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Short communication

Genetic comparisons between *Heteromys desmarestianus* and the recently described *H. nubicolens* (Rodentia: Heteromyidae) in northwestern Costa Rica

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The subfamily Heteromyinae (spiny pocket mice) represents a well-defined monophyletic group within the rodent family Heteromyidae (Hafner 1981; Hafner and Hafner 1983; Wahlert 1991). Two extant genera, *Heteromys* and *Liomys*, are recognized in the Heteromyinae. Species of *Heteromys* inhabit wet (typically evergreen) forests from southern Mexico to western Ecuador (Williams et al. 1993). Present taxonomy recognizes nine species of the genus, including *H. nubicolens*, a species recently described from northwestern Costa Rica (Patton 2005; Anderson and Timm 2006; see also Williams et al. 1993; Anderson and Jarrín-V. 2002; Anderson 2003). Anderson and Timm (2006) provided detailed morphological comparisons between *H. nubicolens* and adjacent populations of *H. desmarestianus* (with which *H. nubicolens* was previously confused) and summarized available information regarding the natural history and biogeography of the new species (see also McCain 2004, 2006). Here, we undertake genetic comparisons between *H. nubicolens* and adjacent populations of *H. desmarestianus*, also including available DNA sequences from other parts of the range of *H. desmarestianus*. Furthermore, we examine samples from two individuals from the contact zone between *H. nubicolens* and *H. desmarestianus* that are morphologically intermediate and may represent hybrids between the two species.

Two mountain ranges lie within northwestern Costa Rica, each oriented diagonally from southeast to northwest (Fig. 1; Castillo-M. 1984; Bergoeing 1998). To the west of the larger Cordillera Central de Costa Rica, a range of Tertiary volcanic peaks (< 2000 m) and ridges forms the Cordillera de Tilarán, which is continuous at an elevation of ca. 1200 m. To the northwest of that range, the Cordillera de Guanacaste is comprised of a series of isolated Quaternary volcanoes, most of which reach 1500–2000 m. Low passes between most of the volcanoes of the Cordillera de Guanacaste connect the Caribbean and Pacific lowlands at elevations of 500–700 m.

The two species of *Heteromys* present in northwestern Costa Rica show clear ecogeographic patterns in their distributions (Fig. 1; Anderson and Timm 2006). In this region, *H. desmarestianus* occurs throughout the wet Caribbean lowlands, as well as at middle elevations on the Caribbean and Pacific

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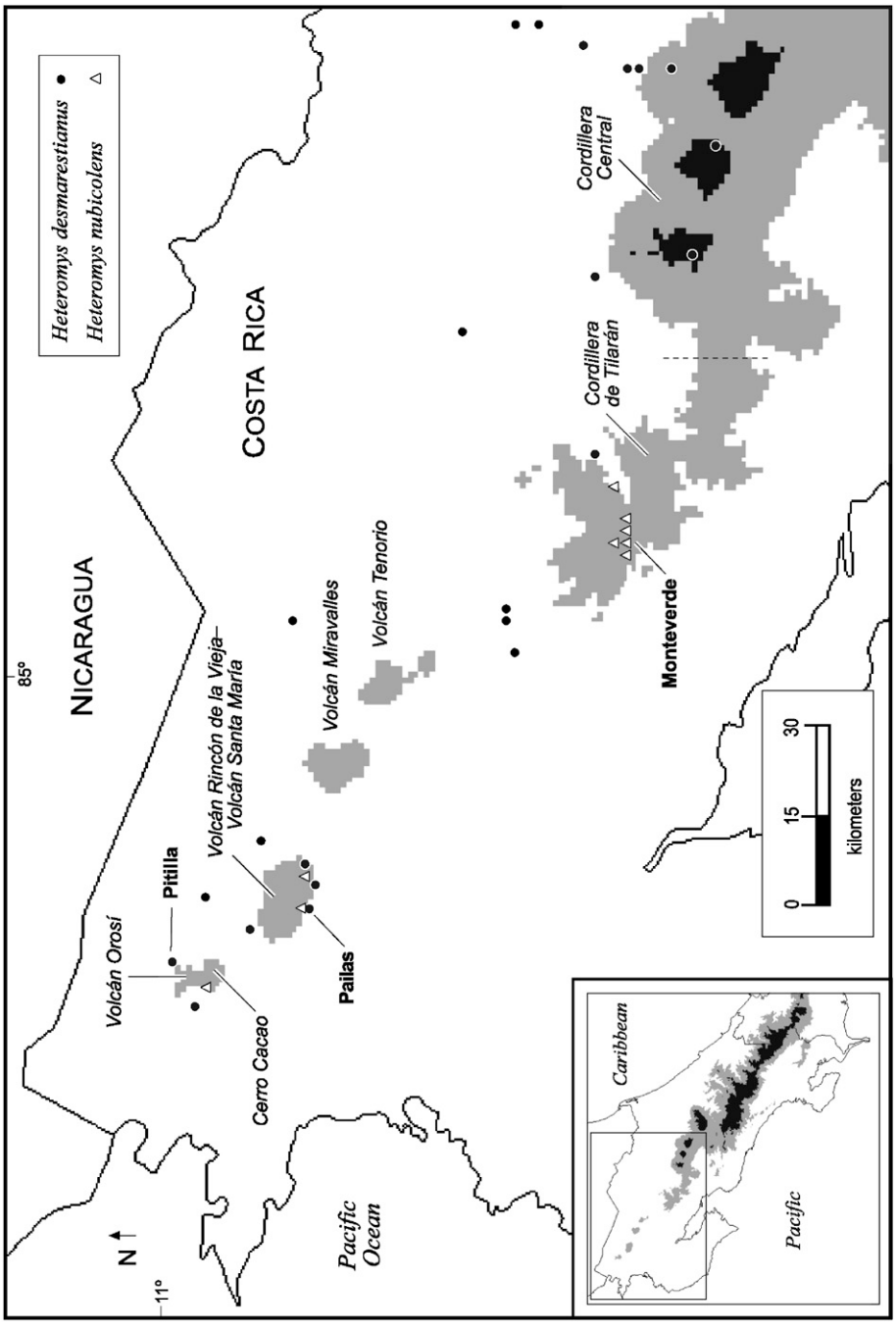
Heteromys desmarestianus
Heteromys nubicolens
NICARAGUA
COSTA RICA
85°
11°
N
Pacific Ocean
Volcán Orosí
Pitilla
Cerro Cacao
Volcán Rincón de la Vieja
Volcán Santa María
Pailas
Volcán Miravalles
Volcán Tenorio
Cordillera Central
Cordillera de Tilarán
Monteverde
0
15
30
kilometers
Caribbean
Pacific

slopes of the Cordillera Central, Cordillera de Tilarán, and Cordillera de Guanacaste. The overall distribution of this widespread species (likely a complex of morphologically similar species; Mascarello and Rogers 1988; Rogers 1989, 1990) extends from Mexico to northwestern Colombia (Williams et al. 1993; Anderson 1999). In contrast, *Heteromys nubicolens* is documented only from the Cordillera de Tilarán and Cordillera de Guanacaste of northwestern Costa Rica. Known populations occur in three highland areas (Monteverde, Volcán Rincón de la Vieja–Volcán Santa María, and Cerro Cacao), but these populations are probably disjunct, being separated by intervening lowlands where *H. desmarestianus* is found. Similar forested montane habitats also exist on Volcán Orosí, Volcán Miravalles, and Volcán Tenorio, and the species may be present on those volcanoes as well. A more-distantly related spiny pocket mouse, *Liomys salvini*, inhabits areas of high seasonality and overall low precipitation in deciduous habitats of the lowlands of northwestern Costa Rica to the west of the Cordillera de Tilarán and Cordillera de Guanacaste (Genoways 1973; McPherson 1985). The other species of *Heteromys* known from Costa Rica, *H. oresterus*, has been collected only at a few localities at high elevations of the Cordillera de Talamanca to the southeast in central Costa Rica (Anderson and Timm 2006).

The data generated here consist of cytochrome-*b* sequences from five individuals of *Heteromys nubicolens*, five of *H. desmarestianus*, and two provisionally ascribed to *H. desmarestianus* by Anderson and Timm (2006). For *H. nubicolens*, we include samples from three localities in the Monteverde Cloud Forest Reserve, ranging from 1250–1840 m (Fig. 1). For *H. desmarestianus*, we use individuals from three localities very close to each other in the Área de Conservación Guanacaste; they range from ca. 600–900 m and lie on the low northeastern slopes of Volcán Orosí, near the Pitilla field station (Fig. 1). We also include samples from two specimens (KU 158614 and 158615) of special interest from the Área de Conservación Guanacaste on the low southwestern slopes of Volcán Rincón de la Vieja–Volcán Santa María. These samples derive from a locality at 800 m near the Pailas field station (Fig. 1) and were provisionally assigned to *H. desmarestianus* (Anderson and Timm 2006). However, these specimens were collected from an elevation where both *H. nubicolens* and *H. desmarestianus* are known to occur. The skulls of these two adult females are smaller than those of *H. nubicolens* from the equivalent age class, but they display only slightly more than half of the cranial characters typical of *H. desmarestianus*. These observations raise the possibility that these two individuals may be rare hybrids that could occur along the contact zone between the two species. Although tissues are not available from these two specimens, embryos from each were preserved in ethanol. We examine mitochondrial DNA from the embryos; this maternally inherited genome thus represents the haplotype of the mother. Unfortunately, tissue samples from other individuals in this contact zone between the two species are not available.

All voucher specimens for sequences produced here were examined by RPA and are deposited at the University of Kansas Natural History Museum (KU), Lawrence; in addition, we provide collector field numbers (CMM = Christy M. McCain; MK = Marion Klaus; and RMT = Robert M. Timm) and GenBank accession numbers for cytochrome-*b* sequences as follows: *Heteromys nubicolens*: Costa Rica: Alajuela: Monteverde, Monteverde Cloud Forest Reserve, Camino a Peñas Blancas, 1250–1300 m [10°18′N, 84°47′W], KU 159102 = CMM 245 = DQ450091, KU

Fig. 1. Map showing known localities for *Heteromys* in northwestern Costa Rica. Triangles represent localities of *H. nubicolens*, and circles denote localities of *H. desmarestianus* (modified from Anderson and Timm 2006). Localities from which we examined tissue samples are indicated by name (in bold). Gray shading denotes regions more than 1000 m in elevation, and areas shown in black lie above 2000 m. To the northwest of the Cordillera de Tilarán, the Cordillera de Guanacaste is comprised of a series of isolated volcanoes (Volcán Tenorio, Volcán Miravalles, Volcán Rincón de la Vieja–Volcán Santa María, Cerro Cacao, and Volcán Orosí).

159103 = CMM 258 = DQ450092; Puntarenas: Monteverde, Monteverde Cloud Forest Reserve, Cerro Amigos, 1800–1840 m [10°19′N, 84°48′W], KU 159101 = CMM 222 = DQ450090; Monteverde, Monteverde Cloud Forest Reserve, Investigator's Trail, 1550 m [10°18′N, 84°48′W], KU 159025 = RMT 4468 = DQ450088 (holotype of *Heteromys nubicolens*), KU 159026 = RMT 4469 = DQ450089; *H. desmarestianus*: Costa Rica: Guanacaste: Área de Conservación Guanacaste, ca. 39 km N Liberia, Pitilla [ca. 700 m, 10°59′N, 85°26′W], KU 158512 = MK 99-093 = DQ450097, KU 158513 = MK 99-094 = DQ450098; Área de Conservación Guanacaste, ca. 39 km N Liberia, Pitilla, Sendero Carica [ca. 600–800 m, 10°59′N, 85°26′W], KU 158514 = MK 99-102 = DQ450099; Área de Conservación Guanacaste, ca. 39 km N Liberia, Pitilla, Sendero Orosilito [ca. 700–900 m, 10°59′N, 85°26′W], KU 158508 = MK 99-088 = DQ450095, KU 158509 = MK 99-090 = DQ450096; *H. desmarestianus* (provisional identification): Costa Rica: Guanacaste: Área de Conservación Guanacaste, ca. 20 km NNE Liberia, Pailas, Sendero Pailas, near Río Colorado, 800 m [10°47′N, 85°21′W], KU 158614 = MK 00-111 = DQ450093 (embryo), KU 158615 = MK 00-112 = DQ450094 (embryo).

We obtained sequences of cytochrome *b* from each of the 12 samples as follows. DNA was extracted from all tissues using a QiaAmp extraction kit (Qiagen Inc.). We attempted to amplify the entire cytochrome-*b* gene using primers MVZ05 and UMMZ04 (Smith and Patton 1993; Jansa et al. 1999) in 20 mL PCR reactions using Taq polymerase (Promega Corp.) and recommended concentrations of primers, nucleotides, buffer, and $MgCl_2$. Resulting PCR products were purified via electrophoresis through a 2% low-melting-point agarose gel; the appropriate band was then excised from the gel and melted in 300 mL of sterile water. To generate products of a suitable size for sequencing, the resulting purified product was used as a template in two subsequent reamplification reactions, one using primer MVZ05 paired with UMMZ12 and one using UMMZ13 paired with UMMZ04 (Jansa et al. 1999). These reamplifications were performed using Taq polymerase (Promega Corp.) in 30 mL PCR reactions. All reactions were performed on a Perkin-Elmer 9700 thermal cycler for 35 cycles using an annealing temperature of 50°C. The resulting products were sequenced in both directions using amplification primers and dye-terminator chemistry on an ABI 3700 automated sequencer. Sequences were edited and compiled using Sequencher 4.1 (GeneCodes). All sequences have been deposited in GenBank (accession numbers DQ450088–DQ450099). Sequences were aligned with reference to the translated amino acid sequences.

Additional cytochrome-*b* sequences from the following *Heteromys* were taken from GenBank (all those available for the genus). To clarify and correct the information available for these samples in GenBank and the original publications, wherever possible we provide full geographic provenience, museum catalog numbers, and numbers used to track the samples in the field and laboratory, in addition to the GenBank accession numbers. Three sequences of *H. desmarestianus* from other parts of the range of the species complex were included in the ingroup along with the sequences generated here, and *H. anomalus* and *H. gaumeri* were designated as outgroups for rooting trees: *H. anomalus*: Venezuela: Miranda: 40 km N Altagracia, TCWC 39720 = MDE 2129 = AK 3482 = DQ168468 (Rogers and Vance 2005; voucher specimen examined by RPA); *H. desmarestianus*: Guatemala: El Petén: Tikal, ROM 99298 = FN 31848 = LVT 5499 = AY926358 (Alexander and Riddle 2005); Honduras: Atlántida: Lancitilla, TCWC 52259 = BEL 865 = AK 9696 = DQ168466 (Rogers and Vance 2005); Mexico: Oaxaca: Vista Hermosa, Distrito Ixtlán, 1000 m, MVZ 161229 = DSR 1685 = DQ168467 (Rogers and Vance 2005; voucher specimen examined by RPA); *H. gaumeri*: Mexico: Quintana Roo: Puerto Morelos, MNHN (CG) 2000-234 = V-238 = T-348 = AJ389536 (Montgelard et al. 2002; see also Catzeflis 1991). Abbreviations follow: AK = Texas A&M University karyotype/tissue numbering series; BEL = Robert D. Bradley, Jan Ensink, and Thomas E. Lee field catalog series; DSR = Duke S. Rogers field catalog series; FN = Royal Ontario

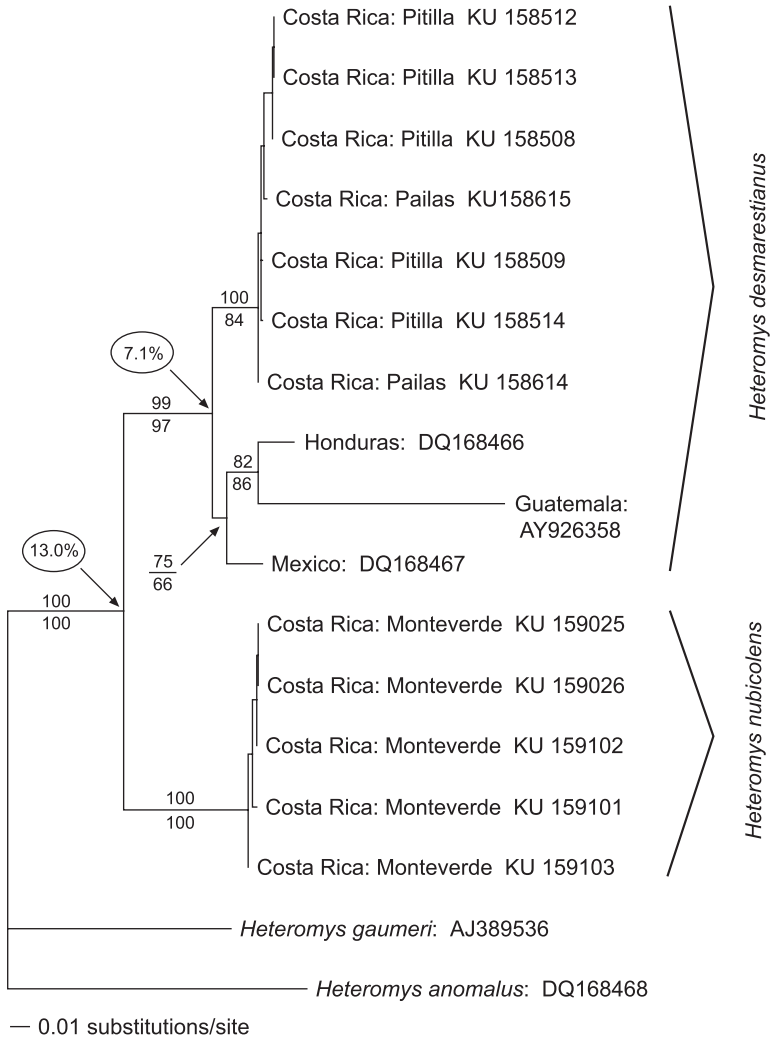

Fig. 2. One of the two equally likely trees obtained from maximum-likelihood analysis of cytochrome-*b* sequences of *Heteromys*. The strict-consensus tree from parsimony-based analysis (not shown) recovered the same principal clades. Bootstrap values (likelihood above the line, parsimony below) for the principal clades are shown. Sequence divergence values (Jukes–Cantor corrected) are provided at respective nodes for comparisons between the Pitilla–Pailas samples and other *H. desmarestianus*, and between *H. nubicolens* and *H. desmarestianus*. Museum catalog numbers are provided for specimens sequenced here, and accession numbers appear for samples taken from GenBank.

Museum Field Number series; LVT = Las Vegas Tissue number series; MDE = Mark D. Engstrom field catalog series; MNHN (CG) = Muséum national d'Histoire naturelle (Catalogue Général); MVZ = Museum of Vertebrate Zoology, University of California, Berkeley; T = Collection of Mammalian Tissues, Laboratoire de Paléontologie, Institut des Sciences de l'Evolution, Montpellier; TCWC = Texas Cooperative Wildlife Collection, Texas A&M University; and V = François M. Catzeflis field catalog series.

Sequences were subjected to phylogenetic analysis using maximum parsimony and maximum likelihood as implemented in PAUP* version 4.0b10 (Swofford 2002). Several sequences were shorter than the full cytochrome-*b* gene; missing bases were coded as unknown. For the parsimony analysis, all characters were treated as unordered and equally weighted, and all parsimony searches were exhaustive. The best-fit model for the likelihood analysis was determined by evaluating the fit of various substitution models on a neighbor-joining tree and applying sequential likelihood-ratio tests as implemented in Modeltest 3.6 (Posada and Crandall 1998). The parameters describing this best-fit model were used in a heuristic search employing 10 random-addition replicates with TBR branch swapping as implemented in PAUP*. Bootstrap values (Felsenstein 1985) were calculated under both parsimony and likelihood criteria using 1000 pseudoreplicates, with heuristic searches employed within each. We also calculated pairwise divergence estimates using Jukes–Cantor corrected distances adjusted for within-species divergence where appropriate (Nei and Li 1979). To estimate the divergence within species, we calculated both nucleotide diversity (π) and haplotype diversity (h) using the program DnaSP 4.10.4 (Rozas et al. 2003).

The Costa Rican haplotypes indicate low intrapopulational variability and substantial interspecific differences. Three unique haplotypes were present among the five samples from Monteverde ($h = 0.70$), and nucleotide diversity (π) among these individuals was 0.003. The samples from Pitilla and Pailas were not reciprocally monophyletic; therefore, we pooled these samples and treated them as a single population for distance-based statistics. Haplotype diversity among the samples from Pitilla and Pailas was 0.81, and π was 0.008. The individuals of *H. desmarestianus* from Pitilla and Pailas differ from conspecifics elsewhere in Central America and Mexico by 7.1% (Jukes–Cantor corrected average distance; 6.8% *p*-distance corrected only for within-species divergence). Parsimony and likelihood analyses recovered two well-supported clades corresponding to *Heteromys desmarestianus* (including the Costa Rican samples) and *H. nubicolens* (Fig. 2). Parsimony analysis yielded 6 minimum-length trees (length = 538, Consistency Index = 0.76, Retention Index = 0.82). Likelihood analysis under the best-fit GTR + Γ model resulted in two equally likely trees ($-\ln L = 3902.14$). Both parsimony and likelihood analyses recovered the same principal clades; the only differences among the various trees concerned relationships among individuals from Pitilla and Pailas and among individuals from Monteverde (Fig. 2). The analyses show large genetic differences between *Heteromys nubicolens* and *H. desmarestianus*. Although boundaries between species cannot be determined by percent sequence divergence alone, the genetic distance between these two species (12% *p*-distance if corrected only for within-species divergence; 13% with Jukes–Cantor correction) falls within the range of uncorrected distances between species of *Liomys* for cytochrome *b* (Rogers and Vance 2005). These differences between the two species corroborate the morphological and behavioral differences detailed in Anderson and Timm (2006), supporting the specific status of *H. nubicolens* relative to *H. desmarestianus*. However, the current limited taxonomic sampling within the genus precludes determination of the phylogenetic positions of these species relative to other *Heteromys*.

The two specimens (KU 158614 and KU 158615) tentatively assigned to *Heteromys desmarestianus* by Anderson and Timm (2006) indeed possess the mitochondrial DNA of that species. Due to the uniparental (maternal) inheritance of the mitochondrial gene cytochrome *b*, our analyses are insufficient to reject a hybrid origin of these individuals. The current results are consistent with these individuals being either: (1) pure *H. desmarestianus* or (2) hybrids between the two species (with maternal lines from *H. desmarestianus*). However, given that the majority of morphological characters match those of *H. desmarestianus*, and the lack of genetic evidence to the contrary, we continue to treat these specimens as *H. desmarestianus*. Future studies including nuclear markers are necessary to examine this possible hybrid zone in detail (cf., Tosi et al. 2003).

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