Manuscript formatted for submission to the journal Molecular Phylogenetics and Evolution]

Molecular phylogenetics of the genus *Handleyomys*

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Highlights

Monophyly of *Handleyomy*s sensu lato was supported.

Handleyomys sensu stricto*, H. alfaroi*, the *H. chapmani* group and the *H. melanotis* group show inter-generic levels of divergence.

Two and three cryptic lineages were identified within *H. alfaroi* and *H. rostratus,* respectively.

The split of *Handleyomys fuscatus-intectus* from the *alfaroi-chapmani-melanotis* groups lineage was estimated to be ~4.8 Ma.

The niche conservatism hypothesis was rejected for most phylogroups pairs despite partially overlapped models.

Canonical functions provide a tool for identifying and quantifying niche differences.

Optimally suitable areas of habitat were predicted collectively by the ecological niche models.

Abstract

Mesoamerica is considered a biodiversity hot spot with levels of endemism and species diversity likely underestimated. Unfortunately, the region continues to experience some of the highest deforestation rates in the world. For mammals, the evolutionary relationships of many endemic taxa are controversial, as it is the case for the some members of the genus *Handleyomys* and closely related genera *Euryoryzomys*, *Hylaeamys*, *Nephelomys*, *Transandinomys* and *Oecomys* (taxa formerly a part of the specious rodent genus *Oryzomys*). Estimation of a time calibrated multilocus phylogenetic hypothesis for these six genera supported a monophyletic *Handleyomys* sensu lato. However, the taxonomic separation of *Handleyomys* sensu stricto, *H. alfaroi*, the *H. chapmani* and the *H. melanotis* species groups is advisable based on amounts of genetic divergence among these four lineages equivalent with inter-generic comparisons. In addition, the divergence of these groups was estimated at ~4.8 Ma, in parallel with of the rest of the genera included herein. Moreover, species delimitation suggested the existence of cryptic species-level lineages within *H. alfaroi* and *H. rostratus*. The divergence times within *Handleyomys* suggest two simultaneous speciation events, likely by contiguous (*H. chapmani* and *H. melanotis*) and long-distance (*alfaroi*) episodes of range expansion followed by long-term isolation. On the other hand, the separation of *H. intectus* and *H. fuscatus* was attributed to vicariance. An in-depth analysis of biogeographic patterns in a hypothesis-testing framework is presented elsewhere.

Keywords

Mesoamerica, endemics, molecular phylogeny, species limits, niche conservatism, *Handleyomys*

Introduction

Mesoamerica is regarded as a biodiversity hot spot, despite the fact that levels of endemism and species diversity likely are underestimated and that only ~20% of the original vegetation remains intact (DeClerck *et al.* 2010; Sarkar *et al.* 2009). Unfortunately, the region continues to experience some of the highest deforestation rates in the world (Mas *et al.* 2009; Sánchez-Colón *et al.* 2009), which will further degrade its biodiversity (Brooks *et al.* 2002). Among mammals, the majority of Mesoamerican endemics are rodents (Musser & Carleton 2005; Reid 2009). This is especially true for Mexico, where the order accounts for 82% of mammalian endemism (Ceballos 2007). Molecular studies dealing with rodents in this region consistently recover the existence of cryptic lineages, usually restricted to different mountain systems and physiographic provinces (Arellano *et al.* 2005; Gutiérrez-García & Vázquez-Domínguez 2012; Hardy *et al.* 2013; León-Paniagua *et al.* 2007; Matson 2012; Ordóñez-Garza *et al.* 2010; Rogers *et al.* 2007; Rogers & González 2010; Vallejo & González-Cózatl 2012). Therefore, species limits, and consequently the geographic ranges of many Mesoamerican taxa, are not well understood.

The rodent genus *Handleyomys,* originally included *H. intectus* and *H. fuscatus*; two taxa endemic to Colombia (Handleyomys sensu stricto; Voss *et al.* 2002). More recently Weksler et al., (2006) broadened the scope of *Handleyomys* to the *alfaroi* group, which is endemic Mesoamerica (Fig. 1A). Although the number of recognized species in the *alfaroi* group has ranged from five (Goldman 1918) to 12 (Allen 1891, 1913; Allen & Chapman 1897; Goldman 1915; Merriam 1901), with another eight forms proposed as subspecies (Musser & Carleton 2005) taxonomic hypotheses recognize *H. alfaroi*, *H. melanotis, H. rostratus, H. chapmani*, *H. rhabdops*, *H. saturatior* and more recently, *H. guerrerensis* as valid species

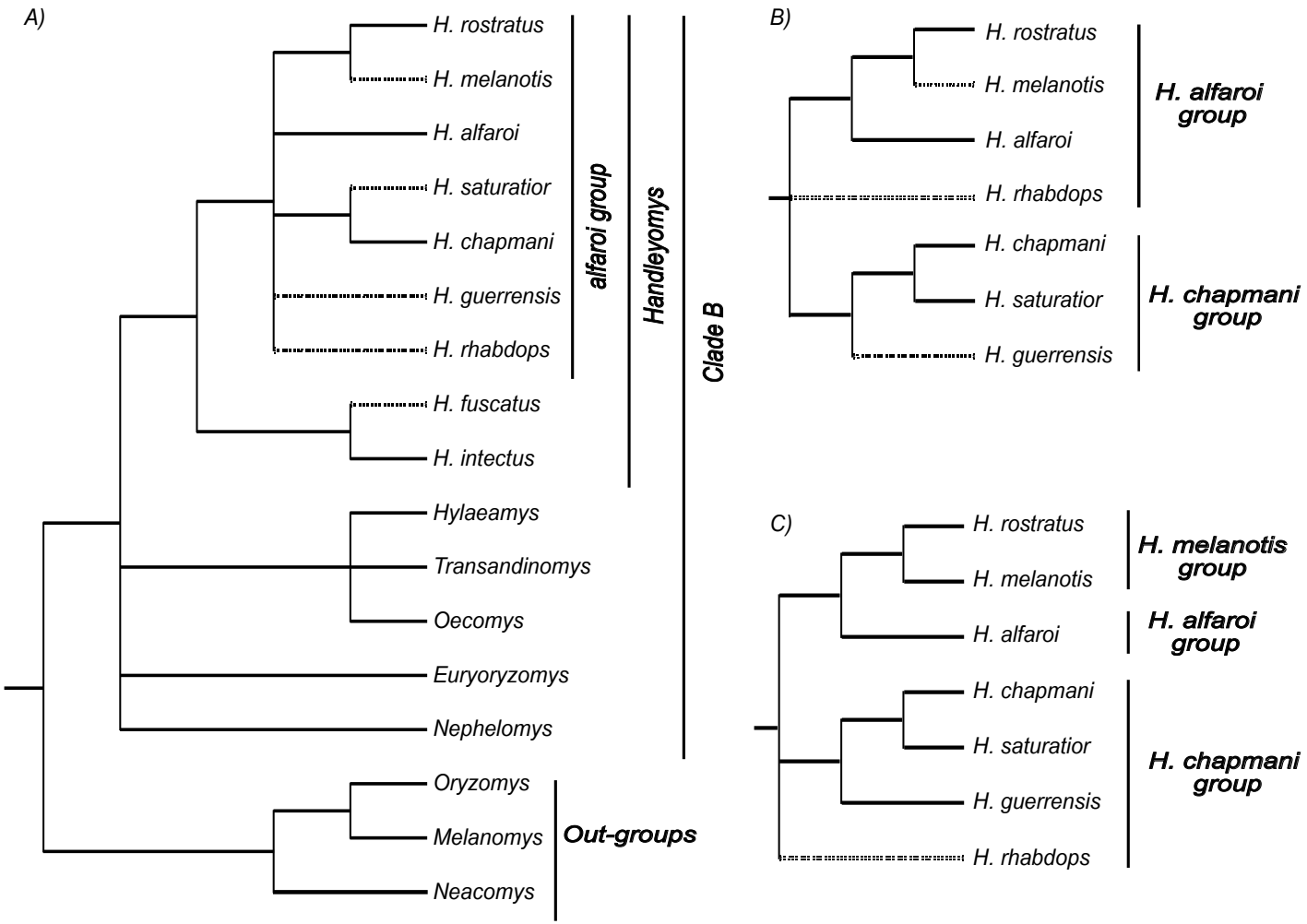

Figure 3. 1. A) Phylogenetic hypotheses for *Handleyomys* and related genera (*Euryoryzomys*, *Hylaeamys*, *Nephelomys, Oecomys*, and *Transandinomys*), and for three other Oryzomine taxa more distantly related to the *Handleyomys* (*Oryzomys, Melanomys* and *Neacomys* (summarized from Weksler, 2006, Weksler et al., 2006 and Pine et al. 2011). Phylogenetic hypotheses for recognized species groups within *Handleyomys* (B— Weksler and Percequillo, 2011; C— Almendra et al. 2014). Stippled lines denote positions that were secondarily inferred by the authors.

(Almendra *et al.* 2014; Ramírez-Pulido *et al.* 2014). Generally, *H. melanotis* and *H. rostratus* have been separated in the *H. melanotis* group (Engstrom 1984), and *H. chapmani*, *H. guerrerensis* and *H. saturatior* in the *H. chapmani* group (Musser & Carleton 2005), which originally included *H. rhabdops* (Merriam 1901). However, alternative demarcations of these species groups have been proposed (Fig. 1B). Therefore, the restricted view of the *alfaroi* group would herein refer the forms synonymized with *H. alfaroi* (Musser & Carleton 2005; Fig. 1C). The complex evolutionary (and corresponding taxonomic) history of the genus *Handleyomys* is not surprising given that species now regarded as members of this genus were formerly placed in the genus *Oryzomys* (Weksler *et al.* 2006), *Aepeomys* and *Thomasomys* [*H. fuscatus*] (Voss *et al.* 2002). Revisionary work using molecular data, and more recently divergence times estimates, are beginning to elucidate the complex genealogic relationships of Sigmodontinae at supra-generic and inter-generic levels (Bonvicino & Martins Moreira 2001; D'Elía 2003; Engel 1998; Gardner 1976; Parada *et al.* 2013; Salazar-Bravo *et al.* 2013; Smith & Patton 1999; Weksler 2003). Nevertheless, evolutionary relationships among some taxa remain unresolved, as is the case for species included within *Handleyomys,* and of this perported clade with respect the genera *Euryoryzomys*, *Hylaeamys*, *Nephelomys*, *Transandinomys* and *Oecomys*, which together comprised one of four supra-generic lineages in the tribe Oryzomyini Clade B (Fig. 1A) (Weksler *et al.* 2006).

The geological history of Mesoamerica has been correspondingly complex and was highlighted by a series of geological events leading the formation of the Panamanian Land Bridge (PLB) and with that, the reconnection of North and South American continents (Coates & Obando 1996; Woodburne 2010). At least 27 physiographic provinces are recognized in Mesoamerica (Cervantes-Zamora *et al.* 1990; Marshall 2007) which underscores the intricate

pattern of fragmentation among mountain and tropical forest ecosystems observed today. This complex physiogeography has been hypothesized to be largely responsible for the elevated levels of endemism observed in Mesoamerica. Coupled with periods of volcanism that followed the tectonic plates involved in the closure of the PLB, and transitory environments and marine incursions during the Pleistocene, this may explain the complex biogeographic patterns observed in the region (Ferrusquía-Villafranca *et al.* 2010; Gutiérrez-García & Vázquez-Domínguez 2013). However, the relationship between endemism and complex physiogeography is based on the assumption of niche conservatism (Peterson et al., 1999; (Wiens & Graham 2005) and this hypothesis has not been rigorously tested in Mesoamerica. Therefore, evaluating the extent to which niches from sister species are constrained could provide insights about the mechanisms that reinforce isolation (Kozak & Wiens 2006; Olalla Tárraga *et al.* 2011; Wiens & Graham 2005).

The genus *Handleyomys* represents an ideal system to test hypotheses of niche conservatism. *H. alfaroi* occupies a relatively wide geographic distribution in evergreen and mountain forests ecosystems (500 – 1400 m) along the Gulf of Mexico, the Mayan and Chortís highlands, the Panamanian Darién, and the eastern slopes of the Ecuadorian Central Andes (Fig. 2). In contrast, *H. melanotis* and *H. rostratus* typically occur at lower elevations (< 800 m; rarely ~1000 m). The former species occupies subtropical and mixed forests of the SMS and the Pacific coast in Mexico, whereas the latter favors deciduous and evergreen tropical forests along the Golf of Mexico, the Yucatán Peninsula, the Guatemalan Petén; the Chortís and Nicaraguan volcanic fronts, and the Chortís highlands (Musser & Carleton 2005; Reid 2009—Fig. 3A). Finally, *H. fuscatus, H. intectus*, *H. chapmani*, *H. guerrerensis*, *H. rhadbops* and *H. saturatior* are limited to high elevation montane forests (>1200 m). The first two are distributed

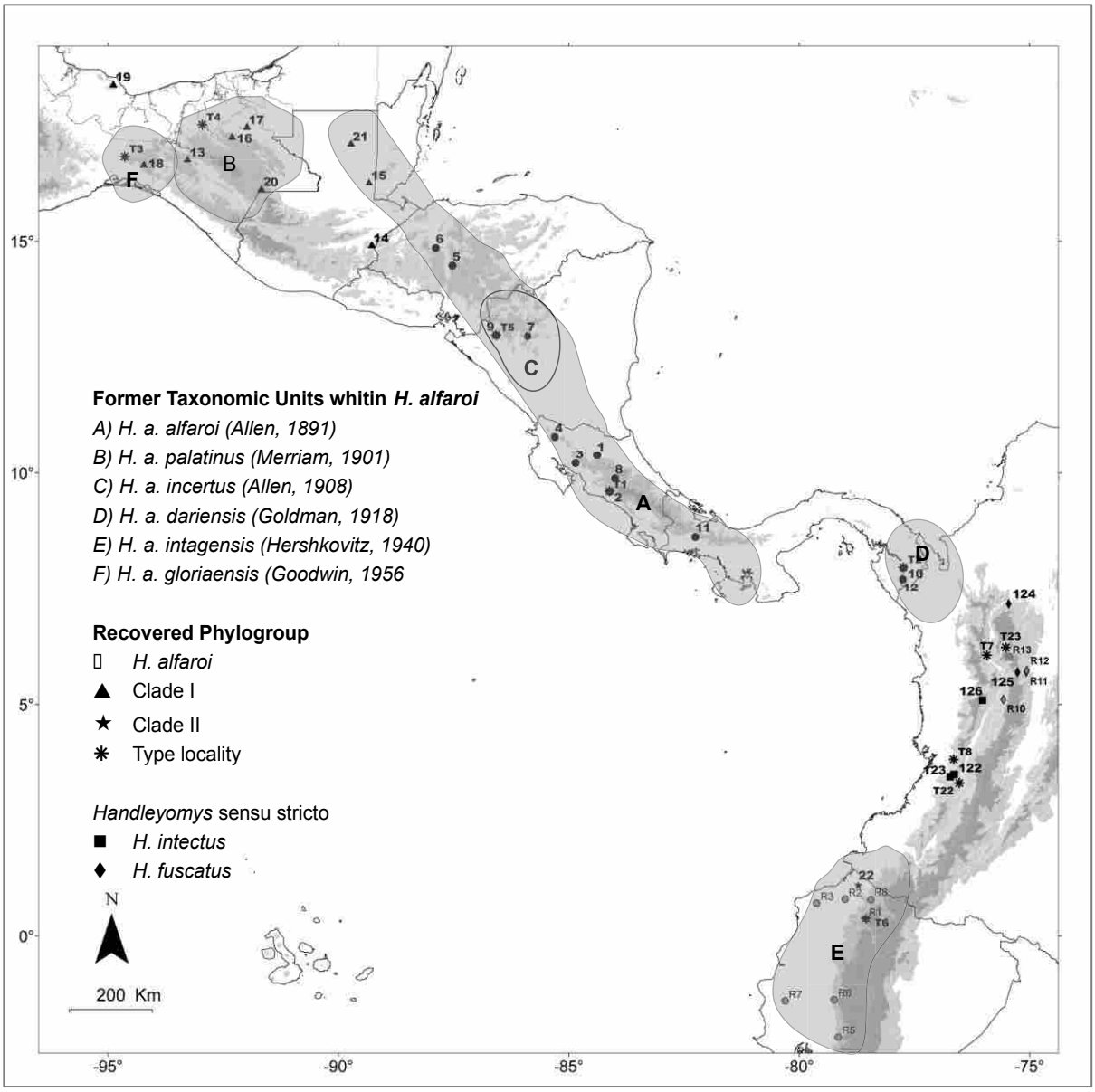

Figure 3. 2. Collecting localities for *H. alfaroi* sensu Musser and Carleton (2005), *H. fuscatus* and *H. intectus*. Symbols correspond to species assignments based on PTP and bGYMC, and letters represent previously recognized species or subspecies. Type localities and additional museum voucher specimen records were used in the ecological niche modeling (ENM) analysis of these three groups. Locality numbers correspond to those in Appendices A and B. The proposed geographic extent of formerly recognized species and subspecies is shown as shaded areas. Elevational gradients are displayed in white = < 800 m, light grey = 800-2,200 m, and dark grey = > 2,200 m, respectively.

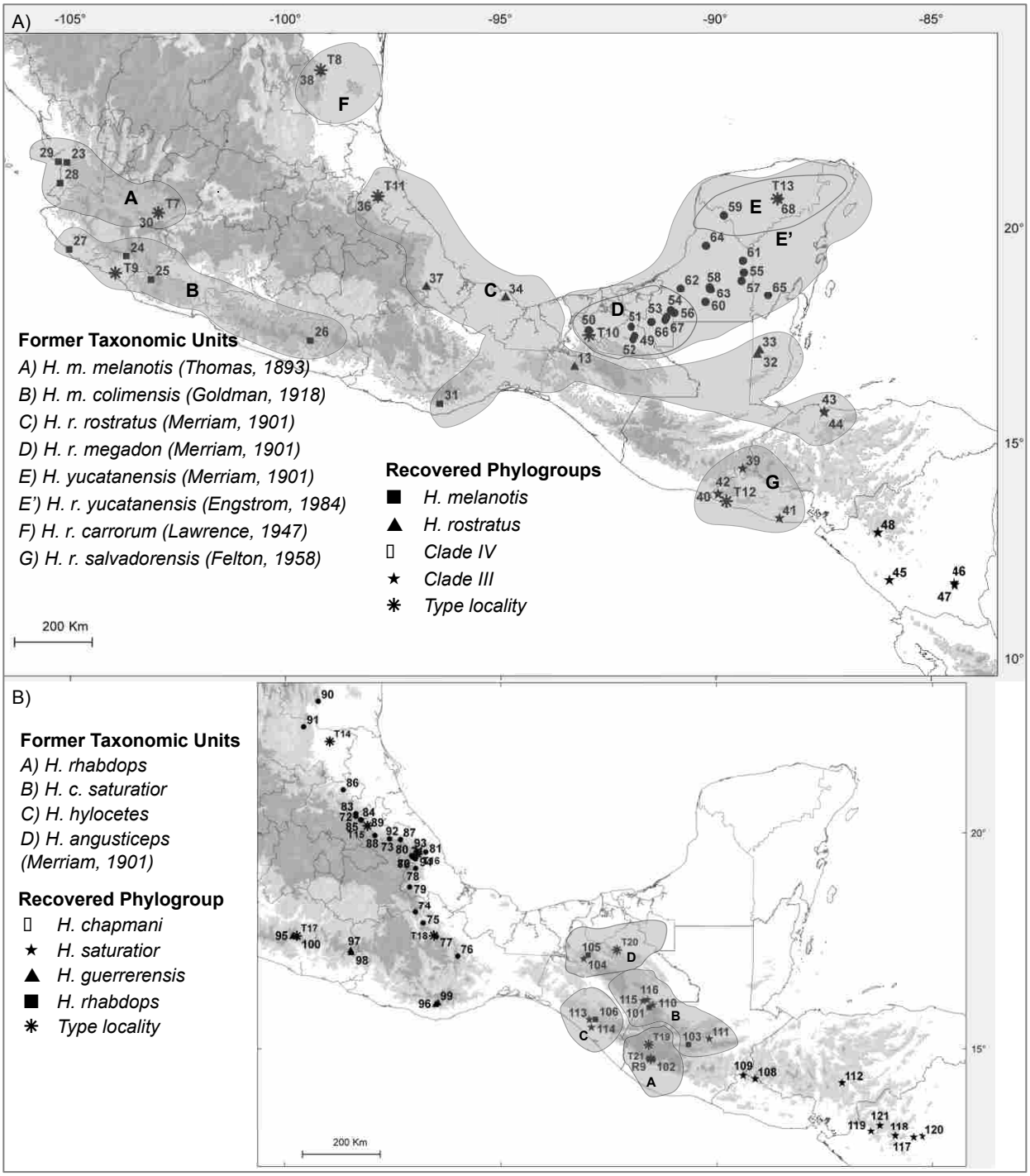

Figure 3. 3. Collecting localities for the *H. melanotis* (A) and *H. chapmani* (B) species groups sensu Musser and Carleton (2005) with taxonomic updates by Almendra et al. (2014). Labels are as in Figure 2.

allopatrically in the Cordillera Occidental and Cordillera Central of Colombia, respectively, *H. chapmani* and *H. guerrerensis* are endemic to the Sierra Madre Oriental (SMO) and Sierra Madre del Sur (SMS) in Mexico, and *H. rhabdops* and *H. saturatior* are restricted to the highlands east of the Isthmus of Tehuantepec in Central America. These latter two species have partially overlapping distributions in the Sierras del Sur de Chiapas, the Sierras del Norte de Chiapas, and in the Sierra de los Chuchumatanes (Fig. 3B).

We used sequence data from four mitochondrial and eight nuclear loci to develop a time calibrated phylogenetic hypothesis for *Handleyomys* to test for monophyly and to evaluate its evolutionary relationships with other Oryzomyini. Then, we defined species limits by integrating our results as operational criteria for the unified species concept (De Queiroz 2007), as an inclusive approach (Leaché & Rannala 2011; Satler *et al.* 2013). Finally, we tested the niche conservatism hypothesis among species-level clades in the genus *Handleyomys*. An in-depth analysis of biogeographic patterns in a hypothesis-testing framework is presented elsewhere (Almendra et al. ms in review).

Methods

Taxon sampling and molecular data

A total of 391 specimens from 161 localities were collected from natural populations or obtained via tissue loan for this study. Specimen identifications to species were confirmed by sequencing the first 800 bp of the mitochondrial gene Cytochrome *b* (*Cytb*). In addition, we included 35 *Cytb* sequences from Genebank representing *Handleyomys* and the proposed closely related genera *Euryoryzomys*, *Hylaeamys*, *Nephelomys*, *Oecomys* and *Transandinomys* (Clade B sensu Weksler *et al.* 2006) (in-group; Appendix A). Finally, we used *Neacomys paracou*, *Oryzomys couesi* and *Melanomys caliginosus* as out-groups (Fig. 1A). We analyzed this data set via Maximum Likelihood (ML) carried out with RaxML v7.4.8 (Stamatakis 2006) for 10000

Table 3. 1. General description of the loci sequenced in this study. Information regarding primer sequences and PCR thermal profiles is included in Appendix D. The probability of inducing noise (P noise) is shown at terminal nodes (t1) and ancestral nodes (t2) (see Taxon sampling and molecular data).

Symbol	Name	Length (bp*)	Inheritance mode	Sample size (n)	Substitution Model	Substitution Rate	P noise t1	P noise t2
Cytb	Cytochrome *b*	801/ 1143	mitochondrial	426 216	HKYγ (1st + 2nd pos.) GTRγ (3rd pos.)	0.1278	0.0010	0.2943
COI	Cytochrome oxidase *I*	656	mitochondrial	142	GTR γ + I	0.1281	0.1243	0.5016
12S	12S ribosomal RNA	396	mitochondrial	216	HKYγ + I	0.0504	0.1448	0.4280
Dloop	D-loop region	637	mitochondrial	197	GTR γ + I	0.1678	0.0470	0.3953
GdX	Housekeeping protein DXS254E	1013	X	173	K80 + I	0.0126	0.1132	0.3692
IRBP	Exon 1 of the interphotoreceptor retinoid binding protein (partial)	1100	autosomal	197	GTR + γ + I	0.045	0.0189	0.2733
CD14	Monocyte and granulocyte surface glycoprotein homolog (partial)	650	autosomal	173	HKY γ	0.0283	0.0402	0.2189
Fgb-I7	Intron 7 of the beta fibrinogen	698	autosomal	173	HKY γ	0.0434	0.0121	0.2651
Fut4	(1,3) fucosyltransferase Intron (partial)	603	autosomal	145	HKY γ + I	0.0243	0.1020	0.2014
Nup160	Intron 15 of the nucleoporin	541	autosomal	147	GTR γ + I	0.0547	0.0883	0.3968
Adh1-I2	Intron 2 of the alcohol dehydrogenase	482	autosomal	145	GTR γ	0.0346	0.0344	0.3000
PRKCI	Protein kinase C iota	465	autosomal	184	GTR γ	0.0324	0.0408	0.3367

*Base pairs.

search replicates and simultaneous bootstrapping. Based on this analysis, the remainder of the *Cytb* (400 bp), two other mitochondrial (*mtDNA*), and eight nuclear (*nuDNA*) loci (Table 1), were sequenced for a genealogically and geographically representative subsample of at least 145 and as many as 203 individuals, depending on our ability to generate sequence data for each locus. In addition, we downloaded 140 *COI* sequences from Genbank for single locus phylogenetic analysis and for multilocus coalescent analysis (*BEAST and Migrate-n—Species delimitation). Specimens examined and accession numbers are provided in Appendix A. DNA sequences were edited and assembled with Geneious Pro v6.1.6. Loci with varying length were aligned with MAFFT v7 [L-INS-i refinement, gap penalty = 3, offset = 0.5] (Katoh & Standley 2013), and refined using MUSCLE [100 iterations, anchor optimization, UPGMB clustering method and gap penalty = -3.0] (Edgar 2004). For non-coding sequences, models of nucleotide evolution were estimated with JModelTest2 (Darriba *et al.* 2012). For coding sequences, the number of necessary partitions was established with PartitionFinder (Lanfear *et al.* 2012).

Because subsequent multilocus phylogenetic analysis already involve a substantial amount of parameters, codon positions with estimated substitution models that differ only in rate heterogeneity parameters (γ + I) were not partitioned (Duchêne *et al.* 2011), although *Cytb* and *COI* codon positions were tested for saturation using DAMBE5 (Xia 2013). The amount of phylogenetic signal supplied to the phylogeny by each locus was assessed with PhyDesign (Lopez-Giraldez & Townsend 2011; Townsend 2007).

Phylogenetic analysis of individual loci

We estimated Bayesian Inference (BI) topologies for each dataset with MrBayes 3.2.2, with two runs (4 chains) of 10 million generations, sampling trees every 1000 and a burn in of 20% of the trees. The resulting topologies were examined individually to identify ambiguous relationships and to detect potential cryptic lineages within currently recognized species.

Monophyletic geographic clades within currently recognized species that were recovered in greater than half of the topologies were subject to migration rates (*xNm*) estimation with Migrate-n 3.3.2 (Beerli & Felsenstein 2001), using FST estimates as starting values to run three replicate chains with adaptive heating (1.0, 1.2, 1.5, 3.0) for 100000 genealogies. Newly recovered phylogroups with unnoticeable or minimum gene flow (Nm < 0.1—Hudson *et al.* 1992) were considered putative species lineages.

Species delimitation

To provide a comparison point of single locus species delimitation, we first demarcated species level clades in a complete *Cytb* (1143 bp) ML topology generated as described in Taxon sampling and molecular data for the partial gene (800 bp), with the Poison Tree Process (PTP) Model (Zhang *et al.* 2013), a non-coalescent method that bases its choice of Operational Taxonomic Units on the Phylogenetic Species Concept. Later, an ultrametric topology was estimated in BEASTv1.7.5 (Drummond *et al.* 2012), with three Markov Chain Monte Carlo (MCMC) runs of 100 million generations sampling every 2,000 trees and a 15% burn-in, under the assumption of a relaxed molecular clock with mean rate 0.017 per million years (Arbogast *et al.* 2002; Li *et al.* 1990). To assess the level of uncertainty in the *Cytb* data, the last 100 *Cytb* trees sampled during the MCMC were analyzed with 1,000,000 generations of the Bayesian General Mixed Yule-Coalescent (bGMYC) model (Reid & Carstens 2012) in the computing environment R (R Core Team 2013). Based on morphologically discernible species, clades suggested with PTP that had a marginal probability of conspecificity (p) < 0.70 with respect to their sister clades in the bGMYC analyses (Table 2 and Fig. 4), were considered species assemblages in subsequent analyses (De Queiroz 2007). Additionally, the *nuDNA* individual locus BI topologies (Phylogenetic analysis) were forced to be ultrametric with the semi-parametric penalized likelihood approach (Sanderson 2002) in the package Ape (Paradis *et al.*

2004) in R. The resulting *nuDNA* topologies were then analyzed with SpeDeSTEM (Ence & Carstens 2011) to assess the relative probability of the species delimitation model that included the *Cytb* supported clades. This validation analysis assumed theta (**θ**)= 0.02, the mean estimate from Migrate-n separate runs.

Species tree estimation

Using multiple loci, we ran the multi species coalescent (Heled & Drummond 2010) method in BEAST v1.7.5 for three Markov Chain Monte Carlo (MCMC) of 1 billion generations each, and trees were sampled every 5,000 generations with a burn in of 10,000. A birth death model prior was set on the speciation rate. Gene trees were assumed to be unlinked for nuclear loci and constant rate birth-death prior on the speciation rate (Heath *et al.* 2012). Analyses were run twice for 10,000,000 MCMC cycles each, sampling every 100 trees and merged with LogCombiner v1.7.5. For comparison with a non-coalescent method for species tree estimation, we applied the Bayesian concordance analysis (Ané *et al.* 2007) implemented in BUCKy (Larget *et al.* 2010) to find the maximum concordance tree and to estimate concordance factors (CF) based on the posterior sample of 8000 trees (after burn in, for each loci) from the BI analyses (Phylogenetic analysis). Analyses run with two chains of 100,000,000 states updates with a prior alpha (α) = 3; assessed using the R script suggested by the authors.

Niche conservatism

For each species clade within *Handleyomys*, we developed present time Ecological Niche Models (ENMs) with MAXENT 3.3.3 (Phillips & Dudik 2008). Correlation between the 19 environmental variables from the WORLDCLIM database (1 km^2 resolution) (Hijmans *et al.* 2005) was calculated with ENMtools v1.4.1 (Warren *et al.* 2010). We also used a selection of 15 environmental grids (correlation = $r \leq 0.8$) (Table 3) in addition to the 90 m resolution Digital Elevation Model (DEM) (Jarvis *et al.* 2008) and its derivative the Compound Topographic Index

Table 3. 2. Species and phylogroup assignments for the *H. alfaroi, H. melanotis*, and *H. chapmani* groups supported by PTP and bGYCM methods. Specimen voucher and locality numbers (in parentheses) correspond to the *Cytb* tree depicted in Fig. 4. Collecting localities are provided in Appendix A.

Species						
H. rostratus	***H. melanotis***	***H. alfaroi***	***H. chapmani***	***H. saturatior***	***H. guerrerensis***	***H. rhabdops***
Clade IV		**Clade II**		***H. saturatior***		
ASK0226 (56)	FXG996 (31)	DSR9028 (13)	BYU15304 (74)	ROM101382 (39)	CMC455 (100	MVZ223318 (101)
ASK2614 (63)	FXG1001 (31)	DSR9056 (13)	BYU15303 (74)	ROM101409 (39)	ASK0750 (107	MVZ223312 (101)
ASK2616 (63)	FXG999 (31)	DSR9032 (13)	CMC772 (79)	ROM101381 (39)	ASK0729 (100	MVZ224809 (102)
ASK0270 (62)	FXG1000 (31)	DSR8954 (13)	FXG827 (84)	ROM101537 (39)	CMC454 (95)	MVZ223313 (101)
ASK0158 (66)	FXG789 (31)	DSR9020 (13)	FXG618 (78)	TTU83742 (112)	ASK0897 (107	ROM97603 (105)
ASK2613 (63)	ASK0896 (26)	ASK0650 (13)	BYU15300 (86)	FN31510 (111)	ASK0895 (107	ROM97604 (105)
FN30675 (61)	BYU1207 (30)	ASK0651 (13)	HBR069 (83)	FN31511 (111)	CMC452 (95)	
FN30674 (61)	ASK1957 (24)	DSR8900 (13)	YHM191 (71)	FN31460 (111)	FXG734 (99)	
ROM95800 (60)	TTU37751 (27	ASK0062 (17)	YHM221 (71)	MVZ223314 (101)	FXG738 (99)	
ASK0214 (54)	BYU1210 (30)	DSR8543 (19)	YHM186 (71)	MVZ223315 (101)	FXG691 (99)	
ASK0227 (56)	ASK1601 (28)	DSR8544 (19)	EAA643 (89)	ECOSCM1229 (18	FXG1044 (98	
ASK2612 (63)	ASK1538 (29)	FXG1342 (16)	CMC740 (91)	MVZ223316 (110)	FXG1041 (98	
ASK0229 (56)		FXG1338 (16)	YHM240 (93)	ECOSCM1228 (18		
ASK2596 (63)		FXG1343 (16)	YHM241 (93)	ECOSCM1231 (18		
ROM95798 (60)		**H1**	YHM223 (71)	MVZ223317 (110)		
ASK0385 (64)		MVZ224808 (14)	YHM238 (93)	**Clade V***		
ROM95799 (60)		MVZ224807 (14)	RMV48 (80)	TTU101644 (117)		
ROM96031 (68)		***H. a. alfaroi***	YHM237 (93)	TTU105140 (118)		
ASK0231 (56)		TTU104273 (5)	CMC775 (79)	TTU105174 (118)		
ASK2597 (63)		TTU104274 (5)	FXG834 (87)	JAGE438 (119)		
FN30673 (61)		TTU104359 (5)	BYU15803 (92)	**H2**		
FN30676 (55)		TTU104336 (5)	TCWC59291 (90)	ASK0588 (104)		
ROM96022		TTU104356 (6)	TCWC59294 (90)	ASK0665 (105)		

(68)
ASK2593 (63)
FN29231 (58)
ASK1043 (62)
ASK0228 (56)
ASK0386 (64)
ASK2592 (63)
ASK2611 (63)
ASK0230 (56)
FN32689 (65)
H. r. megadon*
ASK0064 (17)
ASK0070 (59)
ASK0074 (52)
ASK0081 (50)
H. r. rostratus
DSR8467 (35)
DSR8465 (35)
DSR8952 (35)
DSR8953 (35)
DSR8468 (13)
DSR8464 (35)
TTU44930 (38)
TTU44929 (38)
DSR8560 (34)
FXG1367 (36)
FXG1366 (36)

TTU104337 (5)
LSUMZ605 (1)
LSUMZ603 (1)
H. a. dariensis*
ROM97302 (3)
ROM97303 (3)
TTU39149 (11)
Clade I
TTI102921 (22)

TCWC59289 (90)
RMV84 (81)
CMC741 (91)
CMC739 (91)
FXG949 (75)
CMC105 (77)
CMC103 (77)
FXG943 (75)

Clade III
TTU84376 (44)
TTU84374 (44)
TTU84373 (44)
TTU10393 (43)
TTU10394 (43)
TTU10450 (46)
ROM10228 (40)
ROM10184 (40)

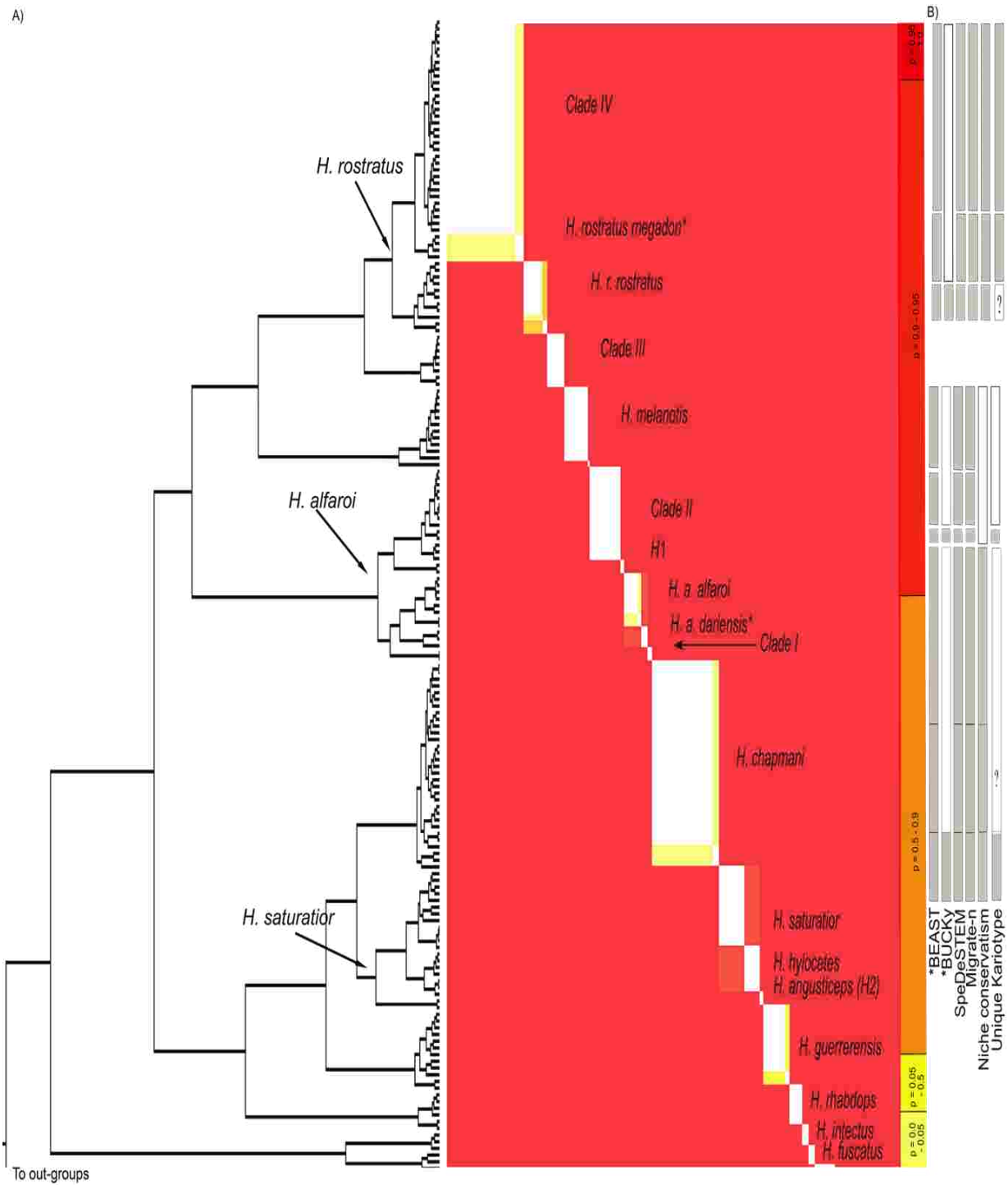

Figure 3. 4. A) *Cytb* Bayesian phylogeny beside the pairwise bGYCM color discontinue probability graph of conspecificity. See Table 2 for the list of samples that correspond to each phylogroup. Labels correspond to the clades depicted in Fig. 1 and Table 2. Groups that were supported by the bGYCM but not by the PTP method are indicated with an asterisk and referenced with former taxonomic names when applicable. B) Summary of the species delimitation methods and additional sources of support for each *a priori* assigned phylogroup (see text for additional information).

(CTI) to assess the ENMs. To homogenize grid cell size, the climatic variables were resampled to 90 m using bilinear interpolation in ArcMap 10.1 (ESRI 2011). Due to the cryptic nature of the phylogroups, we included only presence points that could be confirmed with molecular data for most species with the exception of *H. alfaroi, H. intectus and H. fuscatus* from South America, in which specimen unavailability for certain phylogroups required the use of museum records to increase sample size. In this case, only a subset of museum records used taxonomic revision (Musser *et al.* 1998) of specimens were included (Appendix B). The average model from 20 fold cross-validated replicates was used to assess niche overlap among phylogroups with the Schoener's D (Schoener 1968), intended to represent microhabitat differences; and the *I* statistic, that denotes changes in community composition (Warren *et al.* 2008). We generated a null distribution of these two measurements to test the hypothesis that the ENMs from sister phylogroups were identical, for each pair of within species cryptic lineages by running 100 replicates of the Identity Test (Warren *et al.* 2008) in ENMtools v1.4.1. Likewise, we performed a canonical discriminant functions (CF) to identify the particular environmental variables potentially affecting the extent to which their niches had been conserved, in SPSS v20. For this analysis, we extracted climate data for each variable at each pixel predicted by the individual ENM's using the Spatial Analyst in ArcMap 10.1.

Results

Relationships among selected Oryzomyini genera

We calculated the age of the most recent common ancestor (tMRCA) of *Euryoryzomys, Handleyomys*, *Hylaeamys*, *Nephelomys*, *Oecomys* and *Transandinomys* (Clade B) as an average of both methods at approximately 5.5 Myr with a composite credibility interval (cCrI) between 5.3 and 6.5 Myr (Fig. 5). Monophyly of this clade was recovered across the majority of individual loci with the exceptions of *GdX* that recovered *Oryzomys couesi* and *M. caliginosus*

Table 3. 3. Canonical structure matrix exposing meaningful coefficients (> 0.3—bold numbers) and largest absolute correlations between each variable and each discriminant function (CF1 and CF2).

	H. chapmani		*H. rostratus*		*H. alfaroi*		*H. fuscatus*
Climatic variable	CF1	CF2	CF1	CF2	CF1	CF2	CF1
Mean Diurnal Range	**.438***	**-.379***	.234*	.002	-.146*	.017	0.076
Isothermality	**-.562***	-.197	**-.612***	**.341***	**.771***	**.460**	**0.675**
Temperature Seasonality	**.759***	.100	**.464***	-.285	**-.588***	**-.518**	**-0.893**
Mean Temperature of Wettest Quarter	-.033*	-.110	.113	**.354***	-.016*	.001	0.017
Mean Temperature of Driest Quarter	-.195*	-.107	-.039	**.558***	.003	-.013*	0.019
Mean Temperature of Warmest Quarter	-.033*	-.115	-.137	**.373***	-.018*	.004	0.012
Mean Temperature of Coldest Quarter	-.229*	-.165	-.166	**.528***	.007	.016*	0.022
Annual Precipitation	-269*	**-.334**	-.281*	.121	-.037	**.511***	-0.154
Precipitation of Wettest Month	-.099*	**.459***	**-.319***	.053	-.120	**.422***	-0.224
Precipitation of Driest Month	-.165	-.284*	-.018	.176*	.072	.078*	0.034
Precipitation Seasonality	**.443**	**.571***	-.037	-.267*	-.111	-.168*	0.007
Precipitation of Wettest Quarter	-.272*	**.468***	**-.306***	.019	-.108	**.400***	-0.212
Precipitation of Driest Quarter	-.227	**-.302***	-.036	.170*	.104	.149*	0.019
Precipitation of Warmest Quarter	-.161*	-.072*	-.241*	-.076	.206*	-.182	-0.091
Precipitation of Coldest Quarter	-.220	**-.309***	-.113	.142*	.094	**.706***	-0.164
Percent of explained variance	**88%**	**12%**	**87%**	**13%**	**77%**	**23%**	**100%**

(out-group taxa) in a clade with *Nephelomys*, and *12S,* which grouped *O. couesi* with *H. fuscatus* and *H. intectus*. In addition, some inter-generic relationships were not fully resolved. Rooted trees based on *nuDNA* markers typically recovered *Handleyomys* sensu stricto (*H. fuscatus* and *H. intectus*) as sister to the Mesoamerican *Handleyomys* (*H. alfaroi*, *H. chapmani* and *H. melanotis* groups) and this clade in turn was positioned as sister to the rest of the in-group. The *Cytb* (800 bp ML and 1143 bp BI) phylogeny depicted a clade containing *Hylaeamys—Euryoryzomys* and *Nephelomys—Handleyomys* sensu stricto, and the concatenated *mtDNA* recovered *Nephelomys* as sister to the *H. alfaroi*, *H. chapmani* and *H. melanotis* groups. In turn, *H. fuscatus* and *H. intectus* was placed as ancestral within the in-group. Likewise, analysis of the *COI* data set that included the only DNA sequence available for *Mindomys hammondi* (Accession JF491462), recovered this taxon as sister to *Nephelomys*, and *Transandinomys* as sister to the Mesoamerican *Handleyomys*. Despite these discrepancies, the species tree methods (Fig. 5–6) favored the *nuDNA* hypothesis of monophyly of *Handleyomys* sensu lato. We estimated this early split to be contemporaneous with the split between *Neacomys paracou* and *Oryzomys couesi—Melanomys caliginosus* among the out-group taxa between 4.8-5.4 mya. Diversifications of the *H. alfaroi*, *H. chapmani* and *H. melanotis* species groups were placed nearly simultaneously with the divergence of the other in-group genera at 2.8-3.5 mya. *BEAST also recovered a sister relationship between *Euryzoryzomys* with *Hylaeamys*, and *Transandinomys* with *Oecomys* (Fig. 5) that was unclear with individual loci and in the maximum concordance tree (BUCKy) (Fig. 6). We consistently recovered *Nephelomys* in an ancestral position to these clades, with which it shared a common ancestor about 4.4 mya. Finally, when out-groups were not assigned to root the individual locus topologies, the mid-point of the trees was situated between the *H. alfaroi*, *H. chapmani*, *H. melanotis* groups and the rest

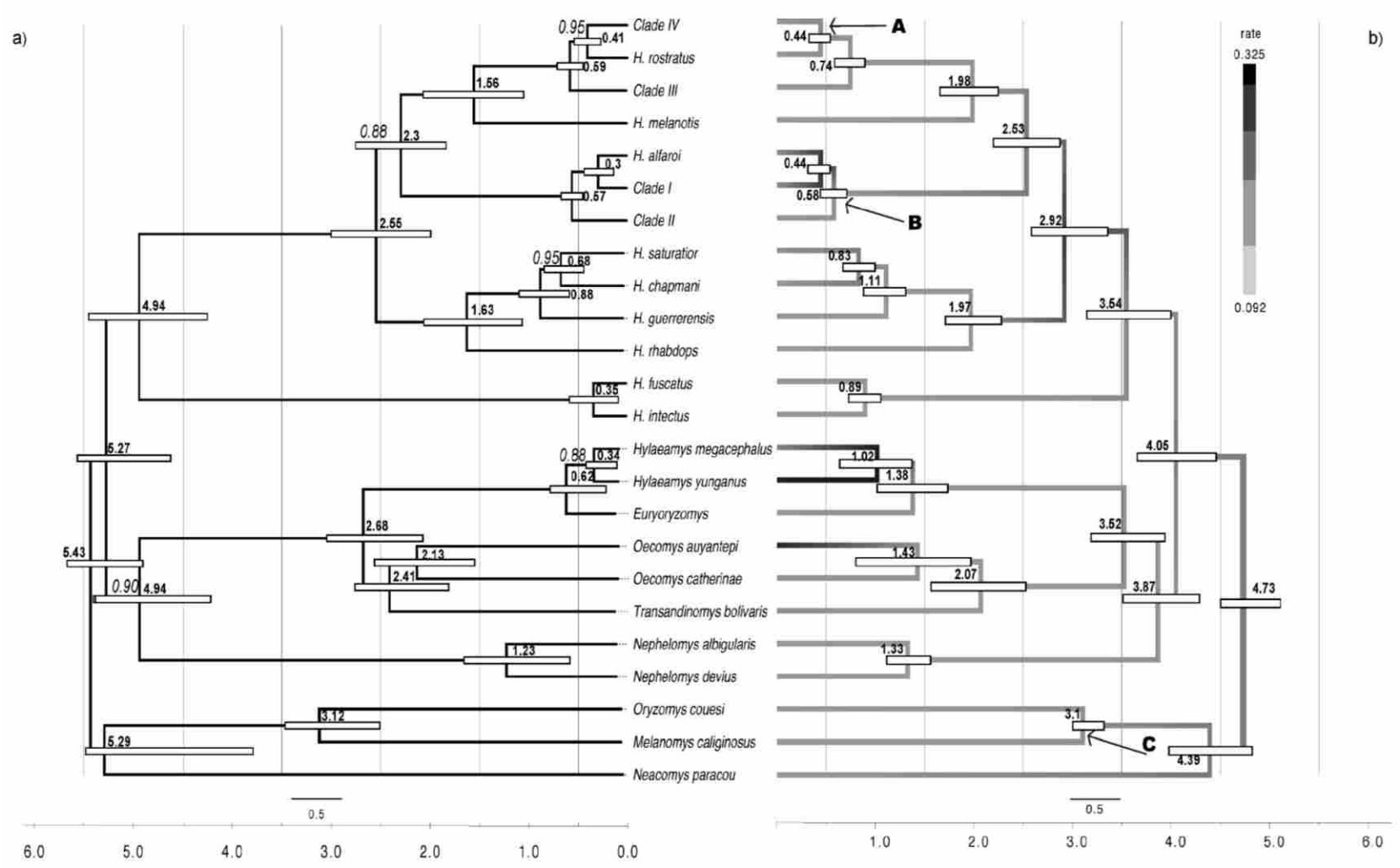

Figure 3. 5. Time calibrated species tree estimated with *BEAST compared tip to tip with DPPdiv divergence times estimates. Posterior probabilities pP = 1.0 are indicated above the node with an asterisk; pP < 1.0 are listed above the node in italics. Black outlined bars denote the 95% Highest Posterior Density for the divergence times parameter. The gray gradient along the branches in the DPPDiv tree [b)] denotes the estimated local clock rate per million years (Myr) (in figure legend). Bold letters in caps represent the fossil calibration points applied as offset values of an exponential distribution; A) 0.3 Myr mean = 0.5 (Ferrusquía-Villafranca *et al.* 2010), B) 0.3 mean = 0.6 Myr (Arroyo-Cabrales *et al.* 2002), C) 1.8 Myr mean = 2.5, D) 3.0 Myr mean = 4.2 (PDB 2011). Terminal taxa for each individual locus are listed in Appendix A.

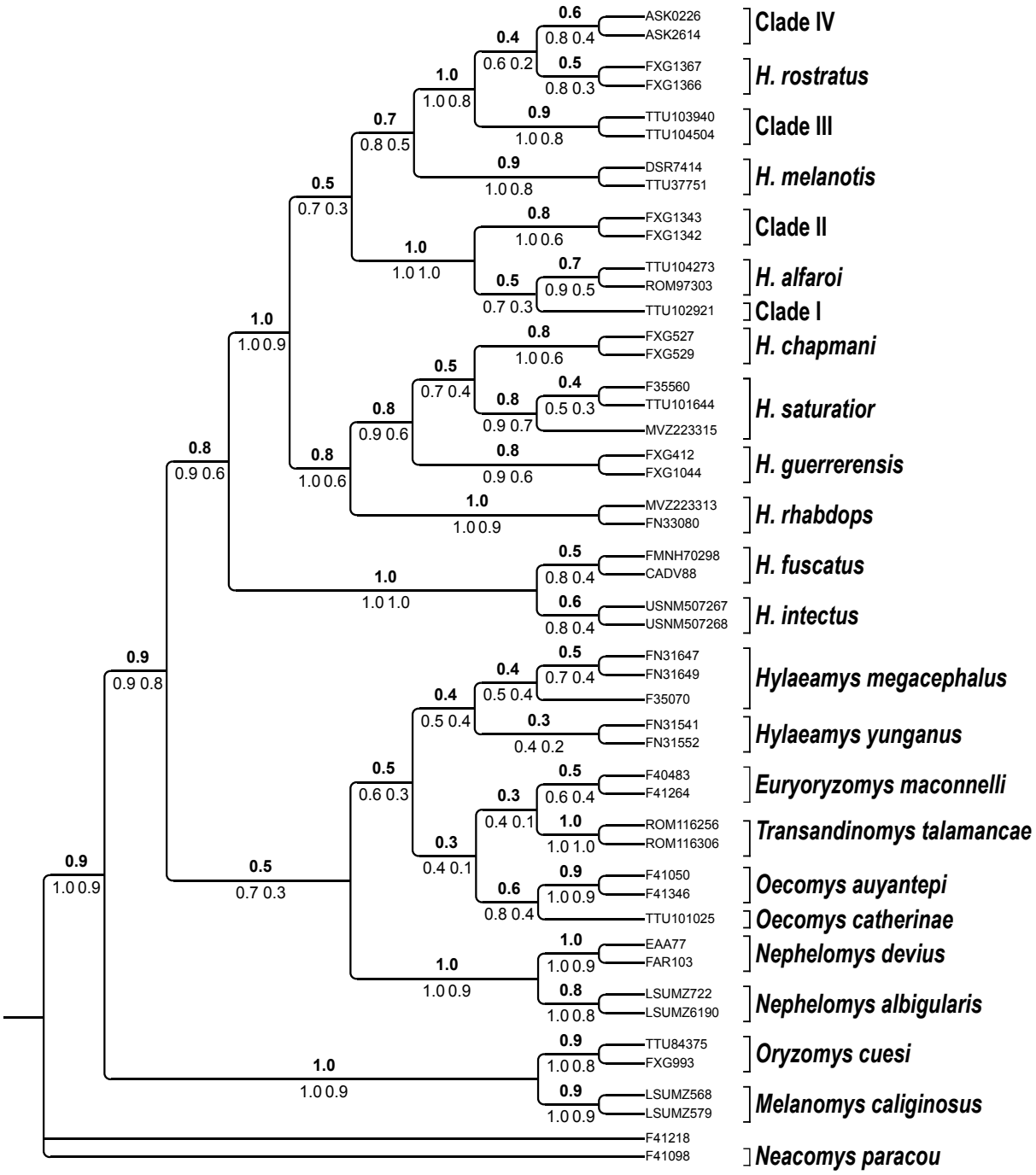

Figure 3. 6. Maximum concordance tree and sample concordance factors (CF) estimated with BUCKy. CF < 0.5 are marked with an asterisk. Terminal taxa labels correspond to those in Appendix A.

of the taxa, including the out-groups, or between *Handleyomys* sensu lato and the rest of the taxa (trees not shown).

Relationships within *Handleyomys*

We recovered the *H. alfaroi*, *H. chapmani*, and *H. melanotis* groups (Mesoamerican *Handleyomys*) as monophyletic clades for all BI analyses of individual loci except for *12S* (Appendix C). In addition, monophyly for the Mesoamerican *Handleyomys* clade was strongly supported with 11 markers and species tree estimation methods (Pp = 1.0, CF = 0.99; Fig. 5–6). The tMRCA for *H. alfaroi*, *H. chapmani* and *H. melanotis* was estimated at 3.6 mya (cCIr = 2.8—4.3). Within the *H. alfaroi* group, monophyly of each currently recognized species, including *H. guerrerensis*, was supported with at least six of the individual data sets. In addition, a clade that included *H. rhabdops, H. guerrerensis, H. chapmani* and *H. saturatior*, within which the later three formed a group relative to *H. rhabdops*, was recovered in all the single locus analyses except for *PRKCI* and *12S*. We also recovered *H. melanotis* and *H. rostratus* as a monophyletic assemblage with all loci except for *Fgb-I7*. The position of *H. alfaroi* was ambiguous. Although five individual datasets (*mtDNA*, *CD14*, *Nup160*, *Fgb-I7* and *PRKCI*) supported a close relationship of *H. alfaroi* with *H. melanotis* and *H. rostratus,* the *IRBP* and *FuT4* data sets positioned it as ancestral to both the *H. chapmani* and *H. melanotis* groups, *Fgb-I7* recovered *H. alfaroi* as sister to only *H. rostratus*, and the *GdX* and *Adh1* data sets inferred a sister group relationship between *H. alfaroi* and the *H. chapmani* group. Other ambiguous relationships involved the *GdX* topology, which did not fully resolve the monophyly of *H. melanotis* and *H. rostratus*, or of *H. chapmani* and *H. guerrerensis*. Finally, the *CD14* and *FuT4* data sets did not recover *H. chapmani* and *H. saturatior* as monophyletic assemblages.

In addition to the seven species delimited as described above, the PTP and bGMYC methods (Fig. 4) revealed three geographically exclusive lineages (phylogroups) within *H.*

Table 3. 4. Relative support for alternative species delimitation models with SpeDeSTEM validation method. Column one specifies the lineages treated as conspecifics for a model with a number of K species.

Taxa arrangement	***K***	**ln(*L*)**	**AIC**	**Delta**	**Model (*L*)**	***W*i[2]**
H. rostratus[1]	22	-116066.787	232175.191	61.565	4.2769E-14	4.24123E-14
H. alfaroi[1]	22	-116040.493	232130.795	32.971	6.92258E-08	6.86487E-08
H. rostratus–Clade IV	23	-116045.499	232144.539	46.715	7.1747E-11	7.114879E-11
Clade III–Clade IV	23	-116041.842	232130.795	32.971	6.92258E-08	6.86487E-08
H. rostratus–CladeIII	*23*	-116031.683	232118.358	20.534	3.476E-05	3.44713E-05
H. alfaroi – Clade II	23	-116030.103	232118.358	20.534	3.476E-05	3.44713E-05
Clade I–Clade II	23	-116027.793	232107.389	9.5656	0.008373	0.00837247
H. alfaroi–Clade I	23	-116026.339	232107.389	9.5656	0.008373	0.00837247
Fully resolved	24	-116020.912	232097.824	-	1	0.9916627

[1]As currently recognized (Musser and Carleton, 2005).
[2]Median model weight.

(*Nm* = 0.29). Migration rates between *H. alfaroi* and Clade I could not be assessed for the lack of appropriate sample size for Clade I. *Cytb* genetic distances between Clades I-II and *H. alfaroi* ranged from 3.0-4.0%, and 4.0-6.0% between Clades III-IV and *H. rostratus*. Species tree reconstruction methods also recognized Clades I-IV as independent species lineages. *BEAST posterior probabilities (pP) ranged from 1 to 0.96 (Fig. 5), whereas BUCKy concordance factors (CF) ranged from 0.7 to 0.8 (Fig. 6). The focal phylogroup within each species (complex) was demarcated geographically based on the species type locality. Thus, *H. alfaroi* is comprised of samples from the Chorotega volcanic arc, the Chortís highlands and Darién (Fig. 2); Clade I included the specimen from western Ecuador, and Clade II included samples from east the Petén Basin, Sierras del Norte de Chiapas and Sierra de los Tuxtlas. Likewise, *H. rostratus* was represented by samples from the Gulf of Mexico, the Sierras del Norte de Chiapas and Sierra de los Tuxtlas. Clade III included samples from the Chortís volcanic front and the Honduras borderlands sub-province (Chortís highlands), whereas Clade IV contained samples from the Yucatan platform (Fig. 3A).

Niche conservatism

ENMs average test specificity and sensitivity (AUC/ROC) values ranged from 0.85 to 0.99, while predictability (the proportion of presence points for a clade correctly predicted by that clade ENM) ranged from 87.5% to 100%. In the case of *H. rostratus* and Clade II, our ENMs failed to predict locality 13 in Los Altos de Chiapas (Fig. 2 and Fig. 3A), and in the case of Clade III, localities in the Honduras Borderlands sub-province also were not predicted (43–44) (Fig. 3A). Interestingly, locality 13 was predicted for *H. chapmani, H. saturatior* and *H. rhabdops,* but these taxa are not known from this region despite confirmed records of the latter two species in the Sierras del Norte and Sur de Chiapas. Similarly, maximum inter-predictability (proportion of presence points for one clade predicted by the ENM of another clade) was

Table 3. 5. Pairwise graph of Schoeners' D (upper triangle) and *I* statistics (lower triangle) measures of niche overlap (Overlap Index—OI). The OI values among currently recognized species and between *H. intectus* and *H. fusctaus* are highlighted in shades of gray where darkness increase denotes greater niche overlap. For comparison, OI values between each species complex and their most closely related lineage are underlined (minimum OI) or in bold letters (maximum OI).

		Schoener's *D*												
	Phylogroup	*H. chapmani*	*H. saturatior*	*H. guerrerensis*	*H. rhabdops*	*H. intectus*	*H. fuscatus*	*Clade I*	*Clade II*	*H. alfaroi*	*H. rostratus*	*Clade III*	*Clade IV*	*H. melanotis*
	H. chapmani	-	0.201	0.051	0.254	0.075	0.016[a]	0.062	0.269	0.110	0.261	0.109	0.083	0.125
	H. saturatior	0.427	-	0.174	0.480	0.254[b]	0.068	0.292	0.439	0.534	0.353	0.465	0.080	0.275
	H. guerrerensis	0.139	0.380	-	0.215	0.053	0.177	0.077	0.112	0.106	0.101	0.152	0.004	0.260
	H. rhabdops	0.542	0.698	0.485	-	0.232	0.180	0.369	0.498	0.400	0.435	0.370	0.046	0.511[c]
	H. intectus	0.223	0.520[b]	0.185	0.495	-	0.638	0.513[b]	0.231	0.353	0.123	0.229[b]	0.010	0.080
***I* statistics**	*H. fuscatus*	0.065	0.191	0.037[a]	0.389	0.876	-	0.477	0.060[†]	0.214	0.067	0.196	0.001[a]	0.027
	Clade I	0.192	0.586	0.214	0.653	0.793[b]	0.741	-	0.329	0.465	0.212	0.467	0.042	0.222
	Clade II	0.555	0.721	0.282	0.840	0.329	0.182[a]	0.644	-	0.398	0.568[d]	0.402	0.186	0.366
	H. alfaroi	0.291	0.801	0.269	0.659	0.648	0.451	0.768	0.761	-	0.342	0.508	0.069	0.224
	H. rostratus	0.470	0.618	0.252	0.749	0.326	0.203	0.487	0.846[d]	0.621	-	0.295	0.211	0.381
	Clade III	0.277	0.734	0.362	0.629	0.564[b]	0.431	0.739	0.698	0.767	0.571	-	0.078	0.316
	Clade IV	0.231	0.219	0.016	0.157	0.036	0.008[a]	0.153	0.427	0.206	0.458	0.235	-	0.070

[a]Smallest and [b]largest niche overlap between each species group and the suggested ancestral lineage (*Handleyomys* sensu stricto– *H. fuscatus* and *H. intectus*).
[c]Largest niche overlap between geographically isolated and [d] geographically overlapped non-sister phylogroup.

represented by the ENM of Clade II, which predicted 40% of the localities for *H. alfaroi*. Whereas maximum niche overlap (OI–Overlap Index) was observed between *H. fuscatus* and *H. intectus* (I=0.876, D=0.638—Table 5), followed by Clade II and *H. rostratus* (I=0.846; D=0. 568), the ENMs of Clade III and IV, and *H. chapmani* and *H. guerrerensis* were essentially detached (I=0.235, D=0.078; I=0.139, D=0.051, respectively). Similarly, the average OI's between lineages within a species complex and its closest relative was considerably higher in the *H. chapmani* complex (with *H. rhabdops*), and more limited in *H. rostratus* (*H. melanotis).* Maximum niche overlap from *H. fuscatus* and *H. intectus* occurred with the ENMs of Clade I (I=0.793, D=0.513), its closest geographic neighbor, and secondarily with *H. rhabdops* (I=0.495, D=0.232) and Clade III (I=0.564, D=0.229), whose distributional ranges are separated by Central America.

We rejected the null hypothesis of niche equivalence for sister clades within *H. rostratus* and the *H. chapmani* groups, although niche equivalence was not rejected for Clade II and *H. alfaroi* or for *H. fuscatus* and *H. intectus* (Fig. 7). However, the Wilk's Lambda (λ) values from the canonical functions analyses resulted in significant probabilities for all comparisons [$(p) \leq 0.000$ ($\lambda = .075$ for *H. rostratus*, $\lambda = .294$ for *H. chapmani* group $\lambda = .792$ for *H. fuscatus* and *H. intectus*, and $\lambda = .190$ for *H. alfaroi*], and correct case discrimination ranged from 92% within *H. rostratus*, 94% within the *H. chapmani* group, to 81% within *H. alfaroi* and 67.5% between *H. fuscatus* and *H. intectus.* Indeed, the scatterplots of the functions coefficients clearly displayed the separation of Clade IV and *H. rostratus* ENMs, and only slight overlap was detected between the ENMs of *H. champani* and *H. guerrerensis*. Meaningful structure coefficients (> 0.3) along the first Canonical Function (CF) were almost entirely correlated with Isothermality and Temperature Seasonality. Particularly for *H. intectus* and *H. fuscatus*, where these two variables

explained all significant variation (0.675 and -0.893, respectively; Table 3). The environmental variables explaining the remainder of the variance in the ENMs were related to precipitation for *H. alfaroi* and the *H. chapmani* group, and to temperature for *H. rostratus*. The Diurnal Range and Precipitation Seasonality had a large effect on the *H. chapmani* group (.658 and .571, respectively), but these variables were irrelevant for the discriminant functions of *H. rostratus* (-.223) and *H. alfaroi* (-.204). Finally, MaxEnt assessment of variables contribution suggested that geological variables (DEM and CTI) affected the ENMs of all phylogroups except for that of *H. melanotis*. Nonetheless, while the DEM was mostly important for *H. fuscatus – H. intectus* and the *H. chapmani* group, the CTI contributed more strongly to the ENMs of *H. alfaroi* and *H. rostratus*.

Discussion

Molecular phylogenetic relationships between *Handleyomys* and closely related genera

We consider Fig. 8 as our working hypothesis based on a consensus of the molecular data analyses. The monophyly of *Handleyomys* sensu Weksler et al. 2006 (Fig. 1A) was strongly supported by the nuclear data in all analyses, although the *mtDNA* data supported a closer relationship between the *H. alfaroi*, *H. chapmani* and *H. melanotis* groups with *Transandinomys* (*COI*) and with *Nephelomys* (concatenated *mtDNA*). Considering that both of these relationships had been argued based on comprehensive morphological analysis (Musser *et al.* 1998; Weksler *et al.* 2006), the fact that *Nephelomys* was positioned alternatively as sister of the Mesoamerican *Handleyomys*, and the close correspondence in the divergence times for the separation of *Handleyomys* sensu lato from the rest of the genera, and the separation of *H. fuscatus–H intectus*, *Nephelomys,* and pending the inclusion of additional markers; *Mindomys*, from the rest of the in-group, would represent a deep three-way split within Clade B during the late Miocene, consistent with the estimated time for the colonization of South America by Sigmodontinae

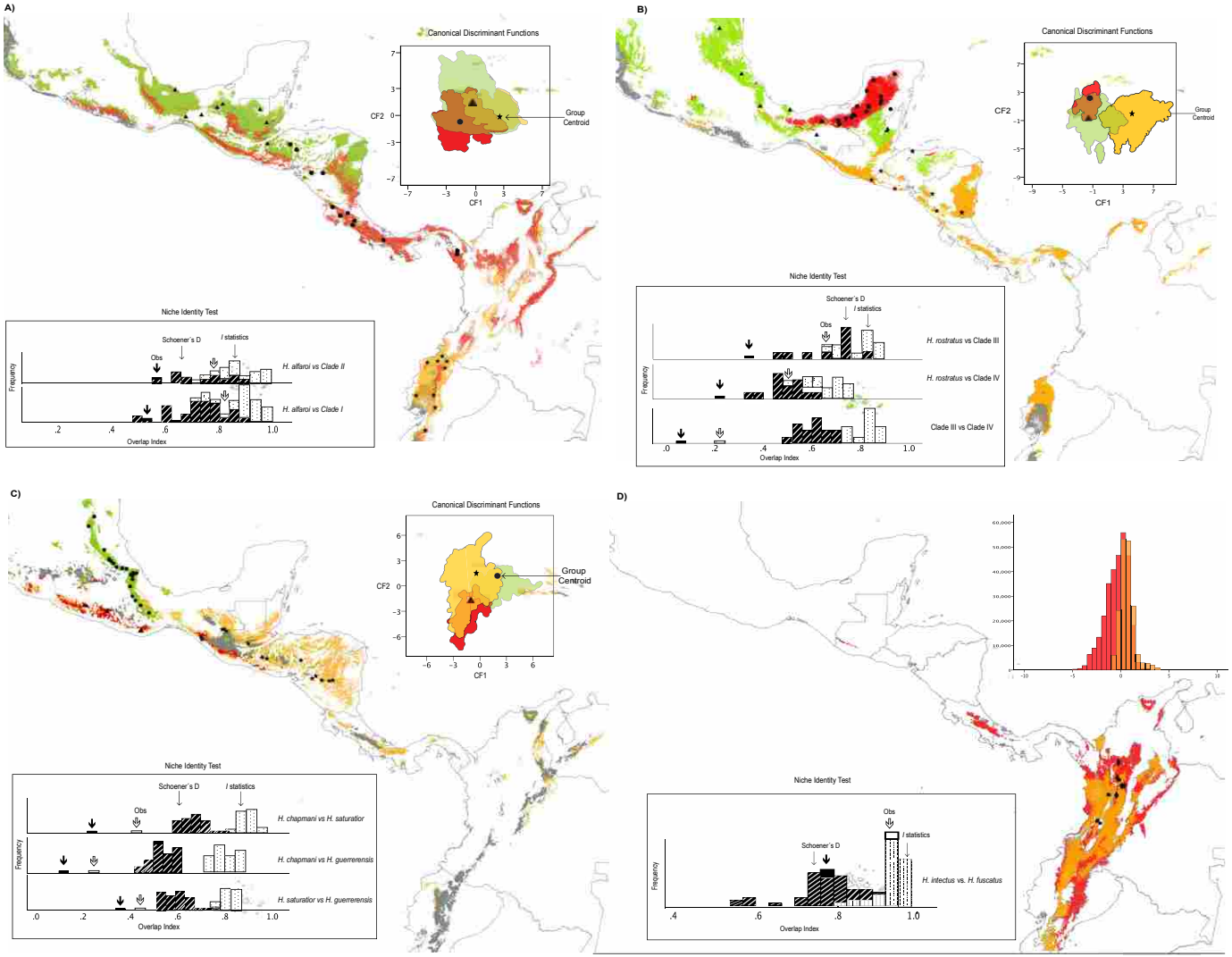

Figure 3. 7. Projected ENMs for the different phylogroups within *H. alfaroi* A), *H. rostratus* (B), the *H. chapmani* group (C), *Handleyomys* sensu stricto (D; *H. intectus* and *H. fuscatus*), and their estimated most closely related lineage [grey—*H. melanotis* (A, B), and *H. rhabdops* (C)]. Locality symbols are as in Fig. 2. A histogram showing the niche identity test results (bottom left) and a scatterplot of the two first discriminant functions (upper right) are embedded within each map (A – D). The corresponding overlap index (OI) and canonical functions structure coefficients are provided in Tables 3 and 5, respectively.

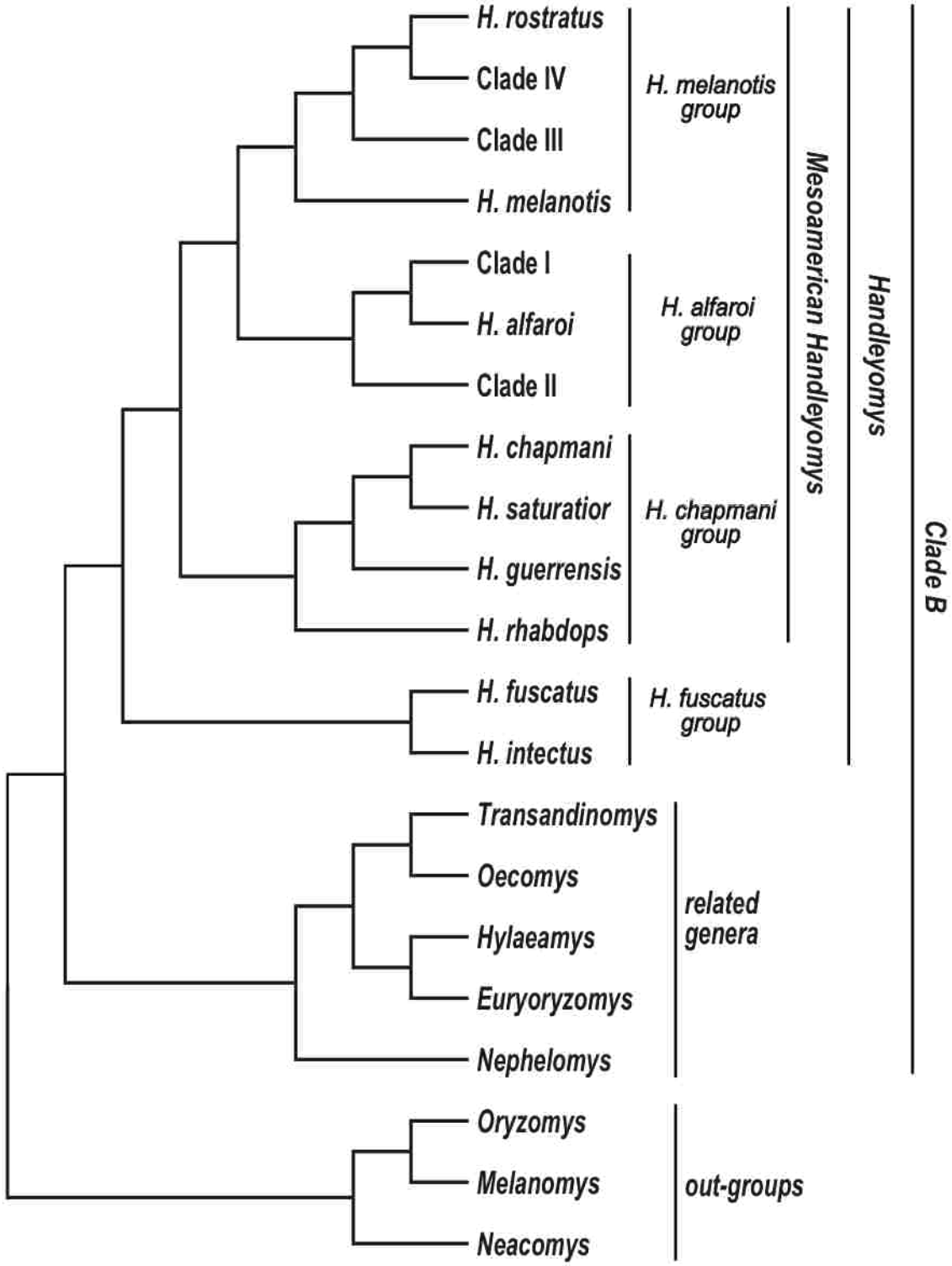

Figure 3. 8. Empirical phylogenetic hypothesis for the genus Handleyomys based on incorporation of all methods.

(Hershkovitz 1966; Leite *et al.* 2014; Steppan *et al.* 2004). The contemporaneous uplift of the Cordillera Oriental in the NE Andes 6.0 Ma (Graham, 2009) during a global period of low sea level 5-7MYA (Marshall 1979), suggests that the separation of *Nephelomys* from *Handleyomys* sensu lato and the subsequent split of *Handleyomys* sensu stricto from the *H. alfaroi–H. melanotis–H. chapmani* species groups, could have involved vicariant events in the northern Central Andes (*Nephelomys*), the western Northern Andes (*Handleyomys* sensu stricto), and north of the Chorotega block in the Mayan region (*Handleyomys* sensu lato). These three regions retain relatively large amounts of ancestral endemism (Morrone 2014b; Patterson *et al.* 2012), and are thought to have been particularly important as permanent refugees for high elevation taxa through the Pliocene –early Pleistocene (Ramírez-Barahona & Eguiarte 2013; Ruiz- Sanchez & Ornelas 2014). This ecological pattern is unlike several other Sigmodontinae rodents in which tolerance to high elevation appeared secondarily (Upham *et al.* 2013), and contrasts with the biogeographic history of other Oryzomyine rodents hypothesized to have recolonized North America (2.5 Ma; *Oryzomys, Melanomys, Oligoryzomys*) (Hanson & Bradley 2008; Machado *et al.* 2013; Palma *et al.* 2010). Moreover, genetic distances (*Cytb, IRBP* and pairwise differences in *nuDNA*) between *Nephelomys* and its closest branch (*H. fuscatus-H. intectus* with *Cytb* and *Oecomys* with *nuDNA*) and between *Handleyomys* sensu stricto and its closest branch (*H. chapmani* group with the *nuDNA*) were the largest within the in-group. For instance, IRBP genetic distances were 4.2%, while they ranged from 2.1% (between *Euryoryzomys* and *Hylaeamys*) to 2.7% (between *Oecomys* and *Transandinomys*, and between *H. alfaroi* and the *H. melanotis* group) among the rest members of the Clade B. Indeed, the diversification of *Oryzomys* and *Melanomys* was estimated to have occurred during the same period of time, despite this clade containing the predicted oldest Oryzomines; *Holochilus* and

Oryzomys palustris (Machado *et al.* 2013). Likewise, previous molecular phylogenies have recovered an early split of Clade B sensu Weksler (2006) within Oryzomine, preceded only by the divergence of *Scolomys* and *Zygodontomys* (Pardiñas *et al.* 2002; Steppan 1996; Zijlstra *et al.* 2010). Furthermore, previous time calibrated phylogenies have recovered the split of *Handleyomys* sensu stricto from the *H. alfaroi – melanotis – chapmani* groups, *Nephelomys* from the rest of Clade B, and *Neacomys* from *Oryzomys – Melanomys,* within the same time interval; although some studies estimated divergence times approximately 0.5 Myr older (D'Elía *et al.* 2006; Martínez *et al.* 2012; Parada *et al.* 2013; Pine *et al.* 2012). However, the estimated divergence times of a multilocus phylogeny of muroid rodents was more comparable with our results (Schenk *et al.* 2013). Consequently, our findings support Weksler and Percequillo (2011) proposal that *H. fuscatus-intectus* and the *H. alfaroi*, *H. melanotis* and *H. chapmani* groups, should be regarded as distinct genera.

Although not the focus of this study, the amount of phylogenetic information contained in the sequence data appeared suitable for an evolutionary scale of relatively recent species-level splits (~1.2 Myr) to inter-generic divergence times (~6. 2 Myr) (Townsend & Lopez-Giraldez 2010). As such, our data provide insights as to phylogenetic relationships among genera thought to be closely related to *Handleyomys* (*Euryoryzomys*, *Hylaeamys*, *Oecomys*, *Nephelomys* and *Transandinomys*). Overall, we found support for a sister relationship between *Euryoryzomys* with *Hylaeamys,* and between *Oecomys* and *Transandinomys*. Despite the position of *Euryoryzomys* in the maximum concordance tree conflicted with the *BEAST topology, the population tree (also estimated by BUCKy), resolved this lineage as did *BEAST. The performance of "gene trees summaries" (the former two) versus "full modeling" methods (*BEAST) has been subject of recent attention (Huang *et al.* 2010; Lacey Knowles *et al.* 2012;

Lanier *et al.* 2014) in part because discrepancy has been shown to arise from several aspects of the methodologies, as well as the natural history of the study group itself (Huang *et al.* 2010; Nakhleh 2013), making both types of methods equally suitable when no prior information on the group is available. In addition to a problematic taxonomic history, the early diversification of Oryzomyalia has been estimated to occur within 1.0 Ma (Schenk *et al.* 2013; Steppan *et al.* 2004). Rapid diversifications usually produce phylogenies where ambiguous relationships among individual locus topologies can be related to nodes with equivalent divergence times (Whitfield & Lockhart 2007). In this context, *BEAST has been shown to incorporate even minor amounts of phylogenetic signal more efficiently, like that contained in slower evolving markers and thus minimizing noise of inflated relationships (Lanier *et al.* 2014). In addition, time calibrated Bayesian coalescent models appear to be more robust to topological re-arrangement than the heuristic best-likelihood methods (DeGiorgio & Degnan 2013).

Phylogenetic relationships within Handleyomys

We found strong support for the monophyly of the *H. alfaroi*, *H. chapmani*, and *H. melanotis* groups. These three clades correspond to the *alfaroi* group *sensu* Weksler et al. (2006). Monophyly of these three species groups was anticipated based on external morphology and its predominantly Mesoamerican distribution compared to other members of the subfamily Sigmodontinae (Goldman 1918; Merriam 1901). Although the inclusion of *H. melanotis* was questioned by Hershkovitz (1966), who proposed *that H. melanotis* and *O. bombycinus* (syn. *Transandinomys bolivaris*) were closely related. However, standard and differentially stained chromosome data have supported a close relationship between the *H. melanotis*, *H. alfaroi* and *H. chapmani* groups (Haiduk *et al.* 1979). In addition, phylogenetic studies based on DNA sequences have consistently recovered representatives of these groups as a monophyletic clade (Pine *et al.* 2012; Voss & Weksler 2009; Weksler 2006). However, the previous studies also

recovered a sister relationship between *H. rostratus* and *H. chapmani*, a finding that disagrees with the position of *H. alfaroi* as sister to the *H. melanotis* group recovered here and that had been hypothesized by Musser and Carleton (2005). However, this discrepancy may result from incomplete taxon sampling (and/or fewer gene loci included) in these previous studies.

The *Cytb* genetic distances between the *H. melanotis*, *H. alfaroi* and *H. chapmani* groups averaged 13.8%; a value comparable to those recovered among the other currently recognized genera within Clade B = 14.8% and the out-group = 15.20% (see Rosa *et al.* 2012). Similarly, uncorrected IRBP genetic distances between these clades averaged 2.4%; also equivalent to those observed between other Oryzomyini genera (D'Elía *et al.* 2006). These findings, along with reported morphological (Weksler 2006; Weksler & Percequillo 2011) and chromosomal differentiation (Engstrom 1984; Haiduk *et al.* 1979; Musser *et al.* 1998), suggests that the *H. alfaroi*, *H. chapmani* and the *H. melanotis* groups could reasonably be classified as separate genera. *H. melanotis* and *H. rostratus* comprise the *H. melanotis* group (Engstrom 1984; Goldman 1915, 1918; Hall 1981; Hershkovitz 1958; Merriam 1901; Musser & Carleton 1993, 2005). *H. chapmani*, *H. saturatior*, and *H. rhabdops* were originally assigned to the *H. chapmani* group (Merriam 1901), however, they were soon relegated as subspecies of *H. alfaroi* (Goldman 1918) until Musser and Carleton (1993) returned them species status. Our results support the *H. chapmani* group. The definition of the *H. alfaroi* group by Weksler and Percequillo (2011) as including *H. alfaroi*, *H. melanotis*, *H. rostratus* and *H. rhabdops* was not supported by our results.

Species limits within *Handleyomys alfaroi*

As many as eight geographic units have been suggested as deserving species or subspecies recognition within *Handleyomys alfaroi* (Musser and Carleton, 2005). These forms can be individually allocated to one of the three clades recovered in this study (Fig. 4). Clade I

or *H. alfaroi* incorporates the extent of the subspecies *H. a. alfaroi* (Allen 1891, 1908, 1910) and *H. a. dariensis* (Goldman 1918). Although each of the former classifications represented a different physiographic province of Central America (the Chorotega arc, the Chortís block, and Darien), an area in which significant lineage differentiation has been observed among populations of rodents with similar ecologies (Anderson & Timm 2006; Arellano *et al.* 2005; Hanson & Bradley 2008; Hardy *et al.* 2013; Rogers & González 2010), populations of *H. alfaroi* from different provinces were not differentiated. Clade I may also represent the subspecies *H. a intagensis* (Hershkovitz 1940), *H. a. gracilis* (Thomas 1894), and *H. a. palmirae* (Allen 1912) based on the ENM for Clade I. Unfortunately, we were unable to sample the later two forms. Clade I of *H. alfaroi* appear to be restricted to the western Central Andes in Ecuador. Although this region is associated with the Chocó-Darién and northeastern Colombia moist forests (Magdalena-Urabá) in a global Ecoregion, the western Ecuadorian moist forest represent a different biogeographic province (Rivas-Martínez *et al.* 2011). The forests in this province are comprised of at least 20% endemic plant species (Gentry 1992) and this level of endemism is consistent with their proposed independent geological histories (Leigh *et al.* 2014). *H. alfaroi* Clade II includes *H. a. palatinus* (Merriam 1901) and *H. a. gloriaensis* (Goodwin 1969). *H. a. gloriaensis* was described from the Chimalapas mountains in Guerrero and Chiapas, Mexico, within the broader range of *H. a. palatinus*. Collected samples outside the distribution of *H. a. palatinus* that could be referred to *H. a. gloriaensis* based on geographic proximity belonged to *H. melanotis* or *H. guerrerensis*. On the other hand, as noted by Engstrom (1984), specimens from Teapa, Tabasco (Loc. 46—the type locality of *H. a. palatinus* and *H. rostratus megadon*) (Merriam 1901), belonged to Clade IV of *H. rostratus*. Nevertheless, we document that representatives of Clade II of *H. alfaroi* and *H. rostratus* co-occur in the Sierra de Los Tuxtlas,

Veracruz, and the los Altos de Chiapas and Sierras del Norte de Chiapas, all within the proposed distribution of *H. a. palatinus* (Hall 1981). Although *Cytb* genetic distances between Clade I and *H. alfaroi* were 3.9%, and between the later and Clade II were 3%, slightly smaller than for other sister species of rodents and bats (Baker & Bradley 2006; Solari *et al.* 2009); they represent a considerable amount of time in isolation, estimated at 0.58 Myr for Clade II and 0.4 Myr for *H. alfaroi* and Clade I. Furthermore, the exclusivity of Clade I is supported by a distinct karyotype (2n=62, FN= 100), that contrasts with that of *H. alfaroi* (2n =60, FN=104; Los Tuxtlas, MX [loc. 19]) (Haiduk *et al.* 1979). Similarly, the taxonomic status of *H. a. palatinus* (Clade II) was reviewed by Goldman (1918); who retained it as subspecies highlighting a narrower zygomatic breath (average = 12.6 mm) than in *H. a. alfaroi* (13.5 mm), or *H. a. intagensis* (13.6 mm). Surprisingly, Clade II was recovered ancestral with the species tree reconstruction methods and largely with nuclear individual loci, whereas the *mtDNA* tree placed this clade and *H. alfaroi* as sister taxa. This would imply that *H. alfaroi* represents a re-colonization event from the south. This hypothesis is supported by the fact that the *mtDNA* of individuals from locality 14, although only 2.1% divergent from Clade II, were closely related to *H. alfaroi.* However, our *nuDNA* data positioned individuals from locality 14 within Clade II. Divergence times estimates would place the age of this putative secondary contact at the end of a cooling period of the middle Pleistocene 0.45 Myr. A range expansion hypothesis that had been proposed for *H. alfaroi* by Goldman (1918) and Hershkovitz (1966) based on its allopatric distribution in Mesoamerica and its ecological preference for forested habitat 500 to 1400 m in elevation. Our data show considerable ecological niche overlap among the three allopatric lineages and indicate a past range expansion of *H. alfaroi* clade. This is in agreement with observations by Reid (2009), who highlighted that Central American populations of *H. alfaroi* are usually found at lower elevations

(< 1,200 m) than in South America. Similarly, the equivalent Central American forests communities in Mexico are also found, on average, to be about 200 m higher in elevation (Islebe & Velázquez 1994).

Pending objective phylogeographic hypothesis tests (see Introduction), a conservative approach would be to consider Clade II as subspecies, however, the hybrid/introgressed population appears considerably divergent from either parent. The average migration we estimated was Nm=0.02 and suggest that populations to the north of locality 14 (La Unión, GT) are reproductively isolated.

Species delimitation in the *Handleyomys melanotis* group

Overall, the distributions of the three clades within *H. rostratus* that we recovered are consistent with the predicted geographic ranges of the three recognized subspecies (Ramírez-Pulido *et al.* 2014). Clade IV closely matches the distribution of *H. r. yucatanensis* sensu Engstrom (1984), and *H. r. megadon,* a form restricted to the Yucatán peninsula, which is an area of high levels of mammalian endemism (Escalante *et al.* 2009; Morrone 2002). *H. rostratus* was recovered as sister to Clade IV, and incorporates the distribution of *H. r. rostratus* and *H. r. carrorum* (Merriam 1901) along the Gulf of Mexico and the Sierras del Norte de Chiapas; areas closely associated with the Yucatán península in a biogeographic context (Ramírez- Barahona *et al.* 2009). Clade III generally corresponds to the anticipated range of *H. r. salvadorensis* (Felton 1958). This clade includes populations of *H. r. rostratus* (Engstrom (1984) from Honduras (loc. 42-43). These localities were not predicted by the ENM of Clade III, or by the ENMs of *H. rostratus* and Clade IV. Instead, they appear to result from a relatively recent period of geographic expansion.

The uniqueness of each of these groups was supported by Engstrom (1984), based on a

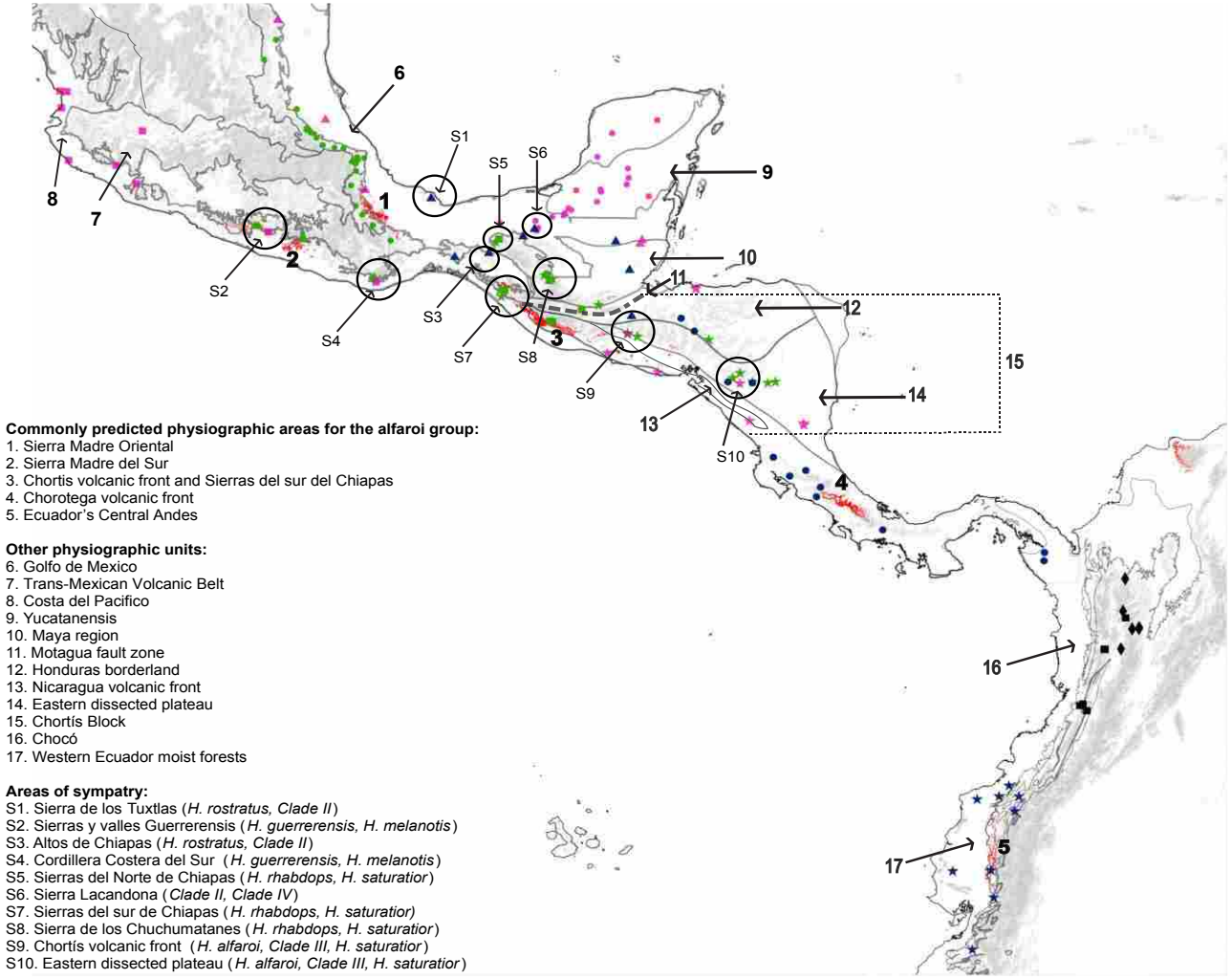

Figure 3. 9. Map of the area used for projection of the ENMs encompassing southern Mexico, Central America and northern South America. The main physiographic regions (adapted from Cervantes-Zamora et al. 1990; Marshall 2007) are outlined and annotated as applicable for this study. The sum of the standardized (each pixel predicted by the ENM re-classified to 1) ENMs for each species clade within Handleyomys is depicted for values ≥ 6. Symbols correspond to different phylogroups in Figs. 2, 3 and 4 within the H. alfaroi (blue), H. chapmani (green) H. melanotis (pink) and H. fuscatus (black) groups. In the background, an elevation gradient is shown in white (sea level to 800 m) and shades of gray (light < 1800 m dark >1800 m).

detailed morphological, molecular and karyotypic analysis. For instance, the karyotype of *H. rostratus* is 2n = 62 FN = 68–70, whereas that of *H. r. yucatanensis* (including *H. r. megadon*) is 2n = 64 FN = 70. Nevertheless, he agreed with Goldman (1918) who regarded these forms as subspecies, in part because of the potential area of sympatry along the Sierras del Norte de Chiapas and the Cordillera Costera del Sur. However, we found no evidence that these two clades co-occur (Fig. 3A and Fig. 9). Moreover, the canonical discriminant function analyses suggest that the ecological niches possessed by Clades III and IV differ markedly, and although to a lesser extent, from the niche of *H. rostratus*. Both lines of evidence would support their demarcation as different species under the general lineage concept (De Queiroz 2007). The ENM of Clade IV is negatively affected by the incidence of temperatures below 19.9°C, contrasting with that for *H. rostratus* projected as 6.7°C and the minimum temperature for Clade III projected as 4.0°C. Thus, the distinctiveness of Clade III was strongly supported with all methods. Indeed, *Cytb* genetic distances between this clade and *H. rostratus* averaged 6%, a value similar to other sister species of rodents with similar geographic distributions (Gutiérrez-García & Vázquez-Domínguez 2012; Ordóñez-Garza *et al.* 2010). Interestingly, the canonical functions revealed higher Isothermality values (max. 91) for the projected distribution of this clade than those predicted for *H. rostratus* (max. 78) and Clade IV (max. 70). This suggesting that Clade IV occupies environments with more extreme daily temperatures, that based on the extracted pixel values tend to get lower (4.0°C). The unique environment each of these three clades occupies has been often noted, for instance, the mountains of El Salvador and Honduras show numerous temperate derived subtropical forest elements that are not present south the Chortís block (Jaramillo-Correa *et al.* 2008; Martin & Byron 1957; Nixon 2006). In addition, these forests show close affinities with the Sierra Madre Oriental and Sierra Madre del Sur in

Mexico, that represent the distributional extent of *H. melanotis* (Torres-Miranda *et al.* 2011). Similarly, the floristic elements of the Yucatan peninsula (Clade IV) are predominantly Caribbean (Chiappy *et al.* 2001), although it also shares important rain forest components with the Gulf of Mexico (Contreras-Medina *et al.* 2007; Ramírez- Barahona *et al.* 2009), that represent the range of *H. rostratus*, with more Neotropical affinities (Challenger & Soberón 2009).

Relationships within the *Handleyomys chapmani* group

A sister relationship of *H. saturatior* with *H. chapmani*, and the species status for *H. guerrerensis*, was recently suggested based on sequence data for two loci (Almendra et al. 2014). Use of additional molecular markers and different methodological approaches used herein strongly support those findings. In addition, we document that *H. chapmani* and *H. guerrerensis* occupy different ecological niches. This likely is a result of the considerable dissimilarities in cloud forest composition between the SMO and SMS (Ornelas *et al.* 2013). Ecological divergence could explain the apparent continued isolation of these forms during the Pleistocene despite the secondary corridors for dispersal reported to have existed (Ceballos *et al.* 2010). Similarly, our study suggests that these three lineages, together with *H. rhabdops*, represent the high elevation monophyletic assemblage initially suggested by Merriam (1901). Finally, the *H. chapmani* group is morphologically diagnosable from *H. alfaroi* and the *H. melanotis* group by the absence of sphenofrontal foramen (Weksler 2006). Therefore, we regard *H. saturatior*, *H. chapmani*, and *H. guerrerensis* as species-level taxa (De Queiroz 2007).

Niche conservatism and geographic limits

The substantial amount of niche variation accompanying speciation in the *H. melanotis* and the *H. chapmani* groups also has been documented for other rodent lineages with similar distributions (Martínez-Gordillo *et al.* 2010). The applicability of niche conservatism

hypotheses as operational criteria for species delimitation has frequently been demonstrated (Kozak *et al.* 2008; Olalla Tárraga *et al.* 2011; Pyron & Burbrink 2009; Raxworthy *et al.* 2007). Similarly, continuous improvement in methods to estimate the ENMs and to better quantify niche divergence (Machac *et al.* 2013; Radosavljevic & Anderson 2014; Warren *et al.* 2008, 2010), have made this method particularly suitable to approximate the potential environments of cryptic lineages. This also highlights the potential risks of not considering this information for developing conservation and management plans (Fuller, T. *et al.* 2006; Sánchez-Cordero *et al.* 2005; Sarkar *et al.* 2009). Indeed, environmental variables potentially reinforcing isolation of allopatric taxa are generally not acknowledged, nor are they tested objectively (Glor & Warren 2011; Gutiérrez-García & Vázquez-Domínguez 2013). Although the majority of ENMs were concordant in identifying mutually optimal distributions within the Sierra Madre Oriental, Sierra Madre del Sur, Chortís volcanic front, Sierras del Sur de Chiapas, Chorotega Volcanic Front and Ecuador's Central Andes (Fig. 9), not all phylogroups realized their potential (modeled) distributions. Our data suggest that although allopatric phylogroups gradually adapted to their diverging environments, they failed to adapt to presumably less suitable environments imposed by geographic barriers (Cooper *et al.* 2010; Wiens 2011). Thus, to a degree, niches have been conserved (Peterson 2011). Although our understanding of the complex biogeographic history of Mesoamerica is in its infancy, the ineffectiveness of existing conservation policies to integrate the varied mosaic of ecosystems, and with that, species with potentially restricted distribution ranges, is readily apparent (Amori *et al.* 2013; Ornelas *et al.* 2013; Sarkar *et al.* 2009).

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Appendix A

Supplementary Materials for Chapter 3

List of specimens

Specimens Examined.— Oryzomyine rodents included in this study. For each voucher specimen we list the museum acronym and catalog number as follows: ASNHC= Angelo State Natural History Collections, Angelo State University; BYU= Monte L. Bean Life Science Museum, Brigham Young University; CIByC= Colección de Mamíferos del Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos; CURN= Centro Universitario Regional del Norte de la Universidad Autónoma de Nicaragua; ECOSCM= El Colegio de la Frontera Sur; FMNH= Field Museum of Natural History; ICN= Instituto de Ciencias Naturales, Universidad Nacional de Colombia; LSUMZ= Louisiana State University Museum of Zoology; MZFC= Museo de Zoología, Facultad de Ciencias, UNAM; ROM = Royal Ontario Museum; TCWC= Texas Cooperative Wildlife Collection, Texas A&M University; TTU= Museum of Texas Tech University; USNM= United States National Museum. Specimens are listed by taxon, country, locality number, collecting location (geographic coordinates are given inside parenthesis) and museum voucher number.

Handleyomys alfaroi.— COSTA RICA: **locality 1**: 7 Km NE Quesada, Alajuela, 433 m (-84.3962, 10.37692), (LSUMZ 603, LSUMZ 605); **locality 4**: Hacienda Santa Maria, Rincon de la Vieja, Guanacaste, 976 m (-85.30107, 10.767), (ROM 113350, ROM 113351); **locality 8**: Cerros de la Carpintera, Iztaru, Cartago (-83.98447, 9.881768), (ROM 113136). HONDURAS: **locality 5**: Comayagua, 600 m (-87.874832, 14.892368), (TTU 104273, TTU 104274, TTU

104336, TTU 104337, TTU 104359, TTU 104356). NICARAGUA: **locality 7**: Selva Negra, Matagalpa, 1066 m (-85.891507, 12.960726), (EU579489 = TK 93700).

*Handeyomys alfaroi dariensis**.— COSTA RICA: **locality 3**: Monteverde, Rio Guacimal, Puntarenas (-84.8097, 10.30816), (ROM 97302= FAR 83, ROM 97303= FAR 84). PANAMA: **locality 11**: 26 Km by road W Volcan, Chiriqui, 617 m (-82.241936, 8.8000002), (TTU 39149); **locality 12**: Cerro Pirre, Darien, 938 m (-77.727242, 7.713033), (ROM 116285).

Handleyomys alfaroi (Clade I).— ECUADOR: **locality 22**: Comuna San Francisco de Bogota, San Francisco de Bogota, Esmeralda (-79.87, 0.549), (TTU 102921); **record 1**, Hacienda Chinipamba, near Pena Herrera, Intag, Imbabura (-78.540573, 0.344866); **record 2**, Rio Cayapas, Sapallo Grande (-78.9833, 0.7833); **record 3**, Majua, 3 Km W Esmeraldas, Esmeraldas (-79.597836, 0.695555); **record 4**, Puente de Moromoro, 1 Km SW El Oro, Esmeraldas (-79.73333, -3.73333); **record 5**, Puente de Chimbo, Chimborazo, Chunchi (-79.13333, -2.2); **record 6**, Limon, Los Rios, Quevedo (-79.216232, -1.398644); **record 7**, Pescado River, Manabi (-80.280018, -1.423613); **record 8**, Hacienda Paramba, Ibarra, Imbabura (-78.430023, 0.773139).

Handleyomys alfaroi (Clade II).— GUATEMALA: **locality 14**: 2.3 Km S 1.7 Km E La Unión by road aldea Jupilingo, La Unión, San Marcos, 1501 m (-89.275713, 14.943904), (MVZ 224808, MVZ 224807); **locality 15**: 1.5 Km S, 1 Km W Poptún, Petén, 622 m (-89.333, 16.3), (ROM 99240, ROM 99241); **locality 21**: Campo Los Guacamayos, 40 km N El Naranjo, biotopo Laguna del Tigre, Petén (-90.81, 17.6) (ROM 99536). MEXICO: **locality 13**: 12 Km N (by

road) Berriozábal, Chiapas, 1120 m (-93.2722, 16.7986), (CIByC 2732= DSR8899, CIByC 2743= DSR8900, CIByC 2745= DSR8937, CIByC 2752= DSR8957, CIByC 2595= DSR8991, CIByC 2597= DSR8993, CIByC 2636= DSR8996, CIByC 2651= DSR9018, CIByC 2652= DSR9019, CIByC 2653= DSR9020, CIByC 2654= DSR9021, CIByC 2687= DSR9023, CIByC 2688= DSR9024, CIByC 2689= DSR9025, CIByC 2691= DSR9027, CIByC 2692= DSR9028, CIByC 2716= DSR9032, CIByC 2717= DSR9033, CIByC 2729= DSR9055, CIByC 2749= DSR8954, CIByC 2730= DSR9056, CIByC 2724= DSR9050, CIByC 2725= DSR9051, CIByC 2690= DSR9026, CIByC 2750= DSR8955, CIByC 2746= DSR8938, ROM ASK0650, ROM ASK0651); **locality 16**: Forest behind Escuela Secundaria Tecnica No.95, Tumbalá, Chiapas, 1390 m (-92.315375, 17.289429), (FXG1342= CIByC 2817, FXG1343= CIByC 2818); **locality 17**: Ruinas de Palenque, Chiapas, 97 m (-91.9939, 17.50412), (ROM ASK062, ROM ASK061, ROM ASK063, ROM ASK064, ROM ASK065, ROM ASK076); **locality 18**: Sustainable forest Los Ocotones, Cintalapa, Chiapas, 1800 m (-94.179611, 16.694079), (ECOSCM2760); **locality 19**: 13.0 Km NW (by road) Sontecomapan, Estación Los Tuxtlas, IBUNAM, Catemaco, Veracruz, 200 m (-94.8856, 18.4132), (CIByC 2246, CIByC 2247); **locality 20**: Paso del Soldado, Lagos de Montebello, Chiapas, 1400 m (-91.783231, 16.13496), (ECOSCM320); **locality 35**: 7 Km N (by road) Tumbalá, Chiapas, 1255 m (-92.322677, 17.341133), (CIBYC 2813= FXG1338).

Handleyomys melanotis.— MEXICO: **locality 23**: 4.6 Km NE Jalcocotán, Nayarit, 711 m (-105.0827, 21.52291), (ROM ASK1705); **locality 24**: Hacienda San Antonio, Comala, Colima, 871 m (-103.7084, 19.3447), (ASNHC ASK1957); **locality 25**: 10.9 Km NW (by road) Coalcoman, Michoacán, 1141 m (-103.1356, 18.8021), (CIByC 1806= DSR7715, CIByC 1807=

DSR7716); **locality 26**: Acahuizotla, Chilpancingo de los Bravo, Guerrero, 1000 m (-99.444551, 17.384634), (ROM ASK896); **locality 27**: 6 Km SE Chamela, UNAM Estación de Biología, Jalisco, 112 m (-105.032056, 19.499038), (TTU 37751, TTU 45123); **locality 28**: 1.8 Km N Peñita de Jaltemba, Nayarit, 100 m (-105.2388, 21.0457), (ASNHC 3418= ASK1538); **locality 29**: 8 Km E San Blas, Nayarit, 100 m (-105.278, 21.5402), (ASNHC 3419= ASK1601); **locality 30**: 3.4 Km W (by road) San Sebastian del Oeste, San Sebastián, Jalisco, 1450 m (-102.9687, 20.363), (BYU 1210, CIByC 1207); **locality 31**: 5.5 Km S Concepcion de Guerrero, Putla, Oaxaca, 936 m (-96.424599, 15.908838), (CIBYC 1668= FXG1000, CIBYC 1669= FXG1001, CIBYC 1670= FXG1002, CIBYC 1664= FXG996, CIBYC 1665= FXG997, CIBYC 1666= FXG998, CIBYC 1667= FXG999, CIBYC 942= FXG789, CIBYC 939= FXG793, CIBYC 1032= FXG794, CIBYC 1033= FXG795).

Handleyomys rostratus.— MEXICO: **locality 34**: 10.0 Km NW (by road) Sontecomapan, Estación Los Tuxtlas, IBUNAM, Catemaco, Veracruz, 200 m (-94.8856, 18.4132), (CIByC 2222= DSR8560); **locality 13**: 12 Km N (by road) Berriozábal, Chiapas, 1120 m (-93.2722, 16.7986), (CIBYC 2747= DSR8952, CIBYC 2748= DSR8953, CIBYC 2241= DSR8464, CIBYC 2242= DSR8465, CIBYC 2243= DSR8466, CIBYC 2244= DSR8467, CIBYC 2245= DSR8468, CIBYC 2719= DSR9045); **locality 36**: Rancho Don Guillen 2 Km S Metlaltoyuca, Francisco Z. Mena, Puebla, 351 m (-97.85, 20.7333), (CIByC 2841= FXG1366, CIByC 2842= FXG1367); **locality 38**: 3.2 Km W Calabazas, Rancho Calabazas (near Ciudad Victoria), Tamaulipas, 732 m (-99.177, 23.6691), (TTU 44929, TK 27527= TTU 44930); **locality 69**: 41.5 Km SW Tuxtepec, Oaxaca, 441 m (-96.348724, 17.725469), (TCWC 34497).

Handleyomys rostratus (Clade II).— EL SALVADOR: **locality 40**: El Imposible, El Refugio, Ahuachapan, 728 m (-89.9458, 13.82516), (ROM102288= F35719,); **locality 42**: El Imposible, Ahauchapan (-89.9451, 13.8331), (ROM 101843= F35718). HONDURAS: **locality 43**: Atlántida, 191 m (-87.460098, 15.745006), (TTU 103939, TTU 103940); **locality 44**: Lancetilla Botanical Garden, Atlántida, 378 m (-87.460098, 15.745006), (TTU 84373, TTU 84376, TTU 84374= TK101717). NICARAGUA: **locality 46**: Nueva Guinea, Atlántico Sur, 164 m (-84.451904, 11.759815), (TTU 104504); **locality 48**: El Tigre, Matagalpa, 930 m (-86.1074, 12.9651), (TK 113553). Belize: **locality 32**: Roaring River, Cayo, 380 m (-88.8048, 17.18118), (ROM 35100).

Handleyomys rostratus (Clade IV).— MEXICO: **locality 49**: 1.2 Km E Ruinas de Palenque by road, Chiapas, 73 m (-91.8853, 17.50304), (ROM ASK067); **locality 50**: 4 Km N Teapa, Tabasco, 40 m (-92.9533, 17.6286), (ROM ASK081); **locality 51**: 19 Km N Palenque, Palenque, Chiapas, 45 m (-91.964595, 17.712049), (ROM ASK154); **locality 52**: 6.6 Km S Palenque (by road), Palenque, Chiapas, 400 m (-91.924769, 17.42467), (ROM ASK074, ROM ASK075); **locality 53**: 27 Km S Candelaria, Palenque, Chiapas (-91.143367, 17.938223), (ROM ASK300); **locality 54**: 11 Km S Candelaria, Campeche (-91.0429, 18.08134), (ROM ASK213, ROM ASK214, ROM ASK215, ROM ASK216, ROM ASK217, ROM ASK218, ROM ASK219); **locality 55**: 18 Km S Xkanha, Campeche (-89.3516, 18.97769), (ROM 30676); **locality 56**: 22 Km S Candelaria, Campeche (-90.9572, 18.025751), (ROM ASK224, ROM ASK225, ROM ASK226, ROM ASK227, ROM ASK228, ROM ASK229, ROM ASK230, ROM ASK231); **locality 57**: 25 Km N Xpujil, Campeche (-89.4054, 18.7848), (ROM 96088= FN29879); **locality 58**: 27.5 Km S Constitución, 70 Km E Escarcega, Campeche (-90.142822,

18.635835), (ROM 29231, ROM 29232); **locality 59**: 44 Km N Hopelchen, Campeche (-89.816, 20.296), (ROM ASK070, ROM ASK001); **locality 60**: 44 Km S Constitución, 70 Km E Escarcega, Campeche, 102 m (-90.2405, 18.2773), (ROM95797= FN29588, ROM95798= FN29589, ROM95799= FN29590, ROM95800= FN29591, ROM95796= FN29587, ROM95801= FN29592, ROM95806= FN29597, ROM95807= FN29598); **locality 61**: 60 Km SE Dzibalchen, Campeche (-89.3746, 19.2488), (ROM 30670, ROM 30672, ROM 30673, ROM 30674, ROM 30675); **locality 62**: 7.5 Km W Of Escarcega, Campeche, 80 m (-90.8164, 18.60631), (ROM ASK1043, ROM ASK269, ROM ASK270, ROM ASK268, ROM ASK271, ROM ASK272, ROM ASK273, ROM ASK274, ROM ASK275); **locality 63**: 9.5 Km S Constitución, 70 Km E Escarcega, Campeche, 117 m (-90.1226, 18.5669), (ROM ASK2591, ROM ASK2592, ROM ASK2593, ROM ASK2594, ROM ASK2595, ROM ASK2596, ROM ASK2597, ROM ASK2611, ROM ASK2612, ROM ASK2613, ROM ASK2614, ROM ASK2615, ROM ASK2616); **locality 64**: Ruinas de Edzna, Campeche, 32 m (-90.2296, 19.5978), (ROM ASK384, ROM ASK385, ROM ASK386); **locality 65**: Kohunlich, Quintana Roo (-88.7881, 18.4347), (ROM 32689, ROM 32688); **locality 66**: 3.8 Km SW Ruinas Acalán, Tabasco (-91.491394, 17.82453), (ROM ASK155, ROM ASK157, ROM ASK156, ROM ASK158, ROM ASK159, ROM ASK160, ROM ASK161); **locality 67**: 6 Km S El Triunfo, Tabasco (-91.1721, 17.87132), (ROM ASK2532); **locality 68**: Cenote Seco, 2 Km E Chichen Itza, Yucatán (-88.5514, 20.68017), (ROM 96021= FN29812, ROM 96022= FN29813, ROM 96023= FN29814, ROM 96030= FN29821, ROM 96031= FN29822).

Handleyomys chapmani.— MEXICO: **locality 70**: 18 Km NW Teocelo, Teocelo, Veracruz, 1721 m (-97.045026, 19.442354), (ROM YHM234); **locality 71**: 300 M NW Cascadas de

Texolo, 1.5 Km SE Xico, Veracruz, 1136 m (-96.994342, 19.402083), (ROM YHM186, ROM YHM191, ROM YHM192, ROM YHM221, ROM YHM222, ROM YHM223); **locality 72**: Rio Chiflón, 9.7 Km NE junction Los Tules road to Zacualpan, Agua Blanca, Hidalgo, 1900 m (-98.363772, 20.389366), (FXG 1141); **locality 73**: 5 Km NE junction Nexpanateno (by road), Zacapoaxtla, Puebla, 1802 m (-97.5902, 19.8688), (CIByC 1712); **locality 74**: 1.5 Km S Puerto de la Soledad , Teotitlán, Oaxaca, 2600 m (-96.99324, 18.150825), (BYU 15303= EAA310, BYU 15304= EAA311); **locality 75**: 14.4 Km NE (road to Santa Flor) , Concepción Pápalo, Oaxaca, 2600 m (-96.8143, 17.8951), (CIBYC 1382= FXG943, CIBYC 1347= FXG944, CIBYC 1352= FXG949, CIBYC 1389= FXG950); **locality 76**: Santa Maria Yacochi, Santa María Tlahuitoltepec, Oaxaca, 2400 m (-96.0161, 17.1325), (CIBYC 106= DSR5701, CIBYC 107= DSR5763, CIBYC 109= DSR5765); **locality 77**: 11 Km SW (by road) La Esperanza, Ixtlán, Oaxaca, 2924 m (-96.5114, 17.5858), (CIBYC 103= DSR5800, CIBYC 105= DSR5827); **locality 78**: (Las Cañadas near the bridge) Xometla, Huatusco, Veracruz, 1340 m (-96.9878, 19.1858), (CIBYC 779= FXG618, CIBYC 780= FXG619, CIBYC 782= FXG621); **locality 79**: 1.2 Km Se Xochititla, Texhuacán, Veracruz, 1670 m (-97.1252, 18.7533), (CIByC 772= FXG578, CIByC 773= FXG579, CIByC 774= FXG580, CIByC 775= FXG581); **locality 80**: Matlalapa, Xico, Veracruz, 2070 m (-97.0814, 19.4796), (CIBYC 1495= RMV48, CIBYC 1497= RMV50, CIBYC 1499= RMV53, CIBYC 1493= RMV46, CIBYC 1494= RMV47, CIBYC 1496= RMV49, CIBYC 1498= RMV51, CIBYC 2177= RMV163, CIBYC 2182= RMV171); **locality 81**: Mesa de la Yerba, 3.4 Km SW exit to Mazatepec (Xalapa-Perote access road), Acajete, Veracruz, 2004 m (-96.7556, 19.5654), (CMC1490= RMV84, CMC1390= FXG872, CMC1353= FXG873, CMC1483= RMV74, CMC1484= RMV75, CMC1485= RMV76, CMC1486= RMV77, CMC1487= RMV78, CMC1488= RMV79, CMC1489= RMV81,

CMC1491= RMV85, CMC1492= RMV86); **locality 82**: Xico Viejo, Xico, Veracruz, 1756 m (-97.060188, 19.451053), (CIByC 1503= RMV64, CIByC= 1501 RMV56, CIByC= 1502 RMV63, CIByC 1504= RMV66); **locality 83**: Zacualpan, Veracruz, 1694 m (-98.3624, 20.4476), (MZFC 8304= HBR069, BYU HBR058); **locality 84**: 22 Km NE (by road) Metepec, Metepec, Hidalgo, 2210 m (-98.252633, 20.309453), (CIByC 1043= FXG823, CIByC 1044= FXG827, CIByC 1079= FXG824); **locality 85**: 26.5 Km NE (by road) Metepec, Hidalgo, 2210 m (-98.2376, 20.3032), (CIByC 1042= FXG804, CIByC 1080= FXG831, CIByC 1081= FXG832, CIByC 1082= FXG833); **locality 86**: 3 Km E (by road) Tlanchinole, Tlanchinol, Hidalgo, 1500 m (-98.65242, 21.00309), (BYU 15300= EAA272); **locality 87**: 4.7 Km NE (by road) Teziutlán, Puebla, 1750 m (-97.3366, 19.8497), (CMC1049= FXG834, CMC1052= FXG837, CMC1054= FXG839, CMC1085= FXG835, CMC1086= FXG836); **locality 88**: Tlatempa 2 Km NE (by road) Zacatlán, Puebla, 2062 m (-97.923295, 19.945596), (CIByC 1679= RMV92); **locality 89**: Rancho El Paraiso, 6 Km SW Huahuchinango, Huauchinango, Puebla, 2000 m (-98.0954, 20.1681), (BYU 15801= EAA643, BYU 15802= EAA644); **locality 90**: San Jose, El Cielo, Tamaulipas, 1329 m (-99.228667, 23.046083), (TCWC 59289= ICA75, TCWC 59291= ICA36, TCWC 59294= ICA69); **locality 91**: 3.5 Km N, 3 Km W Maguey del Oriente, El Naranjo, San Luis Potosi, 1600 m (-99.547634, 22.462516), (CIByC 739= FXG527, CIByC 740= FXG528, CIByC 741= FXG529); **locality 92**: Apulco River, 10 Km N Zacapoaxtla, La Gloria Falls, Puebla, 1500 m (-97.5902, 19.8688), (BYU 15803= EAA642); **locality 93**: Banderillas, 6 Km NW Xalapa, Xalapa, Veracruz, 1600 m (-96.954015, 19.588417), (ROM YHM237, ROM YHM238, ROM YHM239, ROM YHM240, ROM YHM241); **locality 94**: El Haya, old road to Coatepec Km 25 Botanic Garden Francisco Javier, Xalapa, Veracruz, 1235 m (-96.944167, 19.512444), (CIByC 1450= RMV01).

Handleyomys guerrerensis.— MEXICO: **locality 95**: 3.4 Km (by road) Carrizal, Leonardo Bravo, Guerrero, 2480 m (-99.8219, 17.6054), (CMC452= FXG462, CMC453-BYU20647= FXG463, CMC454= FXG464); locality 96: 0.7 Km E (by road) La Soledad, Candelaria Loxicha, Veracruz, 1500 m (-96.529054, 16.03774), (CMC943= FXG682); **locality 97**: 3 Km E El Tejocote, Malinaltepec, Guerrero, 2620 m (-98.4833, 17.2833), (CMC1652= FXG1034, CMC1653= FXG1035); **locality 98**: 4.8 Km S El Tejocote, Malinaltepec, Guerrero, 2455 m (-98.651117, 17.304867), (CMC1655= FXG1041, CMC1656= FXG1043, CMC1657= FXG1044, CMC1654= FXG1040); **locality 99**: Rio Molino, Miahuatlan, San Miguel Suchistepec, Oaxaca, 2353 m (-97.7107, 16.8777), (CMC1013= FXG690, CMC925= FXG691, CMC927= FXG734, CMC930= FXG737, CMC931= FXG738, CMC932= FXG739); **locality 100**: 6.1 Km Sw (by road) Omiltemi, Chilpancingo de Los Bravo, Guerrero, 2490 m (-99.724, 17.597), (ROM ASK729, CIByC 455= FXG412, CIByC 456= FXG423); **locality 107**: Carrizal, Leonardo Bravo, Guerrero, 2400 m (-99.724, 17.597), (ROM ASK750, ROM ASK895, ROM ASK897).

Handleyomys rhabdops.— GUATEMALA: **locality 101**: 2.8 Km S (by road) Yalambojoch on road to San Mateo Ixtatán, Sierra de los Cuchumatanes, Huehuetenango, 2200 m (-91.57038, 15.956625), (MVZ 223312, MVZ 223313, MVZ 223318, MVZ 223314, MVZ 223315); **locality 102**: Fuentes Georginas, Zunil, 2600 m (-91.480272, 14.7467), (MVZ 224809); **locality 103**: 2 Km N San Lorenzo, Sierra de las Minas, Zacapa, 1637 m (-90.667, 15.1), (ROM 99891 , ROM 99889, ROM 99888, ROM 99890). MEXICO: **locality 105**: 5 Km SE Rayon by road, Rayon, Chiapas, 1700 m (-92.988968, 17.187166), (ROM 97603= FN33079, ROM 97604= FN33080, ROM ASK665, ROM ASK653); **locality 106**: Reserva El Triunfo B, Angel Albino Corzo,

Chiapas, 2200 m (-92.82, 15.672), (MZFC 4507, MZFC 4508); **record 9**: Finca Helvetia, El Palmar, Quetzaltenango (-91.56126, 14.759777).

Handleyomys saturatior.— MEXICO: **locality 104**: 9 Km SE Rayon by road, Chiapas, 1800 m (-92.9529, 17.1434), (ROM ASK588); **locality 113**: Reserva El Triunfo, Angel Albino Corzo, Chiapas, 2200 m (-92.82, 15.672), (MZFC 541); **locality 114**: 2.5 Km N Mapastepec, Ejido Nicolas Bravo, Chiapas, 113 m (-92.913965, 15.494956), (ECOSCM 1377); **locality 115**: 2 Km SE La Antela, Lagos de Montebello, Chiapas, 1520 m (-91.711872, 16.115358), (ECOSCM478); **locality 116**: Lagos de Montebello, La Trinitaria, Chiapas, 1529 m (-91.6107, 16.1404), (ECOSCM 1228, ECOSCM 1229, ECOSCM 1231). EL SALVADOR: **locality 39**: Parque Nacional Montecristo, Bosque Nublado, Santa Ana, 1990 m (-89.3781, 14.40999), (ROM101382= F35560, ROM101383= F35561, ROM101409= F35587, ROM101410= F35588, ROM101455= F35633, ROM101537= F35715, ROM101538= F35716, ROM101842= F35717, ROM101381= F35559); **locality 108**: Los Planes, Parque Nacional Montecristo, Santa Ana, 2193 m (-89.107103, 14.314621), (ROM 101506, ROM 101450= F35684). HONDURAS: **locality 43**: Atlántida, 191 m (-87.460098, 15.745006), (TTU 84375); **locality 112**: Parque Nacional La Tigra, Francisco Morazán, 2039 m (-87.104588, 14.224617), (TTU 83742). GUATEMALA: **locality 110**: Finca Ixcansán, 10.3 Km E Yalambojoch by road to Rio Seco, Sierra de Los Cuchumatanes, Huehuetenango, 1368 m (-91.483456, 16.016653), (MVZ 223316, MVZ 223317); **locality 111**: 5 Km E Puruhla, Baja Verapaz, 1692 m (-90.1889, 15.24027), (ROM 31406, ROM 31460, ROM 31510, ROM 31511). NICARAGUA: **locality 121**: 3 Km SE Miraflor, Reserva Miraflor, Esteli, 1436 m (-86.232025, 13.241616), (ROM 112276, ROM 112259).

Handleyomys saturatior (Clade V*).— NICARAGUA: **locality 117**: Selva Negra-Trail, Selva Negra, Matagalpa, 1086 m (-85.453, 12.9477), (TTU 101644= TK 113513); **locality 118**: Selva Negra-Atajo Trail, Selva Negra, Matagalpa, 1099 m (-85.453, 12.9477), (TTU 105140, TTU 105174); **locality 119**: (-86.441272, 13.093189), (CURN JAGE438).

Handleyomys fuscatus.— Colombia: **locality 123**: Peñas Blancas, E slope of Western Andes, Páramo, Valle del Cauca, 2000 m (-76.716667, 3.45), (USNM 507267, USNM507268, USNM 507269); **locality 126**: Vereda Los Planes, Risarada Santuario (-76.0162178, 5.1028016); **record 13**: La Ceja, Antioquia (-75.43333 6.03333)

Handleyomys intectus.— Colombia: **locality 122**: 4 Km S El Retiro, Antioquia, (ICN 16093), **locality 124**: Las Ventanas, Valdivia, Antioquia, 2000 m (-75.45, 7.1833334), (FMNH 70333); **locality 125**: 7 Km E Páramo, NW slope of Central Andes, Valdivia, Antioquia, 1650 m (-75.255103, 5.706352), (FMNH 70298); **record 10**: Rio Negrito, 9 Km E Antioquia, Sonsón (-75.5663, 5.11667); **record 11**: Rio Negrito, 15 Km E Antioquia, Sonsón (-75.06542, 5.71181); **record 12**: Páramo, 7 Km E Antioquia, Sonsón (-75.05642, 5.74296).

Nephelomys devius.— COSTA RICA: **locality 127**: 12 Km N Potrero Cerrado by road, Rio Birris, Cartago (-83.6774, 9.75711), (ROM 97316= FAR103, ROM 97317= FAR104); **locality 128**: Monteverde, Quebrada Maquina, Puntarenas, (-84.8097, 10.30816), (BYU 15209 EAA34, BYU 15208= EAA35, BYU 15210= EAA77, ROM 97301= FAR82).

Nephelomys albigularis.— COSTA RICA: **locality 126**: 1.5 Km Ne Tarcoles, 16 Km S, 9 Km W Orotina, 1.5 Km NE Tárcoles, Puntaarenas, 2086 m (-84.6242, 9.79136), (LSUMZ 1829). PERU: **locality 141**: Batán on Sapalache-Carmen Trail, Piura department, 3400 m (-79.38, -5.0527), (LSUMZ 6189, LSUMZ 6190, LSUMZ 6191, LSUMZ 6193, LSUMZ 6223, LSUMZ 6226, LSUMZ 6228); **locality 142**: 5 Km NE Sapalache, Cerro Chinguela, Piura department, 2400 m (-79.4233, -5.104965), (LSUMZ 722).

Euryoryzomys macconelli.— ECUADOR: **locality 130**: Parque Nacional Yasuni, 38 Km S Pompeya Sur, Napo (-76.014404, -1.024421), (ROM106328= F40483).

Euryoryzomys russatus.— SURINAME: **locality 144**: Brownsberg Nature Park, Jeep Trail, Brokopondo (-55.16884, 5.017972), (ROM 114189= F41264).

Oecomys auyantepi.— SURINAME: **locality 144**: Brownsberg Nature Park, Jeep Trail, Brokopondo (-55.16884, 5.017972), (ROM 113975= F41050, ROM 114059= F41134, ROM 114146= F41221, ROM 114316= F41346).

Oecomys rutilus.— PERU: **locality 143**: Maynas, Loreto (-73.851674, -2.682456), (TTU 101025).

Hylaeamys megacephalus.— Guyana: **locality 131**: Baramita, Old World, Baramita, Barima-Waini (-60.48734, 7.398892), (ROM100908= F34906, ROM100976= F34974, ROM101072= F35070, ROM98723= FN31545); **locality 132**: Kwabanna, Barima-Waini (-59.123739,

7.710891), (ROM98737= FN31559, ROM98739= FN31561, ROM98751= FN31573, ROM98752= FN31574); **locality 133**: Santa Cruz, Barima-Waini, Barima-Waini (-59.237111, 7.675582), (ROM98827= FN31649); **locality 134**: Waikerebi, Barima-Waini (-59.77534, 8.305932), (ROM98868= FN31690, ROM98870= FN31692); **locality 135**: Mapenna Creek, about 6 Km from Corentyne River, East Berbice-Corentyne (-57.361679, 5.398641), (ROM100355= F34596, ROM100356= F34597); **locality 136**: 30 Km NE Surama, Potaro-Siparuni (-58.83728, 4.636655), (ROM98089= FN31091, ROM98091= FN31093, ROM98090= FN31092).

Hylaeamys Yunganus.— Guyana: **locality 132**: Kwabanna, Barima-Waini (-59.123739, 7.710891), (ROM98719= FN31541, ROM98730= FN31552, ROM98738= FN31560, ROM98747= FN31569); **locality 133**: Santa Cruz, Barima-Waini (-59.237111, 7.675582), (ROM98781= FN31603, ROM98797= FN31619, ROM98825= FN31647, ROM98826= FN31648).

Oryzomys couesi.— COSTA RICA: **locality 129**: 6Km N Esparaza, 6Km N Esparza, Puntaarenas, (-84.674, 10.153), (LSUMZ 1831). MEXICO: **locality 137**: Biological Station La Mancha, 7 Km Se Farollon Don Carlos, Veracruz (-96.3889, 19.60192), (LSUMZ 7654); **locality 138**: Chamula, 1.5 Km N Cruzton, Cerro Tezontehuitz, Chiapas, 2394 m (-92.575, 16.8222), (CIByC 2780); **locality 139**: 5.5 Km W Queseria, Colima (-103.6289, 19.38654), (ROM ASK 1915); **locality 140**: 3.6 Km NE Guichicovi (by road), Guichicovi, Oaxaca (-95.071735, 16.967939), (DSR 8442, DSR 8443).

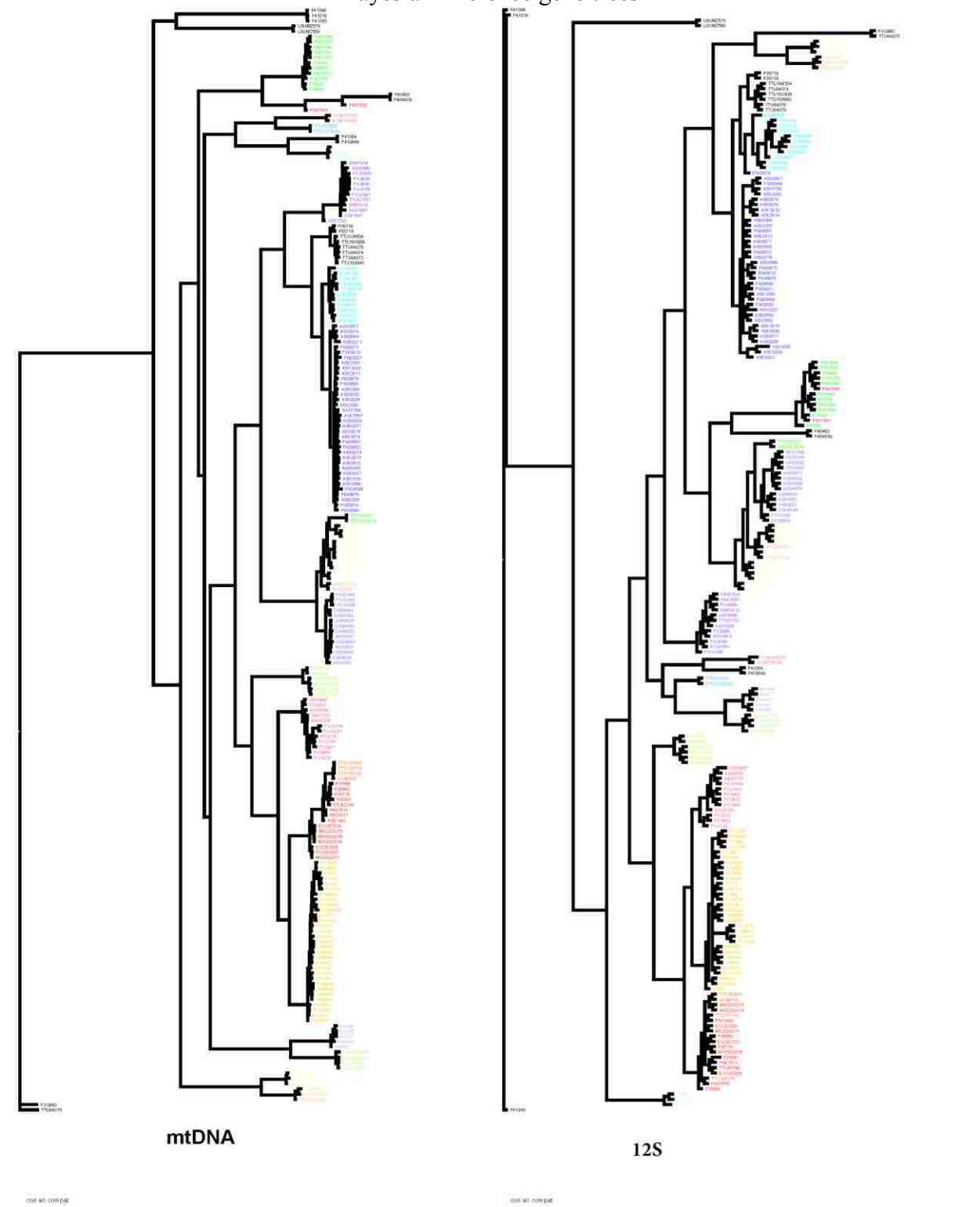
Melanomys caliginosus.— PANAMA: **locality 10**: Cana, 1200 m (-77.693939, 7.739569), (LSUMZ 568, LSUMZ 579).

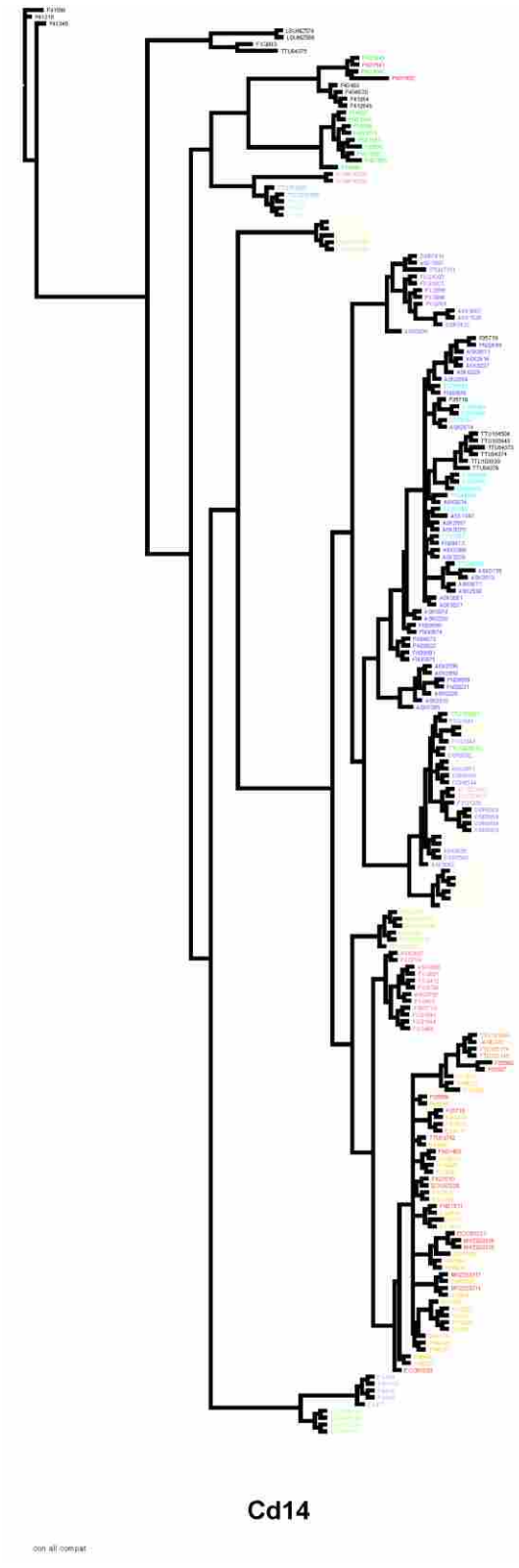
Neacomys paracou.— SURINAME: **locality 144**: Brownsberg Nature Park, Jeep Trail, Brokopondo (-55.16884, 5.017972), (ROM 114150= F41225, ROM 114023= F41098, ROM 114143= F41218, ROM 114315= F41345, ROM 114317= F41347).

Transandinomys talamancae.— PANAMA: **locality 10**: Cana, 1200 m (-77.693939, 7.739569), ROM 116306, ROM 116300, ROM 116256).

Appendix B

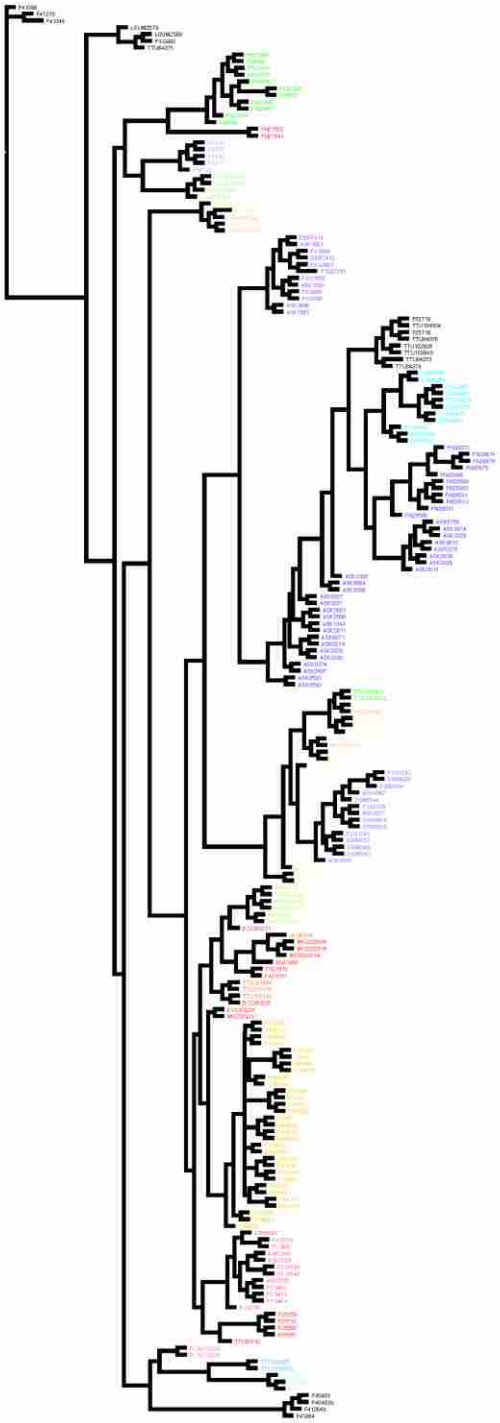
Supplementary Figures for Chapter 3
Bayesian inference gene trees



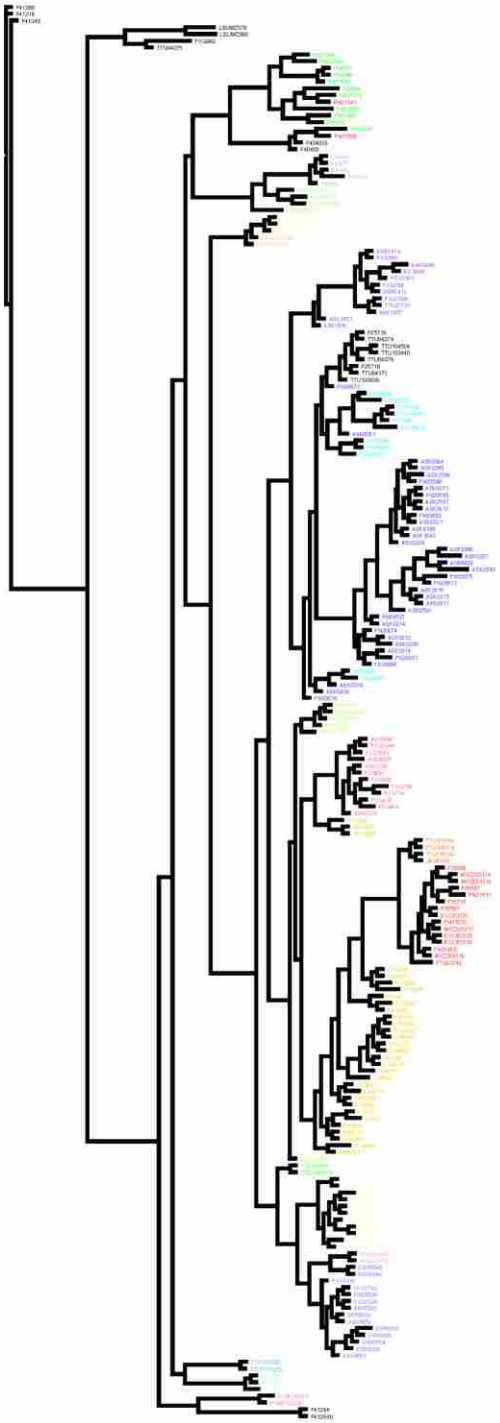


Cd14

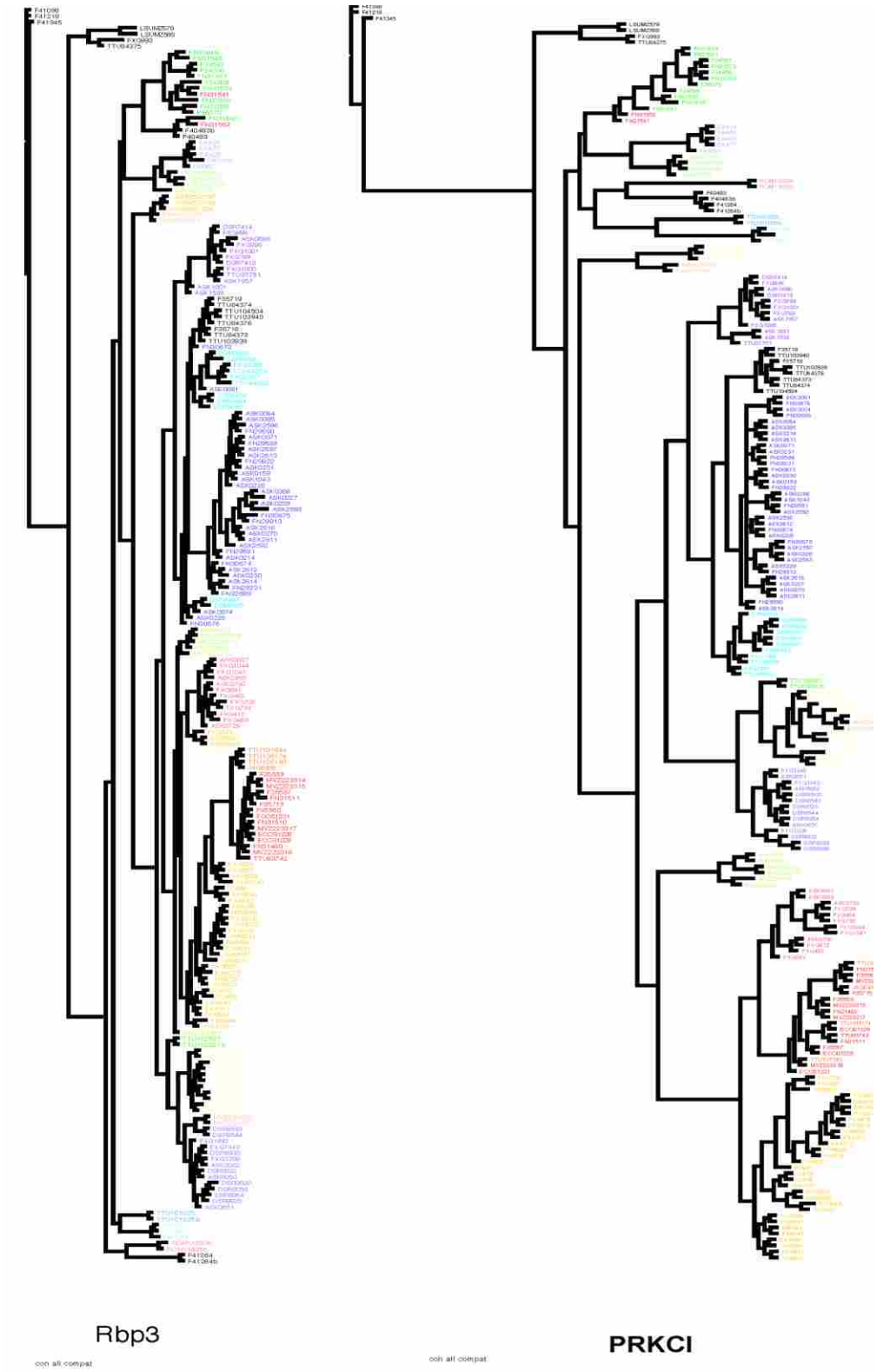
Fgb-I7

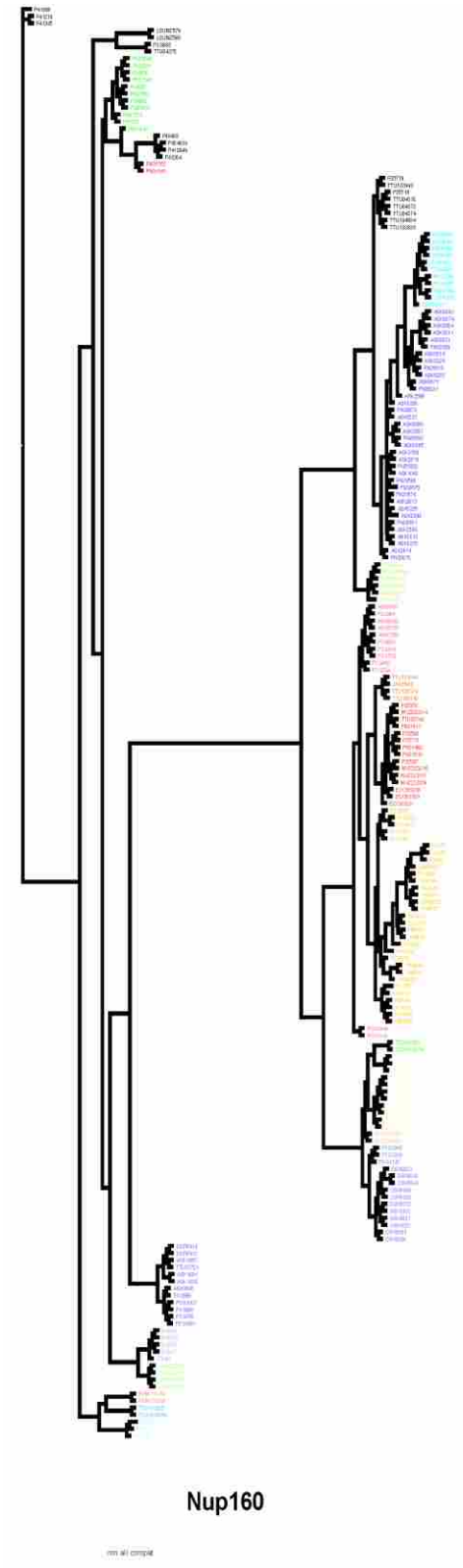


Fut4

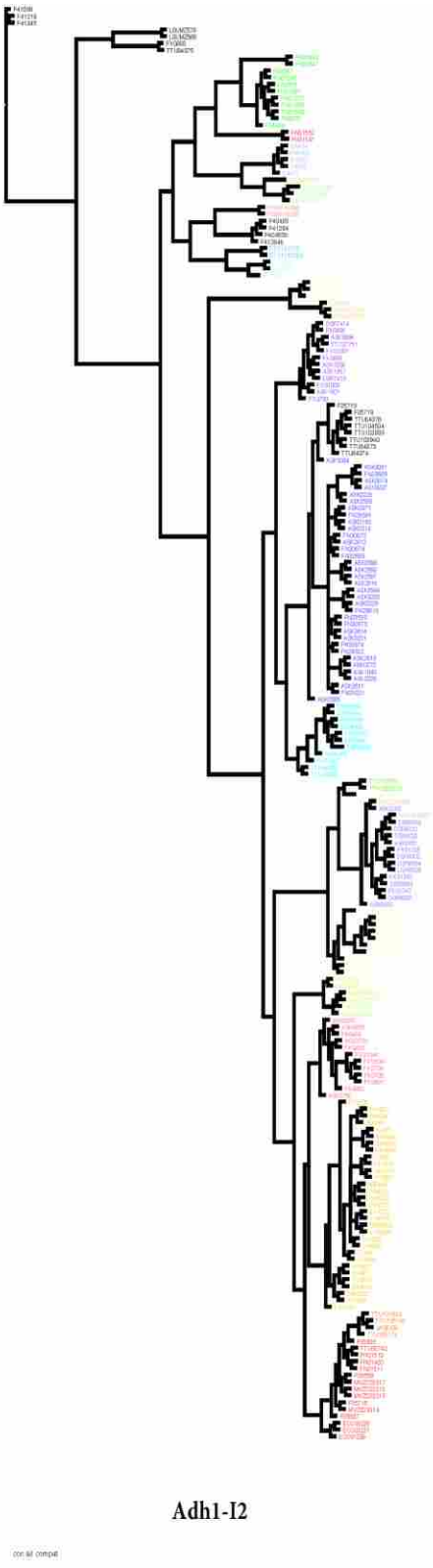


GdX


Rbp3
PRKCI



Nup160



Adh1-I2

Appendix C

PCR and Sequencing Primers

Gene	Description	Primer	Primer Sequence (Forward= F, Reverse= R)			Citation
CD14	Monocyte membrane glycoprotein	mCD14-6-F	AACTGACTCTTGAAAACTTCG	F	*	
CD14	Monocyte membrane glycoprotein	mCD14-4-R	TTACGCAGCGCTAAAACTTG		R	Liu et al. 2008
CD14	Monocyte membrane glycoprotein	mCD14-6-R	YAGTTYCTTGAGGCCRGWAT		R	*
Cytb	Cythocrome b	L14724	CGAAGCTTGATATGAAAAACCATCGTTG		F	Irwin 1991
Cytb	Cythocrome b	L14648	TGAATYTGAGGRGGCTTCTCAGTA		F	Irwin 1991
Cytb	Cythocrome b	L14841	AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA		F	Irwin 1991
Cytb	Cythocrome b	L15513	CTAGGAGACCCTGACAACTA		F	Irwin 1991
Cytb	Cythocrome b	F1	TGAGGACARATATCHTTYTGRGG		F	*
Cytb	Cythocrome b	H14742	GAAACWGGATCHAACAACCC		R	Irwin 1991
Cytb	Cythocrome b	H15149	TGAGGACAAATATCATTCTGAGGGGCTGCAGTTT		R	Irwin 1991
Cytb	Cythocrome b	MVZ04	GCAGCCCCTCAGAATGATATT		R	Smith and Patton 1993
Cytb	Cythocrome b	MVZ16	ATYAAACCAGARTGATAYTTCCTATTT		R	Smith and Patton 1993
Cytb	Cythocrome b	CB40R	CCACCACCAGCACCCAAAGC		R	*
D-loop	Mitochondrial D-loop region	Dloop-F1-H	CCTCAACCGTACATAAAACATTACAGT		F	*
D-loop	Mitochondrial D-loop region	CB40	CCACTAYCAGCACCCAAAGC		F	*
D-loop	Mitochondrial D-loop region	Dloop-R1-H	TGCTGGTTTCACGGAGGATG		R	*
D-loop	Mitochondrial D-loop region	RF1	GCCTTGACGGCTATGGTGAG		R	*
DXS254E	Housekeeping protein DXS254E	mGdx-1-F	TTGGTGTTTGCGTTGGCCGTAG		F	Liu et al. 2008
DXS254E	Housekeeping protein DXS254E	mGdx-3-F	GCTGCAGTGCTTCACTCTGG		F	Liu et al. 2008
DXS254E	Housekeeping protein DXS254E	mGDX-F1-H	AGGGTCCTGGARCAACTACA		F	*
DXS254E	Housekeeping protein DXS254E	mGdx-2-R	ATGAGCCAAACTGCGACATGAG		R	Liu et al. 2008
DXS254E	Housekeeping protein DXS254E	mGdx-4-R	CCAATGTTGTAATCTGACAG		R	Liu et al. 2008
DXS254E	Housekeeping protein DXS254E	mGDX-R1-H	CTRYTGGCATCTGCTACAYT		R	*
Fgb-I7	Betafibrinogen Intron 7	Bfib	CACAACGGCATGTTCTTCAGCAC		F	Wickliffe 2003
Fgb-I7	Betafibrinogen Intron 7	Fgb-F1-H	TCAATTGAAAGCATCCCAACTGG		F	*
Fgb-I7	Betafibrinogen Intron 7	Fgb-F2-H	TTYCCTTTCTTGCCACRGGG		F	*
Fgb-I7	Betafibrinogen Intron 7	B17	ACCCCAGTAGTATCTGCCGTTTGGAT		R	Wickliffe 2003
Fgb-I7	Betafibrinogen Intron 7	Fgb-R1-H	TGAGTAGTTGTCTGGCTTCAGA		R	*
Fgb-I7	Betafibrinogen Intron 7	Fgb-R2-H	CCCYGTGGCAAGAAAGGRAA		R	*
Fgb-I7	Betafibrinogen Intron 7	Fgb-R3-H	CCACCATCCACCACCATCTT		R	*

Fut4	α (1,3) fucosyltransferase	mFUT4-3-F	GTCCTACCGGACCGACTCGG	F	Liu et al. 2008
Fut4	α (1,3) fucosyltransferase	FUT4-F-H	CAGTRCCAGTGGTGTTAGGT	F	*
Fut4	α (1,3) fucosyltransferase	mFUT4-6-R	TGGCCTTATCGCTGGAACCAG	R	Liu et al. 2008
Fut4	α (1,3) fucosyltransferase	FUT4-R-H	ATGGATGAARGARCCACGGG	R	*
Nup160	Intron 15 of the Nucleoporin	Nup160-L3	CATTAAACTATGACCTTTTATATA	F	*
Nup160	Intron 15 of the Nucleoporin	Nup160-L1	GCAGTTTTGATGGAAACCACTTG	F	*
Nup160	Intron 15 of the Nucleoporin	Nup160-H3	AGTATATAAAAGGTCATAGTTTA	R	*
Nup160	Intron 15 of the Nucleoporin	Nup160-H2	GCAGTTTACTACAAATGTCTTCC	R	*
PRKCI	Protein kinase C, iota	PRKCI-F	AAACAGATCGCATTTATGCAAT	F	Matthee et al. 2004
PRKCI	Protein kinase C, iota	PRKCI-F1	TGTCAAGRGAAGTATTYGSRC	F	*
PRKCI	Protein kinase C, iota	PRKCI-R	TGTCTGTACCCAGTCAATATC	R	Matthee et al. 2004
PRKCI	Protein kinase C, iota	PRKCI-R1	GCCACTYACWRTCATGAAGC	R	*
Rbp3	Interphotoreceptor retinoid binding protein exon 1	IRBP-R-RC	CTTGTGTGGGGACTCCTGCA	F	*
Rbp3	Interphotoreceptor retinoid binding protein exon 1	IRBP-F2-H	TGTGCTGGTGGTCACATCTC	F	*
Rbp3	Interphotoreceptor retunoid binding protein exon 1	E2	AGCAGATGCGCAGAGCCATAGTGGT	F	*
Rbp3	Interphotoreceptor retinoid binding protein exon 1	IRBP-R2-H	ATTCTCAGCTTCTGGAGGTCC	R	*
Rbp3	Interphotoreceptor retinoid binding protein exon 1	IRBP-R1-H	GAGATGTGACCACCAGCACA	R	*
Rbp4	Interphotoreceptor retinoid binding protein	D2	TATCCCACATTGCCCGGCAGCA	R	*
Rbp6	Interphotoreceptor retinoid binding protein	IRBP-F1	GAGTCGTGAGATTCTGGGCA	F	*

*Designed for this study.

PCR Temperature Profiles

Gene	Primers	PCR Temperatures profile in Celsius						
Cytb	**L14724**		35 Cycles					
	MVZ16	94	94			72	72	
		04:00	01:00	tm=48		00:45	10:00	4
				01:00				∞
12S	**L1091**		37 Cycles					
	H1478	94	94			72	72	
		04:00	01:00	tm=65		00:45	10:00	4
				01:00				∞
D-loop	**CB40**		35 Cycles					
	RF1	94	94			72	72	
		04:00	01:00	54		00:45	10:00	4
				01:00				∞
			37 Cycles					
PRKCI	**PRKCI-F**	94	94			72	72	
	PRKCI-R	04:00	00:45	tm=54		00:45	10:00	4
				00:45				∞
			37 Cycles					
DXS254E	**mGdx-1-F**	94	94			72	72	
	mGdx-2-R	04:00	00:35	tm=54		00:35	10:00	4
				00:35				∞
Nup160	**Nup160-L2**		35 Cycles					
	Nup160-H2	94	94			72	72	
		04:00	00:45	tm=58		00:45	10:00	4
				01:00				∞
Fut4	**mFUT4-5-F**		37 Cycles					
	mFUT4-L2-R	94	94			72	72	
		04:00	00:45	tm=56		00:45	10:00	4
				00:45				∞
Cd14	**mCD14-5-F**		37 Cycles					
	mCD14-4-R	94	94			72	72	
		04:00	00:45	tm=56		00:45	10:00	4
				00:45				∞
Rbp3	**D2**		35 Cycles					

	E2	94	94				
		04:00	00:45		72	72	
				tm=62	00:45	10:00	
				00:45			4
							∞
Adh1-I2	**ADH1-F**			37 Cycles			
	ADH1-R	94	94				
		04:00	00:45		72	72	
				tm=58	00:45	10:00	
				00:45			4
							∞
Fgb-I7	**Bfib**			37 Cycles			
	B-17	94	94				
		04:00	00:45		72	72	
				tm=62	00:45	10:00	
				00:45			4
							∞

CHAPTER 4

Testing Biogeographic Hypothesis for a Mesoamerican Rodent Clade

Ana Laura Almendra and Duke S. Rogers

[Manuscript formatted for submission to Journal of Biogeography]

Original Article

Testing Biogeographic Hypothesis for a Mesoamerican Rodent Clade

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Aim

To develop and test biogeographic hypotheses regarding the evolutionary history of Clade B of Weksler *et al.* (2006) (Tribe Oryzomyini, Subfamily Sigmondontinae), that includes *Euryoryzomys, Handleyomys*, *Hylaeamys*, *Nephelomys, Oecomys* and *Transandinomys*. This group balanced occurrence in North America (NA) and South America (SA) makes Clade B ideal to test hypotheses about the colonization of Mesoamerica, due to the lack of a taxon-wide applicable biogeographic scenario for the colonization of SA by the ancestors of this and other groups within Sigmodontinae, a subfamily whose North American ancestry is well sustained.

Methods

Using a DNA sequence dataset of three mitochondrial and eight nuclear loci, we developed an empirical biogeographic hypothesis for the evolution of these groups with the continuous-time Bayesian analysis (CTMC) of discrete geographic states in BEAST. The resulting hypothesis was tested against two models of ancestral states combinations. One represented a strictly South American origin and diversification before 3.5 Ma, and one that depicted the vicariance of NA and SA lineages at the root (a polyphyletic group). First, we calculated the global likelihood and the dispersals/extinctions rate for each hypothesis based on

the Dispersal, Extinction and Cladogenesis ML model in Lagrange. Second, the probability of the root geographic distribution postulated under each hypothesis was estimated with the Bayesian Binary MCMC method (BBM) in RASP (Reconstruct Ancestral State in Phylogenies). Additionally, the ancestral geographic extent of *H. alfaroi*, *H. chapmani* and the *H. melanotis* species groups were approximated with the continuous-landscape ML method in Phylomapper. Finally, current climate conditions ENMs for these species were projected on environmental reconstructions of the LIG (120,000-140,000 yr BP) and the LGM (21,000 yr BP) to infer the potential mechanisms involved in their diversification process.

Results

The discrete biogeographic analysis assigned the greatest probability for the ancestral area to the Northern Andes, and placed the most recent common ancestor (MRCA) of Clade B ~5.5 Ma. Next, the long distance colonization of the Maya block by the ancestor of the *H. alfaroi*, *H. chapmani* and *H. melanotis* groups occurred; followed closely by dispersal of the ancestor of *Transandinomys*, *Oecomys, Hylaeamys* and *Euryoryzomys* into the cis-Andes while *Handleyomys* and *Nephelomys* inherited the ancestral range. However, the hypothesis that depicted the vicariance of the *H. alfaroi*, *H. chapmani* and *H. melanotis* groups in the North American plate at the root of the phylogeny, constantly returned better scores in the comparisons. In contrast, a BBM analysis to test the assumption that the composite root distribution (maximum of five areas) could denote a widespread ancestor had the lowest probability. Within NA, the *H. alfaroi*, *H. chapmani* and *H. melanotis* species groups' ancestral ranges were centred along Sierra Madre del Sur, Sierra Madre de Oaxaca and the Sierra Madre de Chiapas. These regions also displayed zones of environmental stability for the three groups through the Pleistocene. Overall, the areas of suitable habitat for eight of 11 clades during interglacial

periods were more constricted and placed in more southern latitudes than their current geographic distribution. Finally, the simultaneous diversification of *H. melanotis* and *H. rhabdops* (2.0-2.3 Ma) and of Clade III and *H. alfaroi*+Clade I (0.8-1.0 Ma) was well supported.

Main conclusions

Although the estimated age for several inter-generic Sigmodontinae clades in SA are like Clade B (~5.5. Ma) recovered within Pliocene boundaries, the timing of the presumptive reintroductions to NA are, on average, 2.0 Myr younger and are part of the Great American Biotic Interchange (GABI) ~3.5 Ma. The more recent migration from SA is supported by the final uplift of the Northern Andes during the early the Pliocene, which could have prevented migrations from cis-Andes areas. Nonetheless, the purported shallowly flooded basins of the Chorotega Block and the Atrato region may have allowed direct entrance of North American lineages to the northern Andes as early as 6.0 Ma. This value predates the estimated time of the range uplift, which instead may have driven the separation of *Nephelomys* in the Central Andes, an area where ancestral splits have been proposed for several mammalian groups, including the proposed origin of other Sigmodontines. The particular environments preferred by *Handleyomys* and *Nephelomys* are unlike most other members of the Subfamily and could explain their conserved pathway into SA. Indeed, Pleistocene projections of the ecological niche models for eight of 11 species level clades within the Mesoamerican *Handleyomys* revealed extended areas of niche suitability during the glacial periods; contrary to the general expectations for tropically adapted taxa. The potential that the Tribe Oryzomyini (that includes Clade B and a number of other SA clades) had begun differentiation in NA in parallel with the process of the colonization of SA by other Sigmodontine tribes has often been suggested. This is because the earliest fossil records of Oryzomyini, its largest tribe, are recorded in SA until the late Pliocene (~3.0 Ma)

despite accounts of other specious rich tribes (Akodontini and Phyllotini) during the early Pliocene (~4-4.2 Ma). On the other hand, the (Oryzomyalia) fossil records in NA are often allocated to Oryzomyini, or considered to be its close relatives. Therefore, Clade B may denote a continuing lineage from the period of Oryzomyini diversification in North America, also supported by the fact that no other lineage of Oryzomyalia displays the amount of endemic lineages in Mesoamerica contained in the *H. alfaroi*, *H. chapmani* and *H. melanotis* species groups, that biogeographically resemble more closely to the Tylomyinae and several Mesoamerican endemic groups of Neotominae, than other Sigmodontinae.

Keywords

Sigmodontinae, Oryzomyini, *Handleyomys*, Mesoamerica, Biogeography, ENM, Pliocene, Pleistocene.

Introduction

The present day geographic distributions of most suprageneric and intergeneric taxa across the American continents are presumed to have been mostly shaped during the last 9 Myr, in response to the combined effects of the plate tectonics leading to the formation of the Panamanian Land Bridge (PLB) 4-3.5 Ma (Coates *et al.*, 2004). In turn, this resulted in the over land migration between North and South America referred to as the Great American Biotic Interchange (GABI) (Woodburne, 2010). In addition, the distributions of some taxa were transformed by the decrease in temperature caused by the altered ocean circulations after the closure of the Central America seaway (Martin & Byron, 1957; Lessios, 2008; Molnar, 2008; Leigh *et al.*, 2014); that continued through the Pleistocene (Hewitt, 1996; Lyons, 2003; Hooghiemstra, 2006). A remarkable example of the effect of these events is observed in the subfamily Sigmodontinae, one of the most complex groups of New World mammals in terms of habitat and morphological diversity and corresponding taxonomy complexity. The group currently includes 82 genera and approximately 400 species (Musser & Carleton, 2005; Salazar-Bravo *et al.*, 2013). The geographic origin of this group has long been controversial. As a consequence, studies have built on wide-ranging molecular data sets along conventional lines of evidence; providing combined support for NA, at approximately 12 Ma, as the likely geographic origin of the common ancestor from which *Sigmodon* and *Rheomys* split from the Oryzomyalia. Furthermore, there is increased support for the subfamily Tylominae as the sister of Sigmodontinae, and Neotominae, in turn as their closest group, both with North American distributions (Rinehart *et al.*, 2005; Erickson *et al.*, 2011; Schenk *et al.*, 2013; Leite *et al.*, 2014). On the other hand, during the estimated ~1.0 Myr Oryzomyalia diversification into at least eight supra-generic lineages (or tribes; Steppan *et al.*, 2004) approximately ~7.5 Ma (Engel *et al.*,

1998; Schenk *et al.*, 2013; Leite *et al.*, 2014), there was a radical inter-continental shift in the geographic distribution of these lineages such that at present, only two (*Sigmodon–Rheomys* and the tribe Oryzomyini) of ten lineages occur in NA. On the other hand, at least one species from the other eight commonly recognized tribes (Smith & Patton, 1999; Musser & Carleton, 2005), except perhaps Ichtyominy (pending resolution of its paraphyletic status; Salazar-Bravo *et al.*, 2013), is accounted in South America (SA). However, the remarkable taxonomic and ecological asymmetry of Oryzomyini (Fabre *et al.*, 2012), accompanied by the northern distribution of several of its representatives, prompted the suggestion that at least Oryzomyini would have entered SA partially diversified (Marshall, 1979; Engel *et al.*, 1998). This hypothesis is supported by the North American Sigmodontinae fossils regarded as the potential migrants to SA from which current tribes originated that appear beginning in the Hemphillian (10.3-4.9 Ma; ~6.7 Ma; †*Antecalomys* and †*Repomys*) in US and northern Mexico along with †*Prosigmodon* (Sigmodontini) (Baskin, 1979; Lindsay *et al.*, 1984; Schultz, 1990). Likewise, most of the NA Sigmodontinae fossil from the Blancan (4.6 Ma), include *Sigmodon,* along †*Bensonomys*, †*Jacobsomys*, †*Symmetrodontomys* (Lindsay & Jacobs, 1985; Czaplewski, 1987; Carranza-Castañeda & Walton, 1992; Pajak III *et al.*, 1996) and the new assemblage of †*Prosigmodon* and *Oryzomys* sp. (Oryzomyini) (Repenning & May, 1986); from which *Sigmodon* and *Oryzomys* survive today. However, the phylogenetic associations of these fossils remain subject of debate (see Pardiñas *et al.*, 2014) and, unfortunately, the SA fossil record for Sigmodontinae is equally scarce. For example, the oldest reference is for †*Auliscomys formosus*, from the upper Montehermosan age (5.0-4.0 Ma) (A. L. Cione & Tonni, 2005; Tomassini & Montalvo, 2013); currently regarded as an extant genus of Phyllotini. However, other age estimations for this fossil indicated a late Pliocene provenance (Pardinas & Tonni, 1998; Deschamps *et al.*, 2012;

Tomassini *et al.*, 2013). In addition, previous taxonomic arrangements of this tribe have been polyphyletic (Salazar-Bravo *et al.*, 2013). The remaining fossil records are placed in the late Pliocene and the Quaternary, and are assignable to the tribes Akodontini or Oryzomyini (†*Agathaeromys, Lundomys*, †*Carletonomys,* †*Megaoryzomys* and †*Noronhomys* (Marshall, 1979; Pardiñas & Teta, 2011).

The fact that the tribe Oryzomyini has a wide distribution in NA and SA makes it an ideal candidate for testing biogeographic hypothesis for Sigmodontinae. Latest taxonomic summaries for the tribe Oryzomyini support the existence of four main inter-generic lineages referred to as "Clades A to D" (Weksler *et al.*, 2006). Individually, each of these clades comprises representatives from both North and South America, although most of North American Oryzomyines belong to Clade B and Clade D. Recently, a biogeographic pattern estimated for Clade D of the aforementioned clades (Martínez *et al.*, 2012), set its origin during the late Pliocene (3.5 Ma) in southern SA, connecting the effect of habitat constraints with the timing and direction of the separation.

Clade B comprise the genus *Handleyomys* sensu lato (Weksler, 2006) and includes nine species (see Chapter 3), found in Mexico, Central America and northern South America. Its purportedly closest relatives are *Transandinomys* and *Nephelomys*, with Andean (west slopes to high elevations) and Central American (CA) distributions respectively, *Hylaeamys* and *Euryoryzomys*, strictly South American, and the more widespread genus *Oecomys,* found widely in SA and as far north as the Chorotega block in CA (Fig. 1). A recently developed molecular phylogeny for this group suggested that *Handleyomys* comprises four major lineages with levels of genetic divergence equivalent to other genera of Oryzomyine rodents (cytochrome *b*–*Cytb*

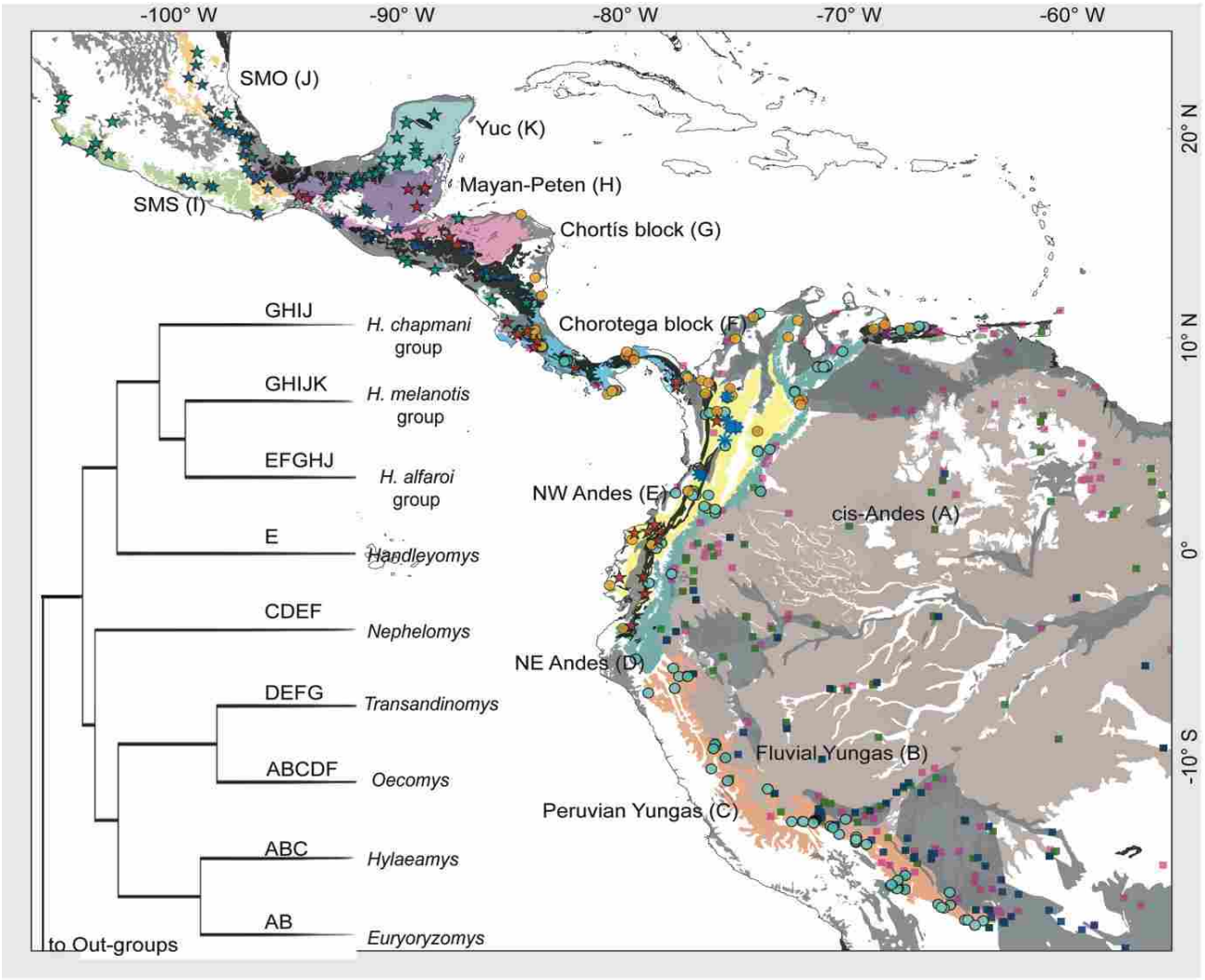

Figure 4. 1. Map showing geo-referenced localities for taxa included in Clade B (Weksler et al. 2006): H. alfaroi (red stars), *H. chapmani* (blue stars), and *H. melanotis* (green stars) groups, *H. fuscatus-H. intectus* (light blue asterisks), *Euryoryzomys* (blue squares), *Hylaeamys* (bright green squares) *Nephelomys* (cyan circles), *Oecomys* (pink squares) and *Transandinomys* (orange circles). The discrete geographic regions that define the distribution limits (letters A to K) of each of these 9 taxa are specified above their corresponding branches in the molecular phylogeny for Clade B (see Almendra et al. 2014). Dark grey areas represent geological features from the late Miocene and the Pliocene (12–2.58 Ma); and light grey areas denote a Quaternary age (2.58–0.1 Ma).

~16%). These lineages are: *Handleyomys* sensu stricto=*H. intectus* and *H. fuscatus* (Voss *et al.*, 2002) the *H. alfaroi* complex; the *H. chapmani* species group (*H. chapmani*, *H. guerrerensis*,*H. saturatior* and *H. rhabdops*); and the *H. melanotis* species group (*H. melanotis*, and the *H. rostratus* complex) (Chapter 3). *Handleyomys* was consistently recovered as monophyletic and sister to the rest of Clade B genera. The most recent common ancestor (MRCA) of this clade and *Handleyomys* was estimated at ~5.7 Ma.

Using this recently developed time calibrated phylogeny for members of Clade B; we generate an empirical biogeographic hypothesis for this clade by reconstructing ancestral ranges and a series of hypothesis tests depicting alternative locations. In addition, we test a Pleistocene diversification hypothesis for *H. alfaroi*, *H. chapmani* and *H. melanotis* groups (Mesoamerican *Handleyomys*) based on simulation of alternative paleodistribution models. Finally, we apply an objective Bayesian method to estimate the probability of the simultaneous divergence of co-distributed taxa across proposed barriers for their dispersal.

Material and methods

Taxa assignments and geographic ranges

DNA sequence data from three mitochondrial and eight nuclear loci from Almendra et al. (see Chapter 3) was obtained for 198 individuals representing Clade B taxa; *Handleyomys* sensu lato (Weksler, 2006) and closely related genera *Euryoryzomys*, *Hylaeamys, Oecomys, Nephelomys* and *Transandinomys* (Appendix S1). The geographic limits for the 13 species level clades comprised in *Handleyomys* were demarcated based on confirmed collecting localities. For the remaining taxa, we followed Musser & Carleton (2005) and Reid (2009), to define countries where the occurrence of these genera has been formally documented. For the selected countries and per species, we downloaded georeferenced localities from the Global Biodiversity Information Facility (GBIF), that were displayed on a simplified physiographic map of Mexico,

Central America and South America, to visually delimit the occurrence of each clade in each discrete region (Fig. 1).

Pliocene biogeography

The discrete areas specified in Fig. 1 were assigned as location characters to each terminal taxon to estimate discrete ancestral geographic areas under a continuous-time Bayesian analysis (CTMC) in BEAST 1.8 (Drummond *et al.*, 2012) for 500 million generations. Partition specific models of nucleotide substitution were assumed uncorrelated with branch independent rates (Drummond *et al.*, 2006). Individual locus topologies were linked with a birth-death tree prior (Gernhard, 2008). Ancestral areas for nodes Posterior Probabilities (pP) above 0.80 were annotated on a maximum clade credibility tree with TreeAnnotator v1.8 (available from http://beast.bio.ed.ac.uk), edited in TreeGraph v2.0.54 (Stover & Muller, 2010). In addition, the most probable location for the centre of the ancestral range of the *H. alfaroi*, *H. chapmani* and *H. melanotis* species groups and of each group, was approximated in the continuous land space with the ML statistical model implemented in Phylomapper (Lemmon & Lemmon, 2008), assigning the collection localities geographic coordinates as characters (Appendix S1). For this analysis, we used the maximum clade credibility tree estimated by BEAST (Fig. 2). *Oecomys*, *Hylaeamys*, *Euryoryzomys*, *Transandinomys* and *Nephelomys* were transferred to the out-groups.

Alternative biogeographic hypotheses

The geographic diffusion process implied by the BEAST analysis (H_0) was visualized in space-time using SPREAD (Bielejec *et al.*, 2011), by assigning a geographic reference to denote each discrete area. To compare this hypothesis against alternative scenarios for the diversification of *Euryoryzomys*, *Hylaeamys*, *Handleyomys Transandinomys*, *Oecomys* and *Nephelomys* (H_1 and H_2; Table 1), we calculated the dispersal/extinction rate and global likelihood of each hypothesis with the Dispersal, Local Extinction and Cladogenesis (DEC) (Ree

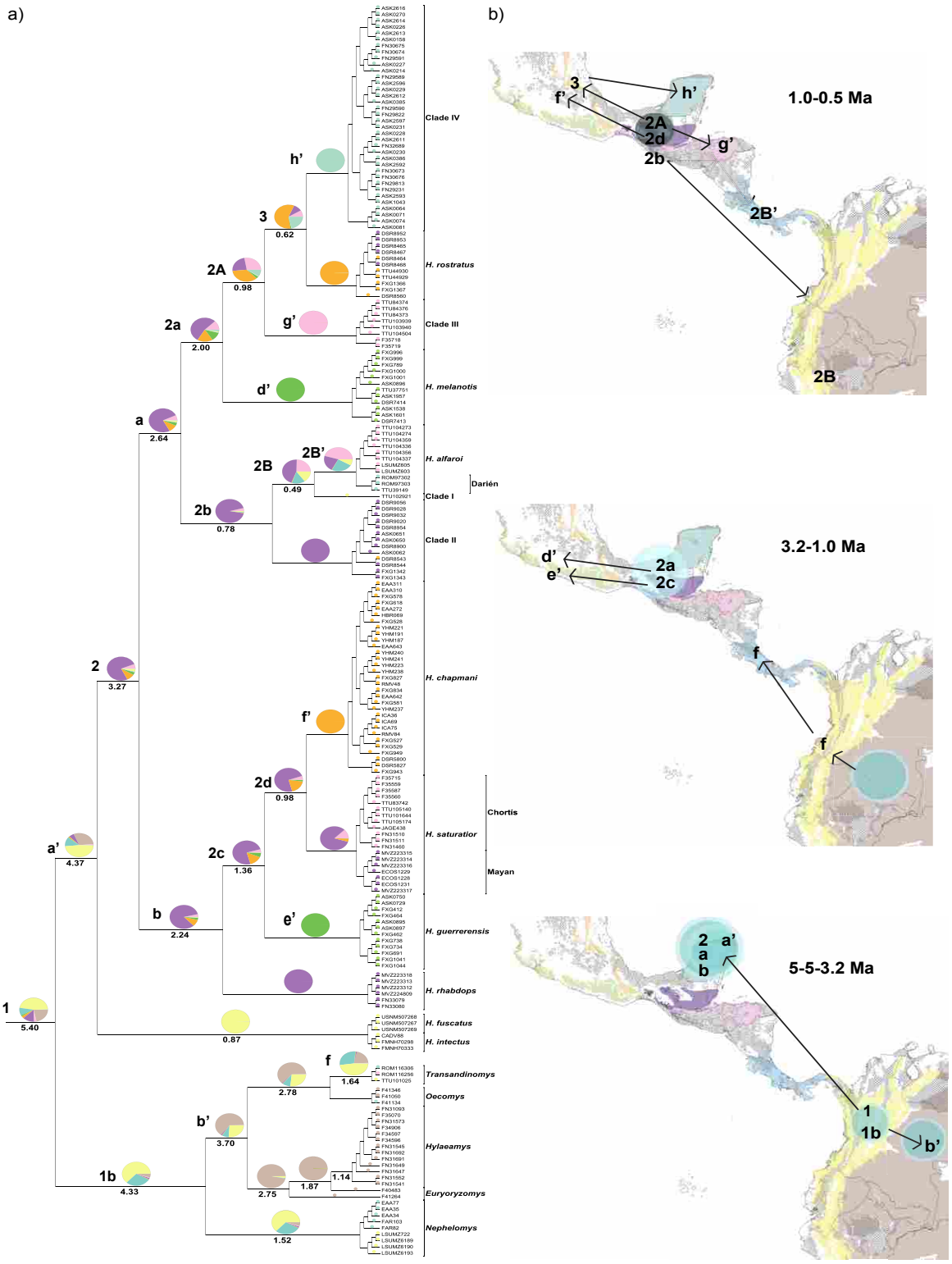

Figure 4. 2. a) Bayesian coalescent estimation of discrete ancestral areas with BEAST for taxa included in this study. Pie charts on the nodes represent the posterior probabilities of each discrete area as the ancestral state. b) Inferred continuous time diffusion process, adapted from the graphic output produced by SPREAD. Alpha and numerical codes to the upper right of each node reference its location on the maps.

& Smith, 2008) model for maximum likelihood (ML) estimation of competing hypothesis in Lagrange (Ree & Smith, 2007). In addition, the relative probability of different states combinations at the root node was estimated with the Bayesian binary MCMC (BBM) analysis in RASP (Reconstruct Ancestral State on Phylogenies) (Yan et al., 2011). Ten MCMC chains were run for 100000 generations sampling every 100 generations. For both these analyses, the species tree estimated by Almendra et al. (2014) was reduced to include a single terminal per clade (Fig. 1). The biogeographic settings of these clades covered two time periods, the Pliocene before the formation of the PLB (t_1= 5.5-3.5 Ma), and the Pliocene–Lower Pleistocene, after the PLB formation (t_2 = 3.5-2.0 Ma) (Table 1).

Locating Pleistocene refuges for *Handleyomys*

Ecological Niche Models (ENMs) were estimated through 20 fold cross-validated replicates in MAXENT 3.3.3 (Phillips & Dudik, 2008) under current climate conditions defined by 15 uncorrelated climatic variables from the WORLDCLIM database (1 km2 resolution) (Hijmans et al., 2005) using the 90 m resolution Digital Elevation Model (DEM) and the Compound Topographic Index (CTI) (Jarvis et al., 2008). Current ENMs were projected on climate reconstructions of the Community Climate System Model (CCSM3) for the Last Glacial. Maximum (LGM; 21,000-20,000 yr bp) (Collins et al., 2006) and for the Last Interglacial (LIG; 120,000-140,000 yr bp) (Otto-Bliesner, 2006). Each set of climatic variables was resampled to 90 m using bilinear interpolation in ArcMap 10.1 (ESRI, 2011) to standardize pixel resolution among grids. To quantify differences in the ENMs from different time periods, the amount of range overlap (Overlap Index; OI) was calculated with ENMTools 1.3 (Warren et al., 2010). Likewise, areas of environmental stability for each of the three species groups was inferred by calculating a suitability sum raster of the ENMs from the three time periods and all the species

Table 4. 1. Empirical (H_0) and alternative biogeographic hypothesis from the literature for the colonization of Mesoamerica by members of Clade B (H_1 and H_2; Fig. 4), and the predicted results from the DEC (Dispersal, Local extinction and Cladogenesis) and Bayesian Binary MCMC (BBM) analyses if each given hypothesis is supported.

Hypothesis	**Predictions**
H_0. The ancestral range of Clade B was situated in the N Andes of SA ~5.7-5.8 Ma. Colonization of CA and NA occurred before the final closure of the PLB ~4.4 Ma.	-DEC results will support a long-range dispersal from the N Andes (D, E) to the Maya-Peten (H), and a range expansion from the N Andes to the cis-Andes (A, B) during the early Pliocene (t_1= 5.5-3.2 Ma). Followed by a range expansion from cis-Andes into CA (F, G) and a stepping stone model among areas in NA (G, H, I, J) (t_2= 3.2-1.5 Ma). -The assumption that the ancestor of Clade B was restricted to the N Andes (D, E) will have the largest probability with the BBM method.
H_1. The ancestral range of Clade B was situated in SA ~5.7-5.8 Ma. Colonization of CA and NA occurred after the final closure of the PLB ~3.5 Ma.	-DEC results will support a stepping stone model among areas in SA during the early Pliocene (t_1= 5.5-3.2 Ma). Followed by range expansions from the SA to the N Andes and lower CA (F), and stepping stone dispersals to NA (t_2= 3.2-1.5 Ma). -The assumption that the ancestor of Clade B had a wide distribution in SA (A, B, C, D, E) will have the largest probability with the BBM method.
H_2. The ancestral range of Clade B included allopatric areas of SA, and CA–NA (a polyphyletic group).	-DEC results will support stepping stone model among the Andes (C, D, E) and lower CA (F), and between northern CA (G) and NA (H), during the early Pliocene (t_1= 5.5-3.2 Ma). Followed by a stepping stone model of range expansion (t_2= 3.2-1.5 Ma). -The assumption that the ancestor of Clade B had an allopatric distribution in NA (G, H or J), and in lower CA–northern SA (C, D, E and F), will have the largest probability with the BBM method.

lineages in each group. Areas predicted by half or more models were assumed to have persisted (e.g. ecologically stable) during the Pleistocene.

Evolutionary dynamics during the Pleistocene

To assess the correspondence between inferred stability areas and the detected potential barriers for dispersal, we ran 100 replicates for two types of the Range Breaking Tests (RBTs). (Glor & Warren, 2011) in ENMtools 1.3 (Warren *et al.*, 2010). The linear RBT determines if the disruption in the ENMs of two lineages is consistent with the existence of a major geological feature (Central Valleys of Oaxaca, Isthmus of Tehuantepec and Motagua–Polochic system). Second, the blob RBT examines whether or not the disruption between the ENMs of two sister groups could be caused by an irregular cline of suboptimal habitat for both species (Centla Marshes). In addition, we tested the hypothesis of simultaneous divergence across these barriers for *H. melanotis* and *H. guerrensis* (i.e. across the Isthmus of Tehuantepec) and for species-level clades within the *H. alfaroi* complex (through the Motagua–Polochic fault system). These tests were carried out with the hierarchical approximate Bayesian computation (HABC) coalescent model implemented in MTML-msBayes (Huang *et al.*, 2011). The equivalence between divergence times hyper-parameters (mean=E(t); variance=Var(t) and dispersion index of t = omega; Ω) from the posterior (empirical) sample and a uniform prior distribution of hyper-parameters (all possible values for Ω) determines the empirical number of divergence times (Wakeley' s (Ψ) statistics), which equals Ψ=1 when divergence occurred simultaneously (Ω=0.0, cut-off < 0.02; Lawson, 2010).

Results

Geographic Distributions

Nine discrete areas were found to delineate the geographic extent of the nine groups we evaluated (Fig. 1): The cis-Andes territories (cis-Andes) defined the distribution of

Euryoryzomys and *Hylaeamys*; the West Northern Andes (NW Andes) comprised the distribution of *Handleyomys* sensu stricto; the Chorotega block, represented by *H. alfaroi*; the Chortís block, where only *Transandinomys* occurs; the Mayan and Petén regions (Maya block), which was exclusive for *H. alfaroi*, the *H. chapmani* group and *H. melanotis* group; the Sierra Madre Oriental (SMO) and the Oaxacan highlands (OH), where only the *H. chapmani* group was distributed; the Sierra Madre del Sur (SMS) and the western portion of the Trans-Mexican Volcanic Belt (TMVB) (W NA; western North America), where only the *H. chapmani* and *H. melanotis* groups are found; and the Yucatán peninsula (Yuc), which was exclusive for the *melanotis* group (Fig. 1). In addition, the North-eastern Andes (NE Andes), the Peruvian Yungas, and the Bolivian Yungas (Yungas), each held a unique combination of *Hylaeamys, Euryoryzomys*, *Oecomys*, *Transandinomys* and *Nephelomys*, which were included for hypothesis tests.

Empirical Biogeographic Hypothesis

The continuous-time Bayesian biogeographic analysis estimated the time of the most recent common ancestor for Clade B (tMRCA) approximately ~5.4 Ma, and placed the largest posterior probability (pP) for the location of the MRCA in the NW Andes (pP = 0.47), followed by the Maya block (pP = 0.23), and then the Chorotega block (pP = 0.16; Fig. 2). The split involving the separation of *Handleyomys* sensu stricto from *H. alfaroi*, and the *H. chapmani* and *H. melanotis* groups was ~4.4 Ma. The ancestral area was estimated in the Maya block (pP = 0. 76), and of *Nephelomys* from the MRCA of *Transandinomys–Oecomys* and *Euryoryzomys–Hylaeamys*; that migrated to the cis-Andes (~4.3 Ma; pP = 0.64; Fig. 2), followed by a recolonizing event by *Transandinomys* to the N Andes (pP = 0.62) at the beginning of the Pleistocene (~2.7 Ma) and from there to the Chorotega and Chortís blocks (pP = 0.47). After the colonization of the Maya block, results from BEAST suggested the in-situ divergence of the

alfaroi–melanotis lineage and the *chapmani* group, followed by colonization of the SMO and the SMS ~0.6 Myr. Based on the continuous landscape analysis in Phylomapper, the MRCA of these three groups was placed in the Chimalapas–Zoque Forests of the Sierra Madre de Chiapas (~3.3 Ma; Fig. 3). The in situ divergence event involved the spread of the *chapmani* group to the Chimalapa Mountains, and then to the Sierra Madre de Oaxaca, the estimated ancestral range for the MRCA of *H. chapmani* (SMO-OH), *H. saturatior* (Maya block) and *H. guerrerensis* (SMS) lineages. Similarly, the ancestral area for the *H. melanotis* group was placed east of the Sierra Madre de Oaxaca. The ancestral range of *H. alfaroi* was estimated near the El Salvador volcanic front (Fig. 3). Later events included the colonization of the Yucatán peninsula (0.6 Ma) and the separation of Clade III and *H. alfaroi* south of Maya block between 1.0 and 0.8 Ma. The NW Andes lineage (Clade I) separated approximately 0.3 Myr after the initial spread of *H. alfaroi* (Fig. 2).

Alternative biogeographic hypothesis

A likelihood ratio test of the global likelihood values for each hypothesis rejected H_0; that restricted the ancestral area to the Northern Andes (N Andes and NW Andes) (lnL = 70.84; see above), although the lowest global likelihood (lnL = 72.36) was estimated for the hypothesis that restricted the ancestral range to the Northern Andes and the cis-Andes H_1. H_2 had the best likelihood (lnL = -64.76; Fig. 4), which proposed an ancestral area including the Maya and Chorotega blocks together with the Northern Andes. This hypothesis (H_2) reduced the global dispersal rate (0.4795) compared to the Northern Andes (0.5034), or to the Northern Andes and cis-Andes (0.6365) hypotheses. Nonetheless, extinction rates were comparable between this (H_2= 0.1468) and the Chorotega–trans-Andes hypothesis (H_1= 0.1448), whereas the trans-Andes only hypothesis yielded the lowest extinction rate (H_0= 0.1015). Likewise, a constrained ancestral distribution in the Northern Andes (H_0) had a lower probability 69.19% with the BBM

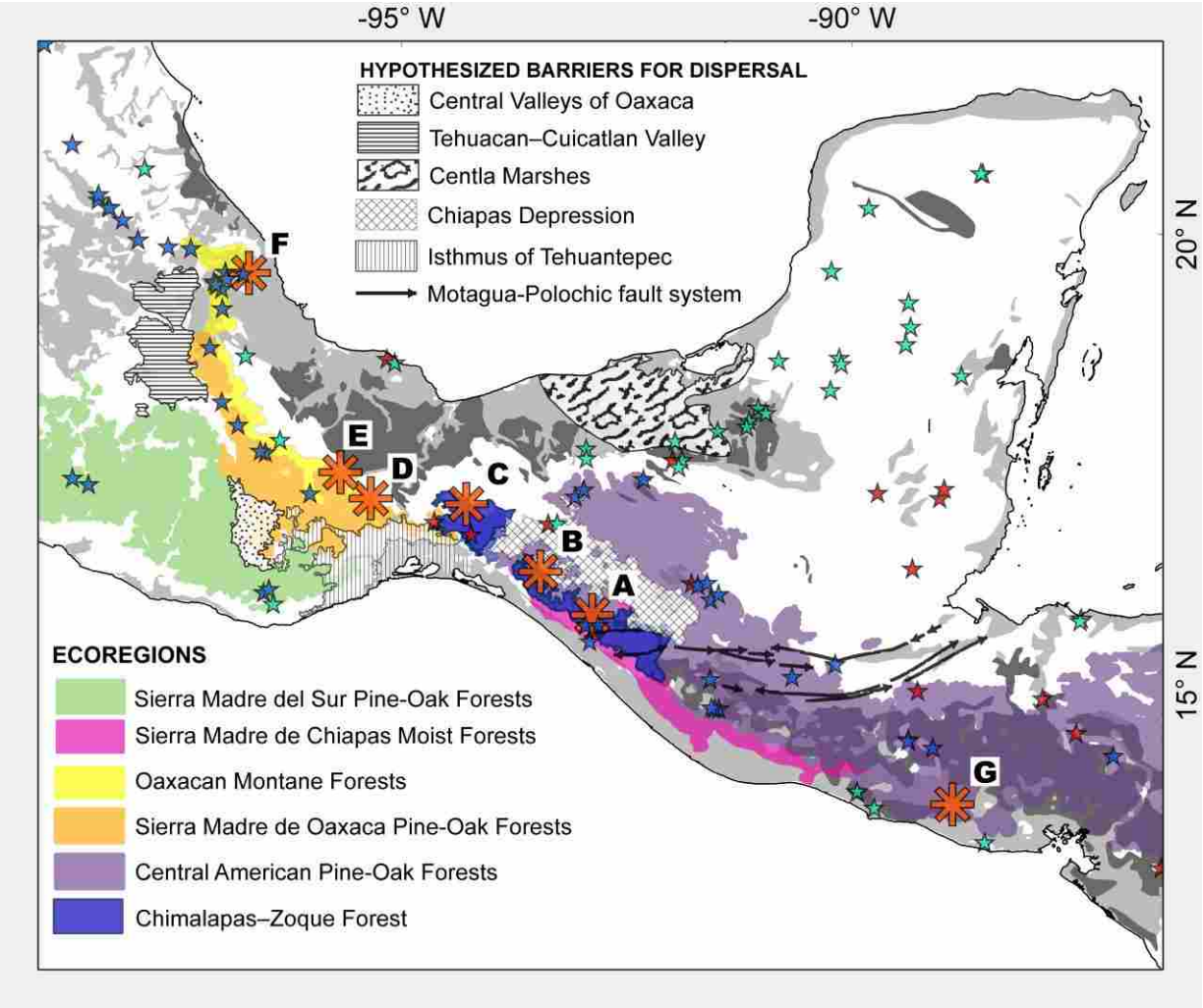

Figure 4. 3. Map of SE Mexico and northern Central America showing Phylomapper ML approximation of the center of the ancestral distribution (orange asterisks) for the *H. alfaroi* (red stars), *H. chapmani* (blue stars) and *H. melanotis* (green stars) species groups: (A); *H. alfaroi–H. melanotis* group (B), the *H. chapmani* group (C), *H. guerrerensis–H. chapmani–H. saturatior* (D), *H. melanotis* group (E), *H. rostratus*–Clade III–Clade IV (F), *H. alfaroi*–Clade I–Clade II (G). Superimposed on the main biogeographic provinces and hypothesized barriers for dispersal in the area.

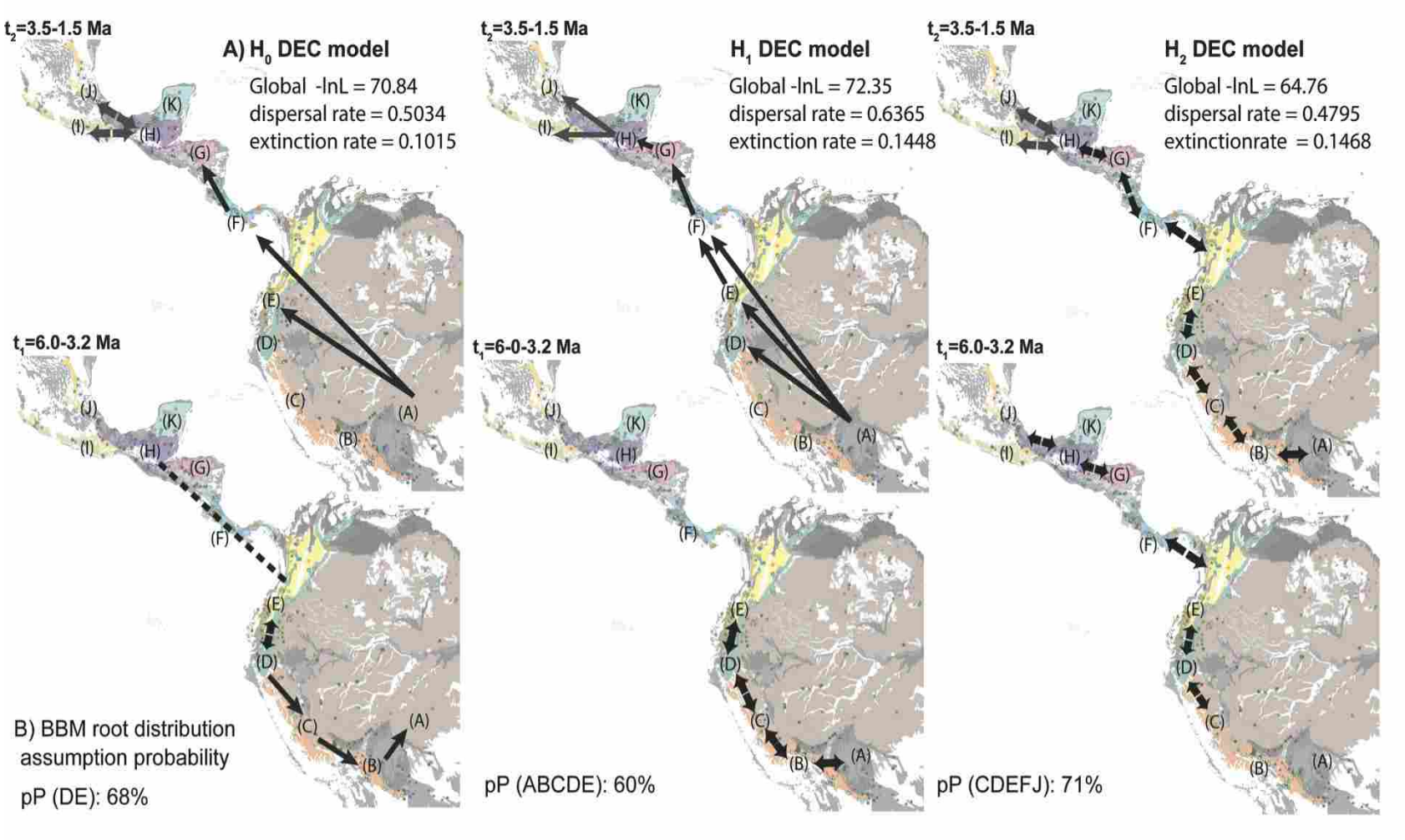

Figure 4. 4. Alternative biogeographic scenarios for the evolution of Clade B. H0 represented the empirical scenario estimated with BEAST; H1 imposed the ancestral range in SA, and H2 suggested two different biogeographic origins (see Table 4. 1). Arrows indicate the origin and direction of the migration process on the taxa distributions and discrete areas map from Fig. 4. 1. For each hypothesis, the DEC model global likelihood, dispersal and extinction rates are compared at the top (a), and the BBM distributions probabilities at the root are indicated at the bottom (b).

analysis than a root that included the Peruvian Yungas, the NW and NE Andes, the Chorotega block and the SMO (BBM optimization of a prior set to include the Chortís block) (H_2=71.61%). Also, a constraint to the ancestral distribution to the five areas in SA (H_1) yielded an even lower probability (53.41%). Finally, a wide root assumption (all areas are represented in the virtual out-groups) of maximum 5 contiguous areas had the lowest probably (15.13%). Nevertheless, the highest probability area array reproduced the root distribution constraint for H_2 (Fig. 4).

Paleodistributions and simultaneous divergence

H. chapmani, H. rostratus, Clade II and Clade IV current conditions ENMs showed their largest overlap when projected on climate conditions of the LIG, whereas the ENMs for the rest of the taxa showed larger overlap with the LGM models (Figs 5 & 6). Overall, niche models tended towards northern latitudes when projected onto the LGM environment, containing suitable areas in the Sierra Madre Occidental (SMOc) for *H. melanotis* and *H. rhabdops*, Trans-Mexican Volcanic Belt (TMVB) for *H. chapmani*, *H. guerrerensis* and Clade II, and the Sierra Madre del Sur SMS, and the Maya and Chorotega blocks for *H. intectus*, where those particular species are not currently found. Conversely, the *alfaroi* complex and *melanotis* group LIG ENMs, included areas of the Northern Andes (*alfaroi*) and moist forests of the northern Amazon (*melanotis)*. On the other hand, areas of stability for the three groups (*alfaroi*, *chapmani* and *melanotis*) converged in the Central American volcanic front (Central American Pine-Oak Forests) and the Sierra Madre de Oaxaca (Fig. 3). The SMS was stable only for the *melanotis* and *chapmani* groups. In addition, the test of simultaneous divergence suggested that the colonization of this area (SMS) by *H. melanotis* and *H. guerrerensis* occurred in parallel ~2.0 Ma (Ω 95%HDP = 0.0-0.01; Fig. 7). Similarly, the separation of Clade I + *H. alfaroi* and Clade III south the Motagua–Polochic fault system (Fig. 3) was supported as a synchronised event ~0.8 Ma (Ω 95%HDP = 0.0-0.0245). However, there was no support for the simultaneous split

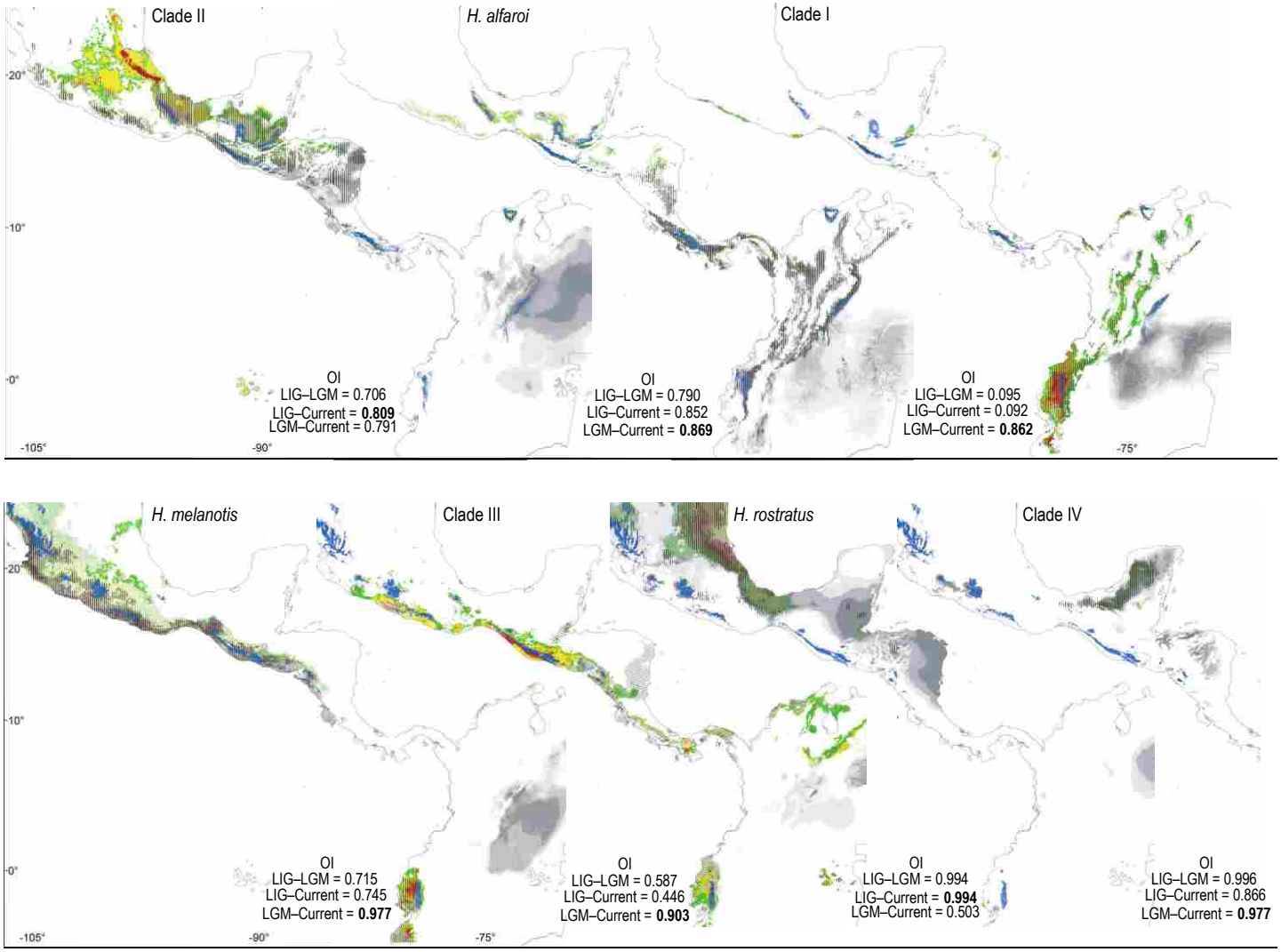

Figure 4. 5. ENMs for the *H. alfaroi* and *H. melanotis* species groups under current climatic conditions (striped areas) projected on climatic reconstructions of the LIG (gray gradient) and the LGM (color gradient). The overlap index (OI) between models from the three time periods is shown for each taxon. Predicted areas of environmental stability for each species group are shown in blue.

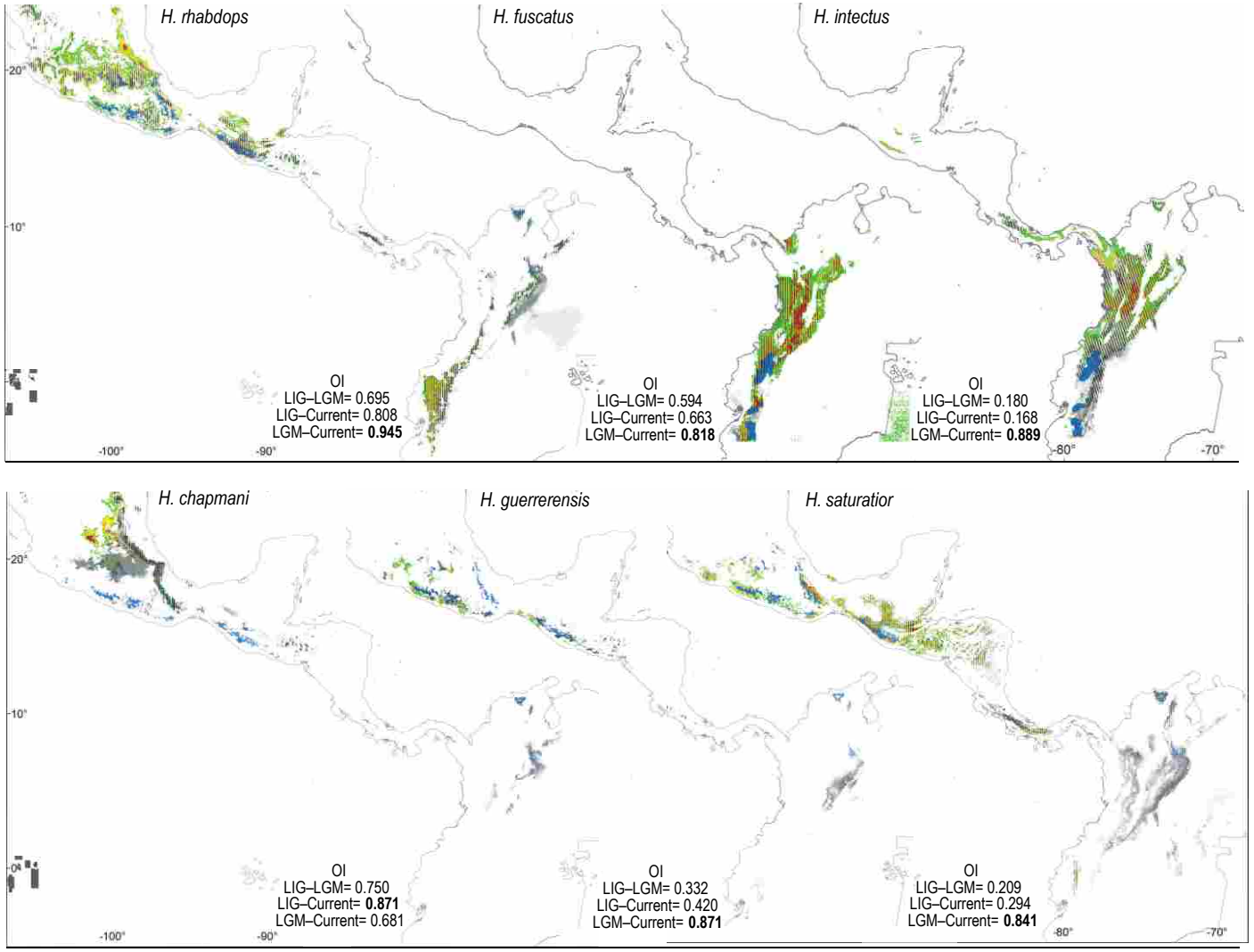

Figure 4. 6. ENMs for the *H. chapmani* group and for *H. fuscatus* and *H. intectus* under current climatic conditions (striped areas) projected on climatic reconstructions of the LIG (gray gradient) and the LGM (color gradient). The overlap index (OI) between models from the three time periods is shown for each taxon. Predicted areas of environmental stability for each species group are shown in blue.

between *H. saturatior* and *H. chapmani* and Clade I + *H. alfaroi* with Clade III (Ω 95%HDP = 0.087-0.308; Fig. 7). Additionally, the linear RBT rejected the hypothesis that the Isthmus of Tehuantepec–Central Valleys of Oaxaca and the Motagua–Polochic fault (Fig. 3), each do not impose a disruption for the ENMs of species that are actually separated by these geological features. Similarly, the blob RBT supported the Centla Marshes as a potential ecological barrier for the separation of *H. rostratus* and Clade IV in the Yucatan peninsula (Yuc). Finally, the hypothesis of a barrier of suboptimal habitat enforcing the separation of Clade I + *H. alfaroi* from Clade II was not supported (Fig. 7).

Discussion

Pliocene biogeography

The separation of *Nephelomys* and *Handleyomys* and the subsequent split of *Handleyomys* sensu stricto from the *H. alfaroi–H. melanotis—H. chapmani* species groups can be best explained as vicariance events impacting ancestral lineages in the central Andes (*Nephelomys*), and the NW Andes, the Chorotega block and the Maya block (*Handleyomys* sensu lato) 5.5 Ma. This was followed by separation of *Handleyomys* sensu stricto in the NW Andes (4.4 Ma) and the extinction of *Handleyomys* lineages that remained in Central America, while the *H. alfaroi–H. melanotis—H. chapmani* species groups persisted and diversified in the Maya block. The estimated time of these events corresponds to a period of geological activity in the Motagua–Polochic fault systems (from 10 to 3 Ma) after the connection of the Chortís block and the North American plate (Marshall, 2007), and the attachment of the Costa Rica–Panama micro plate to the Chocó block 6.0 Ma (Coates et al., 2004; Kirby et al., 2008), such that today, the Chorotega block corresponds biogeographically to the Pacific dominion region of SA (Morrone, 2014). In addition, the west to east migration of the Central American volcanic arc throughout

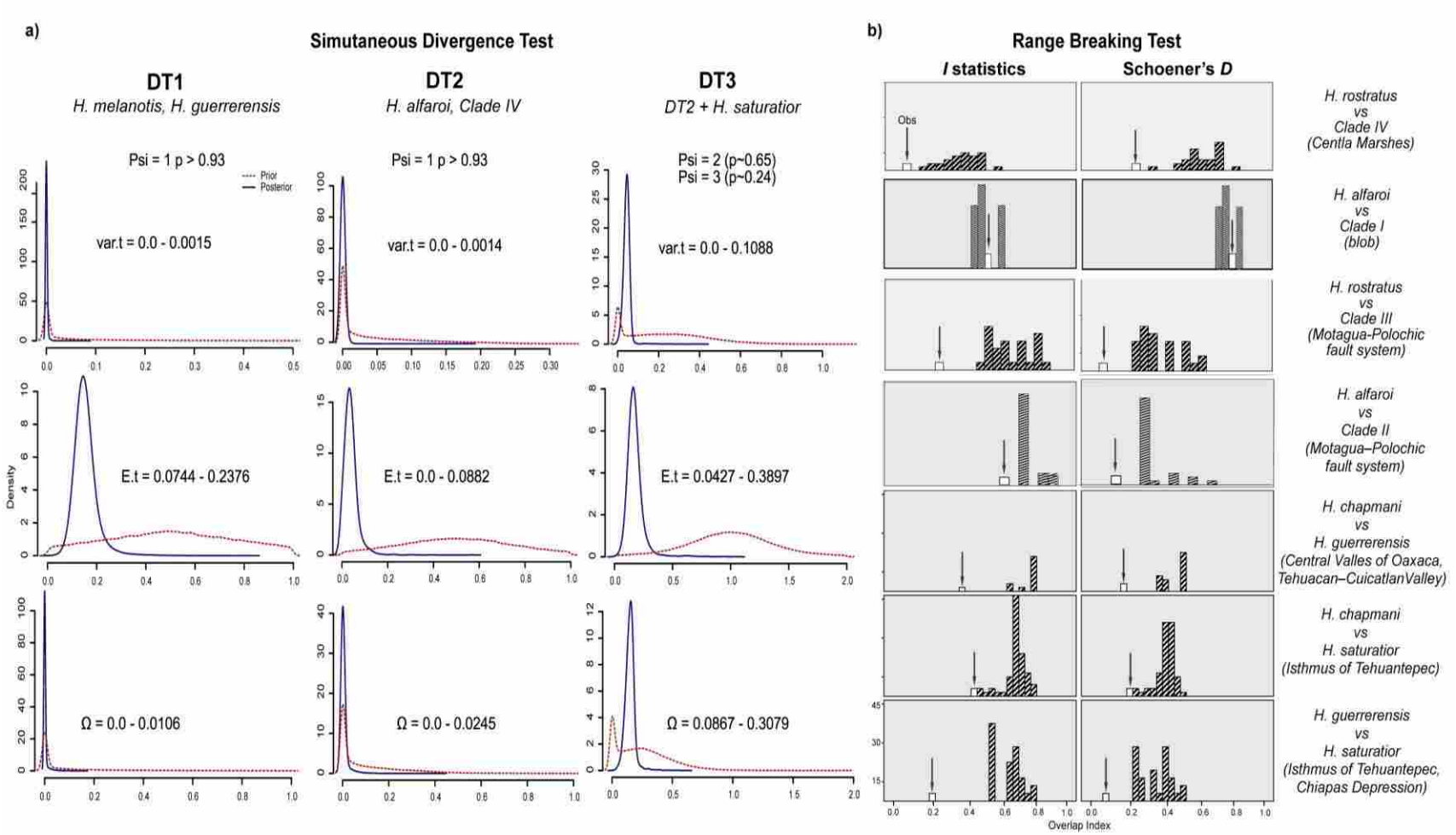

Figure 4. 7. a) Test for the simultaneous split of *H. melanotis* and *H. rhabdops* across the Central Valleys of Oaxaca and the Isthmus of Tehuantepec ~2.3 Ma (DT1); and *H. alfaroi* and Clade III south the Motagua-Polochic fault system ~1.0¬–0.8 Ma (DT2), and a test if the divergence *H. chapmani* and *H. saturatior* could have occurred simultaneously with the expansion of *H. alfaroi* and Clade III (DT3). b) The Schoener's *D* and *I* statistics histograms Range Breaking Test. Implied geological barriers are shown in parenthesis**.**

the Pliocene resulted in the geological and ecological instability of the Chortís and Chorotega blocks until 1.5 Myr after the final closure of the PLB (Saginor et al., 2011).

The contemporaneous uplift of the Cordillera Oriental in the NE Andes 6.0 Ma (Graham, 2009), support the recovered dispersal path of *Euryoryzomys*, *Oecomys*, *Hylaeamys* and *Transandinomys* from an ancestor in the central Andes Peruvian Yungas (ancestral area of *Nephelomys*), a region that contains a relatively large amount of ancestral endemism, including the proposed basal groups of Sigmodontinae (Patterson et al., 2012). In addition, the ecological constraints to high elevation forests with Nearctic elements (Eckert & Hall, 2006; Nixon, 2006; Wang & Ran, 2014) observed in recovered ancestral lineages (*Nephelomys*, *Handleyomys* sensu stricto and the *H. chapmani* group), along with the tolerance to high elevations observed in *Hylaeamys*, *Oecomys* and *Transandinomys*, suggests that the transition to the cis-Andes occurred gradually. This ecological pattern is unlike several other SA rodents in which tolerance to high elevation appeared secondarily (Upham *et al.*, 2013), and contrasts with the biogeographic history of other Oryzomyine rodents hypothesized to have recolonized NA (2.5 Ma). For example, *Oryzomys* and *Oligoryzomys* appear unaffected by reported ecological/geographic barriers for high elevation taxa in Mesoamerica (Rogers *et al.*, 2009; Hanson *et al.*, 2010; Palma *et al.*, 2010), and *Melanomys* and *Sigmodontomys* that do not occur north of the Chorotega block (Hanson & Bradley, 2008; Pine *et al.*, 2012). Moreover, our estimated CA reintroductions (*Nephelomys, Oecomys* and *Transandinomys*) are concordant with this biogeographic pattern. Nonetheless, some of the purportedly reintroduced Mesoamerican lineages of Oryzomyini exhibit substantial in situ genetic variation that has not been taken into account in biogeographic reconstructions. *Cytb* distances between *Oryzomys couesi* (east Mexico), *O. palustris* (eastern USA), and a putative species from Panamá average ~12.0% (Clade D; Hanson *et al.*, 2010), and

distances among *Oligoryzomys fulvescens* and strictly SA species of *Oligoryzomys* range from 10 to 13.1%, and from 7.5 to 9.0% between unnamed lineages of *O. fulvescens* within CA (Rogers *et al.*, 2009), similar to the *Cytb* distances among species of *Sigmodon* (12.8% between *S. hirsutus* in CA and *S. toltecus* and *S. hispidus* in the north) (Bradley *et al.*, 2008); whose initial diversification in NA is supported with at least four species of †*Prosigmodon* from the late Hemphillian (Carranza Castañeda & Wolton, 1992) and the Blancan of NA (Lindsay *et al.*, 1984). In contrast, the average divergence among clades whose distribution only marginally reach CA are considerably lower (*Melanomys caliginosus,* 7.2% – Hanson & Bradley, 2008), and between the two species of *Nephelomys* included herein (6.5% between *N. devius* from Costa Rica and *N. albigularis* from Ecuador). This would imply considerable early diversification of ancestral Oryzomyini in NA. The hypothesis of an early diversification of this tribe in NA (Marshall, 1979; Engel, 1998) is supported by a distinctive Sigmodontinae-like assemblages in the fossil records of the late Miocene and early Pliocene containing proposed ancestors of Oryzomyini (†*Copemys,* †*Bensonomys* and †*Jacobsomys*) (Lindsay & Czaplewski, 2011). Furthermore, they are recovered along extinct *Sigmodon* species during the Pliocene in Mexico and CA (Woodburne *et al.*, 2006).

A potential land connection between NA and SA along the present day Isthmus of Panamá and the Darién region is thought to have existed since the end of the Miocene (Montes *et al.*, 2012) and separated from the Atrato basin in the Northern Andes by sea levels of 100-150 m (Duque-Caro, 1990; Coates *et al.*, 2004). Although waif dispersals across the Antilles could have occurred, the fossils of this region date from the Pleistocene and nest phylogenetically within extant Oryzomyini lineages (Steadman & Ray, 1982; Dowler *et al.*, 2000; Turvey *et al.*, 2010; Zijlstra *et al.*, 2010). Elucidating the phylogenetic position of †*Cordimus* could clarify the

legitimacy of this alternative route of dispersal (Zijlstra *et al.*, 2014). On the other hand, the fact that the late Pliocene-early Pleistocene fossil record of South America contains extant members of Phyllotini, Akodontini and Thomasomyini (Reig, 1987; Engel, 1998; Pardiñas *et al.*, 2002; Salazar-Bravo *et al.*, 2013), while the NA fossils of that period are largely represented by extinct lineages, may indicate severe climate conditions that dominated NA during these periods (Rolland *et al.*, 2014). This inference, coupled with the start of a 4 Myr period of mafic pulses of the TMVB 11 Ma (Ruiz-Martínez *et al.*, 2010; Ferrari *et al.*, 2012), justifies the ~3.5 Myr diversification gap between age estimates for the origin of the subfamily (~12-11 Ma) and the diversification of its extant supra-generic diversity (~7.5-7.0 Ma). Later, silicic volcanism episodes along the now established TMVB 7.0-3.0 Ma (Gómez-Tuena *et al.*, 2007) and the coinciding subsidence of the Isthmus of Tehuantepec 6.0 to 4.0 Ma (Barrier *et al.*, 1998), could have constrained the northern limits of the ancestor of the *H. alfaroi*, *H. melanotis* and *H. chapmani* species groups to the Maya block. The in-situ diversification of these three lineages is supported by fossil records of the three groups in the Pleistocene of Mexico (Arroyo-Cabrales *et al.*, 2002; Ferrusquía-Villafranca *et al.*, 2010), while fossils of equivalent age in SA and Panama incorporate *Nephelomys*, *Oecomys*, *Transandinomys* and *Euryoryzomys*, but not *Handleyomys* (Leigh Jr & Wright, 1990; Terborgh, 1990).

The commonly observed polytomy at the base of Oryzomyalia (Steppan *et al.*, 2004; Schenk *et al.*, 2013; Leite *et al.*, 2014) (Weksler, 2003; D'Elía *et al.*, 2006; Martínez *et al.*, 2012; Parada *et al.*, 2013), containing several apparent relict taxa (*Reithrondon*; *Chinchillula*; *Zygodontomys; Abrawayaomys; Andinomys, Punomys*) indicates that the current biogeography of SA Sigmodontinae are the result of numerous independent histories (Guillermo D'Elía *et al.*, 2007; Salazar-Bravo *et al.*, 2013). This emphasises the need for methodologies that account for

the bias imposed by taxonomic asymmetry, a phenomenon that has been shown to affect the accuracy of discrete ancestral states reconstruction (Cook & Crisp, 2005).

Late Pliocene-Pleistocene biogeography

The late Pliocene diversification of the *H. alfaroi*, *H. chapmani* and *H. melanotis* species groups covered four of the five recognized biogeographic provinces in the Mexican transition zone (between the Nearctic and Neotropical regions), the SMO, SMS-OH, MTVB, and the SMCh (Escalante *et al.*, 2013). These provinces form a natural affiliation with temperate forests (Contreras-Medina *et al.*, 2007; Espinosa & Ocegueda, 2008; Torres-Miranda *et al.*, 2011). However, the SMCh was isolated as a result of the subsidence of the Isthmus of Tehuantepec (ITH) from 5.0 to 3.0 Ma (Barrier *et al.*, 1998), an event that marked the onset of the most striking geologic barrier for Nearctic cenocrons in the transition zone (Morrone, 2010). For example, *Habromys lophurus* (León-Paniagua *et al.*, 2007; Rogers *et al.*, 2007), *Peromyscus mayensis* (Bradley et al., 2007), *Nyctomys sumichrasti* (Corley *et al.*, 2011), *P. aztecus* (Sullivan et al., 1997) and cryptic species lineages within *Reithrodontomys microdon* (Arellano et al., 2005) and *R. sumichrasti* (Hardy *et al.*, 2013) are restricted to the SMCh (Weber *et al.*, 2006) and their closest relatives are found in the SMO, SMS-OH, MTVB and the Sierra Madre Occidental (SMOc). Likewise, *P. grandis, P. guatemalensis*, *P. nudipes* and *P. zarhyncus* form an exclusively trans-isthmian clade; however, their sister lineage remains unknown (Ordóñez-Garza *et al.*, 2010). Although *Handleyomys* is not known to occur in the SMOc, this pattern is also observed in other Mesoamerican groups, including *Nyctomys, Tylomys*, *Habromys* and *Megadontomys* (Vallejo & González-Cózatl, 2012). On the other hand, the SMOc contained areas of environmental stability for *Handleyomys* despite its generally drier and colder environment (Challenger & Soberón, 2009). Likewise, the ENM's of eight species were augmented under the climatic conditions of the LGM, consistent with the documented

downslope migration of pine-oak forests ecosystems in Mexico and northern Central America (Torres-Miranda *et al.*, 2011; Ramírez-Barahona & Eguiarte, 2013). Some stable populations of the species in the *H. chapmani* group and *H. melanotis* are presently found in pine-oak forest localities between 2150 m and 2450 m in the SMS and the OH (Sanchez-Cordero, 2001; Santos-Moreno *et al.*, 2007), and up to 3250 m in the case of *H. rhabdops* in the TIH (Reid, 2009). The constrained forest-dwelling ecology of *Handleyomys* (Engstrom, 1984; Ceballos, 1990; Reid, 2009) and their conserved preference for high elevation environments could explain the enforced allopatric distribution of most species in this clade. This biogeographic pattern is shared by most groups of mammals recognized to have diversified in the transition zone (Munguía *et al.*, 2008), and differs biogeographically from the Mesoamerican region; which runs in parallel at lower elevations (Morrone, 2014). The three younger lineages of *Handleyomys* (*H. alfaroi*, *H. rostratus* and Clade IV) occur along this region, correspondingly, the distributions of these three lineages were predicted to have decreased under conditions of the LGM, consistent with the expansion of rain forest ecosystems (Ceballos *et al.*, 2010). This implies that adaptation to the lower elevations occurred independently in *Euryoryzomys, Oecomys* and *Hylaeamys*. However, the three lineages tolerate ~1500 m in Mexico and CA (*H. rostratus* and *H. alfaroi*) and occur at localities up to ~2200 m in SA (*H. alfaroi*). In addition, some species of *Oecomys* and *Hylaeamys* are restricted to environments above 1200 m (Prado *et al.*, 2014). The timing of the southern migration of *H. alfaroi* and *H. rostratus* of 0.8-1.0 Ma, is in accordance with the western migration of the CA volcanic arc (MacMillan *et al.*, 2004; Saginor *et al.*, 2011), an active Motagua fault, and a permanently flooded Nicaraguan Depression (Marshall, 2007; Woodburne, 2010). Together, these geologic factors maintained most of the Chortís block as unstable during the late Pliocene and early Pleistocene.

The Mexican transition zone and the Mesoamerican region both hold priority for conservation due to their relatively high β diversity (Koleff & Soberon, 2008). In addition, these regions form part of the critical geographic extent for the protection of mammals with large ranges (García-Marmolejo *et al.*, 2008; Agosta & Bernardo, 2013), and serve as primary dispersal corridors (Gutiérrez-García & Vázquez-Domínguez, 2013). In particular, the SMCh, that was the inferred ancestral range for the *H. alfaroi*, *H. chapmani* and *H. melanotis* groups, exhibits a continuity in fossil deposits that denote high ecological stability since the Pliocene and though the Pleistocene (Toledo, 1982; Ferrusquía-Villafranca et al., 2010). Nevertheless, the naturally fragmented ecosystems (Visconti *et al.*, 2011; Amori *et al.*, 2013; Ornelas *et al.*, 2013), along with a socioeconomic complex region (Vaca *et al.*, 2012), continue to challenge conservation policies as it faces the impact of decades of vegetation loss (Flores-Villela & Gerez, 1994; Brooks *et al.*, 2002; DeClerck *et al.*, 2010).

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