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5 **High genetic structure of the Cozumel Harvest mice, a critically endangered island**
6 **endemic: conservation implications**

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34 **Abstract**

35
36 We assessed the genetic structure and diversity of *Reithrodontomys spectabilis*, a critically
37 endangered, endemic rodent from Cozumel Island, México. A total of 90 individuals were
38 trapped from September 2001 to January 2005. Microsatellite data analysis revealed high
39 genetic diversity values: a total of 113 alleles (average 12.5 per locus), $H_o = 0.78$, $H_e = 0.80$.
40 These high values can be related to Cozumel's size (478 km²) and extensive native vegetation
41 cover, factors that could be promoting a suitable population size, high heterozygosity and the
42 persistence of rare alleles in the species, as well as some long-term movement of individuals
43 between sampling localities. A strong genetic structure was also observed, with at least four
44 genetic groups, associated with a pattern of isolation by distance. We found a strong allelic and
45 genetic differentiation shown between localities, with negligible recent gene flow and low
46 inbreeding coefficients. The species life history and ecological characteristics –being nocturnal,
47 semi-terrestrial, a good tree climber, having lunar phobia and significant border effect– are likely
48 affecting its genetic structure and differentiation. The high genetic diversity and population
49 structure award *R. spectabilis* a significant conservation value. Our results can serve as a basis
50 for future research and conservation of the species, particularly considering the problems the
51 island is facing from habitat perturbation, urbanization and introduction of exotic species. In
52 view of the structure and genetic variability observed, it is essential to establish and reinforce
53 protected areas and management programs for the conservation of the endemic and
54 endangered Cozumel Harvest mice.

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56 Keywords: Cozumel Island; genetic diversity; Mexico; microsatellites; *Reithrodontomys*
57 *spectabilis*

58

59 **Introduction**

60

61 Genetic diversity is one of the most basic components of biodiversity and its preservation is of
62 fundamental concern for conservation biology. Indeed, genetic diversity is of prime importance
63 for the long-term survival and evolution of species, and even more so for populations and
64 species that are threatened or endangered (Booy et al. 2000; Vázquez-Domínguez and Vega
65 2006; Allendorf and Luikart 2007). On islands, populations are often small and isolated, and
66 have ecological and genetic characteristics that render them as ideal natural experiments in
67 which to address questions about population genetics and evolution (White and Searle 2007).

68 Oceanic islands in particular are geographically and genetically isolated and represent closed
69 biological systems with limited area, low species numbers and high rates of endemism.
70 Endemism is usually associated with the genetic differentiation of populations after their
71 establishment from a reduced number of mainland migrants (Grant 1998). Other genetic factors
72 related to geographic isolation that make island populations particularly vulnerable are founder
73 effects, genetic bottlenecks, loss of genetic and allelic variability, genetic drift and inbreeding.
74 The interaction of these factors with environmental and ecological processes, i.e. habitat
75 perturbation, introduction of exotic species, reduced competitive ability, high disease
76 susceptibility and lack of biological mechanisms to avoid introduced predators, are all associated
77 with the higher risk of extinction that island populations have compared with their mainland
78 counterparts (Frankham 1997; Hinten et al. 2003).

79 Among insular mammals, rodents deserve special attention because of their relatively
80 high degree of representation in the insular fauna and high endemism. Additionally, there is a
81 general lack of information regarding their population status and less attention is paid to their
82 conservation in comparison with other non-endemic, continental rodents (Amori et al. 2008).
83 Ironically, rodent species on island ecosystems are often considered under a negative context,
84 associating them with the well-known ecological damage caused by a few widely introduced
85 pest species (e.g. *Rattus rattus* and *Mus musculus*), while the fate of endemic species is
86 frequently overlooked. In fact, islands maintain an enormous and largely irreplaceable heritage
87 of rodent biological diversity (Ceballos and Brown 1995). In Mexico, 27 islands harbor endemic
88 mammal species, 23 of which are rodents, and many are considered threatened (Ceballos et al.
89 1998; Semarnat 2010). In addition, there is still rather limited knowledge regarding their genetic
90 status (Vázquez-Domínguez and Vega 2006).

91 Our study was carried out on Cozumel Island, Mexico, an oceanic island that harbors at
92 least 31 taxa of endemic animals (crustaceans, fishes, reptiles, terrestrial mammals and birds;
93 Cuarón et al. 2009). Although the island retains much of its natural vegetation, in recent years
94 serious threats such as exotic species, hurricanes and habitat fragmentation have jeopardized
95 its native biota (Walton 2004; Cuarón et al. 2009). Three endemic rodent species inhabit
96 Cozumel, *Oryzomys couesi cozumelae*, *Reithrodontomys spectabilis* and *Peromyscus*
97 *cozumelae*, although the latter is probably extinct (Vega et al. 2007; Fuentes-Montemayor et al.
98 2009). We have gathered, through the long-term study of their populations, detailed information
99 about the ecology and demography of the former two species (Fortes-Corona 2004; Fuentes-
100 Montemayor et al. 2009; Vázquez-Domínguez et al. 2012), although the population genetics has
101 only been studied for *O. c. cozumelae* (Vega et al. 2007; Sunny 2010).

102 The Cozumel Harvest mouse, *Reithrodontomys spectabilis* (Jones and Lawlor 1965) is
103 slightly bigger than its closest relative, the geographically adjacent *R. gracilis*. The tail is scantily
104 haired, long in relation to the head and body, with an overall brownish ochraceous coloration on
105 the upperparts and brighter on the sides, while the underpart is grayish white (Jones 1982). It is
106 nocturnal, semi-terrestrial, a good tree climber; it shows lunar phobia, i.e. little individual
107 dispersal during full moon days, a strategy to avoid predators (Fuentes-Montemayor et al. 2009).
108 A significant border, or edge, effect has also been observed for this rodent, directly associated
109 with age and reproductive condition (e.g. the proportion of adults and reproductive individuals is
110 higher near edges and juveniles are only found far from edges; Fortes-Corona 2004; Fuentes-
111 Montemayor et al. 2009). *R. spectabilis* is extremely scarce on the island as a result of a
112 significant population decline over the last decades (3.3 ind/ha; Fuentes-Montemayor et al.
113 2009), mainly as a consequence of introduced species (boas, feral dogs and cats, house mouse
114 and black rat) as shown by our group's research (Fortes-Corona 2004; Bautista-Denis 2006;
115 González-Baca 2006; Romero-Nájera et al 2007; Sotomayor-Bonilla 2009; Vázquez-Domínguez
116 et al. 2012). It is currently classified as Threatened by Mexican law (Semarnat 2010) and as
117 Critically Endangered by the IUCN red list (Cuarón et al. 2008).

118 Considering the necessity of incorporating genetic information into conservation efforts,
119 our aim in this study was to assess the genetic structure and diversity of *R. spectabilis*. We
120 expected, based on the species life history, ecological characteristics and island endemic status,
121 that it will show low genetic variability levels, high genetic structure and differentiation and
122 reduced gene flow. Our results provide crucial information for the conservation of this endemic
123 species and more generally for efforts aimed at the long-term preservation of the genetic wealth
124 of Cozumel's native biota.

125

126 **Material and methods**

127

128 Population sampling and DNA extraction

129

130 Cozumel Island is the largest island in the Mexican Caribbean (ca. 486 km²), located 17.5 km off
131 the Yucatán peninsula in the Caribbean Sea (20°16'18.2' ' - 20°35'32.8" N; 86°43'23.3' '-
132 87°01'31.1" W). The island was formed between the Oligocene and the Pleistocene, for which
133 no land bridge is recognized, thus it is classified as oceanic (Weidie 1985). It has a vegetation
134 gradient, formed mainly by semi-evergreen tropical forest and less extended subdeciduous
135 tropical forest and mangroves (Romero-Nájera et al. 2007; Vázquez-Domínguez et al. 2013). It

136 is practically flat with no geographical barriers or superficial water bodies except for a few
137 cenotes (sinkholes).

138 Intensive fieldwork was done throughout the island between September 2001 and
139 January 2005, during which we systematically performed trapping sessions with Sherman and
140 other kinds of traps, as a part of an ongoing study on the ecology, evolution, genetics and
141 conservation of the Cozumel biota (e.g. Cuarón et al. 2004, 2009; Romero-Nájera et al. 2007;
142 Vega et al. 2007; Vázquez-Domínguez et al. 2012). In each site, we placed two quadrants,
143 each with 49 Sherman live traps separated by approximately 8.5 m from each other in a 7 x 7
144 trap-grid arrangement. They were baited with a mixture of rolled oats, peanut butter and vanilla
145 extract. Despite the geographically extended sampling effort we successfully trapped *R.*
146 *spectabilis* only at six localities distributed in the north (MANGLAR and POTABILIZADORA
147 localities), northwest (PALMAS), east (MEZCALITOS) and central regions (CAPA4.4 and
148 CAPA4.8) of the island (Fig.1). Nonetheless, the distribution of these six localities covers almost
149 all the island, with the exception of the southernmost part. A total of 90 *R. spectabilis* individuals
150 were obtained. Tissue samples from each individual were ethically obtained, with the
151 corresponding collecting permits (Semarnat-FAUT-0168), and stored in labeled Eppendorf tubes
152 with 90% ethanol until later use. Techniques used are in compliance with guidelines published
153 by the American Society of Mammalogists for use of wild mammals in research (Gannon et al.
154 2007).

155 We performed DNA extraction with the QuickGene DNA Tissue Kit (Fujifilm Life
156 Sciences), following the manufacturer's protocol. We assessed DNA quantity and quality with
157 1% agarose gels stained with 0.5 µg/ml ethidium bromide and visualized with UV light.

158
159 **Microsatellite typing**

160
161 The genotypes of each individual were characterized with 10 microsatellite loci, using
162 fluorescently labeled microsatellite primers developed specifically for *R. spectabilis* (Vázquez-
163 Domínguez and Espindola 2013). We performed DNA amplification by Polymerase Chain
164 Reaction (PCR) in a 5 µl total volume as described in Vázquez-Domínguez and Espindola
165 (2013). Microsatellites products were multiplexed and run on an ABI Prism3730xl and 3100
166 Genetic Analyzer (Applied Biosystems), with ROX-500 as internal size standard and allele size
167 determined with the software GeneMarker v.1.97 (SoftGenetics). We included negative controls
168 in all runs and sized multiple samples at least twice to assure reproducibility and correct
169 readings.

170

171 Statistical analysis

172

173 We evaluated the presence of null alleles and stuttering with the program Micro-Checker v.2.2.3
174 (Van Oosterhout et al. 2004), using a 95% confidence interval and 1000 repetitions. Significant
175 frequency of null alleles was found at locus mexr32, thus it was removed from analyses. For
176 each sampling locality and for the entire Cozumel population, we examined possible departures
177 from Hardy-Weinberg equilibrium (HWE) with an exact test and linkage disequilibrium (LD) by a
178 log-likelihood ratio statistic (G-test). Allelic frequencies and F_{IS} statistics were estimated to
179 evaluate heterozygote deficit or excess, using GenePop v.4.0 (Raymond and Rousset 1995),
180 and significance tests were done using the Markov chain method implemented in GenePop,
181 using 10,000 as dememorization steps, 1,000 batches and 10,000 iterations per batch. Where
182 necessary, α value was adjusted for multiple comparisons applying a Bonferroni correction (Rice
183 1989). We assessed genetic variability for each sampling locality and for Cozumel by
184 calculating the observed (n_o) and effective (n_e) number of alleles, observed (H_o) and expected
185 (H_e) heterozygosity and Nei's unbiased expected heterozygosity (H_{NEI} ; Nei 1978), using the
186 program GENALEX v.6 (Peakall and Smouse 2006).

187 We used several approaches to assess the degree of genetic structure and
188 differentiation between sampling localities. First, we defined the population subdivision on the
189 island with a Bayesian clustering method that uses multilocus genotype data and identifies the
190 number of K clusters (genetic groups), with the software STRUCTURE v.2.3 (Pritchard et al.
191 2000). STRUCTURE was run with values of $K = 1$ to 8, using the admixture and the correlated
192 allele frequencies models. Twenty runs were performed for each value of K , based on 100,000
193 Markov chain Monte Carlo iterations and a burn-in period of 50,000. In order to evaluate if the
194 STRUCTURE results differed, we also performed the analysis using the sampling localities as a
195 priori. For both cases, we determined the number of genetic groups (K) by comparing mean
196 values and variability of log likelihoods from each run, using the ΔK method of Evanno et al.
197 (2005), which is an ad hoc statistic based on the rate of change in the log probability of data
198 between successive K values and that accurately detects the uppermost hierarchical level of
199 structure (Evanno et al. 2005). The majority of individuals were assigned to a genetic group
200 based on a 70% or higher probability of membership in the STRUCTURE analysis. The genetic
201 group of origin of individuals with a lower probability was established by performing an
202 assignment test with GeneClass v2.0 (Piry et al. 2004), using the programs' default settings.

203 Next, we used GenePop v.4.0 to evaluate the allelic and genotypic differentiation

204 between sampling localities with a G exact test (Goudet et al. 1996). F_{ST} was estimated within
205 sampling localities and at the global level (Cozumel), based on Weir and Cockerham's (1984)
206 approach using FSTAT v.2.9.3 (Goudet 1995). Finally, Nei's genetic distance (D_{NEI}) between
207 sampling localities was estimated with GENALEX v.6.

208 In order to explore if gene flow was present, we estimated the migration rate between
209 sampling localities with BIMr V.1.0 (Faubet and Gaggiotti 2008), a program that makes
210 inferences about recent proportions of immigrant genes in subdivided populations. As opposed
211 to other methods that estimate migration rates over an evolutionary scale, BIMr estimates
212 migration during the last generation. We used a burn-in period of 100,000, a sample size of
213 100,000 and a thinning interval of 50 iterations, with the F -model and default values of pilot runs,
214 priors and incremental values. To verify convergence we performed three independent runs.
215 Inbreeding coefficient per locality was also estimated with the BIMr analysis. We also performed
216 a Mantel test to evaluate the fit of the data to a pattern of isolation by distance, based on F_{ST}
217 pairwise estimates and linear geographic distances between sampling localities, using the
218 program IBDWS v3.15 with 30,000 permutations (Jensen et al. 2005). Finally, distribution of the
219 genetic variance considering different hierarchical levels (sampling localities, genetic groups and
220 individuals) was examined using a molecular analysis of variance (AMOVA), based on F_{ST} as
221 implemented by ARLEQUIN v.3.01 (Excoffier et al. 2005). Significance was calculated using a
222 non-parametric test with 30,000 permutations.

223 In order to test if geographic location may have an effect on genetic differentiation we
224 used a hierarchical Bayesian method (GESTE v.2.0; Foll and Gaggiotti 2006). This analysis was
225 based on the estimated sampling localities' specific F_{ST} s that can be interpreted as a measure of
226 genetic differentiation between each local population and the migrant pool. We considered
227 sampling locality coordinates (latitude and longitude) as geographic factors and estimated the
228 posterior probability of five alternative models: 1) geographic location does not have an effect on
229 genetic differentiation, 2) effect of latitude only, 3) effect of longitude only, 4) effect of both
230 latitude and longitude, and 5) interaction effect. In addition, we used connectivity (i.e., average
231 distance from one locality to all others) to test its effect, while the null model was with no effect.
232 The method provides posterior probabilities for each model tested using a Reversible Jump
233 MCMC approach, where the model with the highest posterior probability is the one that best
234 explains the data. We used 100,000 iterations with a burn-in period of 10,000 and a thinning
235 interval of 100. Three independent runs with these settings were performed to verify
236 convergence.

237 As another indicator of differentiation at the individual level, we evaluated relatedness
238 among individuals with the program ML-RELATE (Kalinowski et al. 2006), which has the
239 advantages that it is designed for microsatellites, is based on maximum likelihood tests and
240 considers null alleles. Also, to explore demographic information of *R. spectabilis*, we estimated
241 the effective population size applying a method that uses linkage disequilibrium (LD) and a
242 random mating system to estimate effective population size (N_e) values (NeEstimator v.1.3; Peel
243 et al. 2004). In addition, we used MSVAR 1.3 (Storz and Beaumont 2002) to infer its historical
244 demography, an approach that allows detecting recent changes in population size (Girod et al.
245 2011). Simulation runs consisted of 100,000 steps with a thinning interval of 20,000 iterations
246 and a 10% of burn-in; two independent runs were performed to ensure consistency of estimates.
247 We used Gelman and Rubin's (1992) convergence diagnostic to check MCMC convergence.
248 Because we found population structuring (see Results), both effective population size and
249 historical demography were estimated for each genetic group, an analyses that ought to be
250 based on panmictic units.

251

252 **Results**

253

254 Genetic diversity

255

256 After Bonferroni correction, only the locus mexr19 showed significant deviation from Hardy-
257 Weinberg equilibrium at CAPA4.8, due to an excess of homozygotes ($p=0.006$). When
258 considering the entire population (Cozumel), only one pairwise locus, out of the 36 possible
259 comparisons, was in linkage disequilibrium. Given that no significant deviation was associated
260 with any locus in particular, we considered the nine loci as independent markers.

261 Regarding genetic diversity values, a total of 113 alleles across the nine loci were
262 obtained, with a range of 3-18 (average 12.5) alleles per locus for Cozumel (Table 1). Mexr13
263 was the locus with the highest observed and effective number of alleles (18 and 9.5,
264 respectively) and the locus with the lowest number was mexr23 (3 and 1.4, respectively).
265 Observed and expected heterozygocities showed high values in all sampling localities ($H_o =$
266 0.773 to 0.838; $H_E = 0.659$ to 0.765; $H_{NEI} = 0.754$ to 0.796). MEZCALITOS was the only
267 sampling locality that did not have private alleles (Table 1).

268

269 Population structure

270

271 STRUCTURE results defined four genetic groups ($\text{LnP}(K = 4) = -3542.8$) (Fig. 2), with 60% of
272 individuals assigned to a particular group ($Q = 0.7$) (Fig. 1). We were able to assign the rest of
273 individuals, in accordance with the STRUCTURE subdivision, using the assignment analysis. The
274 genetic groups did not differ when based on sampling localities as *a priori* in the STRUCTURE
275 analysis. Each genetic group was dominated by a sampling locality, although there was
276 admixture in some: MANGLAR and POTABILIZADORA had a similar genetic composition
277 (pairwise $F_{ST} = 0.008$) and the same was observed for PALMAS and MEZCALITOS (pairwise
278 $F_{ST} = 0.024$). On the other hand, CAPA4.4 and CAPA4.8 differentiated more clearly from the
279 rest (pairwise $F_{ST} > 0.042$) and between each other (pairwise $F_{ST} = 0.099$). We named the four
280 genetic groups as NORTH, NORTHWEST, CAPA4.4 and CAPA4.8, respectively (Fig. 1).

281 A strong allelic and genetic differentiation was found in the pairwise comparisons (G
282 exact test; $p < 0.05$). Similarly, the global F_{ST} value (estimated with GESTE) was 0.060, while F_{ST}
283 values per sampling locality were highest for CAPA4.8 (0.124; 0.072-0.179, 95% C.I.) and
284 CAPA4.4 (0.066; 0.036-0.099, 95% C.I.), followed by MANGLAR (0.054; 0.0003-0.130),
285 PALMAS and POTABILIZADORA (0.038; 0.021-0.057 and 0.038; 0.020-0.059, respectively),
286 and 0.07 (0.011-0.135) for MEZCALITOS. Nei's genetic distances showed the highest values
287 between CAPA4.4 and MANGLAR (0.934) and the lowest between PALMAS and
288 POTABILIZADORA (0.288) (Table 2).

289 Results of the migration rate between sampling localities showed values that are
290 essentially zero for all locality pairs, indicating that migration (gene flow) during the last
291 generation was negligible between them, whereas inbreeding coefficients were also very low
292 (average 0.017 to 0.086). In relation with the AMOVA results, genetic variation resided mainly
293 within individuals (96.1%; $p > 0.05$), while genetic variation among sampling localities within
294 genetic groups and among genetic groups was 2.5% and 3.56%, respectively ($p < 0.01$).

295 Regarding the analyses used to test if geographic location had an effect on genetic
296 differentiation, posterior probability results for the five models tested showed that the null model
297 (geographic location does not have an effect on genetic differentiation) had the highest posterior
298 probability (0.64). The other models (effect of latitude only, effect of longitude only, effect of
299 both latitude and longitude, and interaction effect) had 0.205, 0.123, 0.029 and 0.005 posterior
300 probability values, respectively. When we tested the connectivity scenarios, results indicated no
301 effect, with a 0.87 posterior probability. The Mantel test showed a significant albeit low
302 association between pairwise estimates of F_{ST} and linear geographic distances ($R^2 = 0.155$,
303 $r = 0.394$, $p = 0.029$), which can be associated with a weak pattern of isolation by distance.

304

305 Population size, relatedness and bottlenecks

306
307 The effective population size (N_e) estimated for each genetic group was 70.2 (43.2-165.7, 95%
308 C.I.) for CAPA4.4; 11 (8.2-15.8, 95% C.I.) for CAPA 4.8; 84 (50.6-215.3, 95% C.I.) for
309 NORTHWEST; and 178.7 (103-573.4, 95% C.I.) for NORTH. Relatedness results showed a
310 high proportion of unrelated individuals (86.9%), followed by half-siblings (11.8%), siblings
311 (0.9%) and parent/offspring (0.4%) for Cozumel. Proportion of relatedness of individuals within
312 each sampling locality was similar (data no show); however, in the case of CAPA4.8, we found a
313 higher percentage of parent/offspring and siblings (7.2 and 7.2%, respectively). Finally, MSVAR
314 results suggested that there has not been a significant population size reduction or expansion in
315 *R. spectabilis* (Table 3). Change ratio (N_{e1}/N_{e0}) for each genetic group was: 0.86 for CAPA4.4;
316 0.90 for CAPA4.8; 1.02 for NORTHWEST; and 1.07 for NORTH, which suggest that effective
317 population size has remained stable.

318

319 Discussion

320

321 Information about the genetics of species, e.g. diversity levels, structure, bottlenecks and
322 inbreeding, among others, is of fundamental value for the preservation of biodiversity, and it has
323 been recognized that genetic variability within and between populations should be evaluated and
324 considered in conservation and protection plans (Frankham et al. 2005; Vázquez-Domínguez
325 and Vega 2006; Allendorf and Luikart 2007); nonetheless, it has been regularly ignored in
326 conservation strategies worldwide. Moreover, the long-term survival and evolution of
327 populations and species often depends on their genetic variability, given that genetic loss or
328 erosion can reduce the potential of populations to adapt to new or changing environments and
329 can also diminish individual fitness, increasing the risk of extinction (Gibbs 2001; Frankham et al.
330 2005).

331 *Reithrodontomys spectabilis* showed high levels of genetic and allelic diversity ($H_o =$
332 0.789 and $n_a = 12.5$), in contrast with what is expected for island populations. Many studies of
333 small and medium sized island mammals using microsatellites show a pattern characterized by
334 low levels of genetic and allelic diversity (Eldridge et al. 1999, 2004; Hinten et al. 2003;
335 Abdelkrim et al. 2005; Wang et al. 2005), mainly associated with small population size, isolation,
336 founder effect, genetic bottlenecks, inbreeding and small island size. On the other hand, it has
337 been observed that species with high dispersal capacity, like bats (Rossiter et al. 2000;
338 Vázquez-Domínguez et al. 2013), or those inhabiting islands of great size (Hinten et al. 2003;

339 White and Searle 2007), are able to maintain high genetic diversity levels. It is notable that, as
340 observed for *R. spectabilis*, relatively high genetic variability has also been found for *Oryzomys*
341 *couesi cozumelae*, despite the small population size also shown by this species in the island
342 (Vega et al. 2007). *R. spectabilis*' genetic diversity can be related, as with *O. c. cozumelae*, to
343 Cozumel's size (478 km²) and extensive native vegetation cover, factors that could be promoting
344 a suitable population size, high heterozygosity and/or the persistence of rare alleles for these
345 species.

346 We found genetic structuring and high genetic differentiation for *R. spectabilis* on the
347 island, revealed as four distinct genetic groups and high genetic distance values between
348 sampling localities, suggesting certain degree of isolation. Also, negligible recent gene flow
349 between sampling localities and a small albeit significant isolation by distance were detected. It
350 is likely that we are detecting a relatively recent genetic structure, in particular because this
351 species historically had a more continuously distributed population throughout the island (Jones
352 and Lawlor 1965; Gutiérrez-Granados 2003). The fact that admixture was observed, in which
353 individuals from different sampling localities are clustered together (i.e. they share allele
354 frequencies) supports this. Capture-recapture studies in Cozumel (see Vega et al. 2007,
355 Fuentes Montemayor et al. 2009, and references therein) have shown that rodents on the island
356 move only short distances and rarely cross the c. 3-m wide gravel roads, whereas the main
357 transversal 20-m wide paved road that crosses the island in a southeast-northwest direction
358 (Fig. 1), somehow fragmenting it in a north and a south segment, is related with significant
359 genetic structuring in *O.c. cozumelae*, and likely with the structuring and observed pattern of
360 isolation by distance for *R. spectabilis*. Also, it is important to consider the biological and life
361 history features that characterize *R. spectabilis*. It is semi-arboreal with good climbing abilities
362 and has specific habitat requirements, favoring undisturbed sites with dense vegetation and far
363 from habitat edges (Fuentes-Montemayor et al. 2009). These characteristics can promote little
364 movement between patches or throughout long distances, facilitating genetic differentiation
365 between localities along the island. However, there is scarce information about the relationship
366 between habitat requirements and genetic structure in mammals (Loew et al. 2005), thus this will
367 need to be evaluated directly. Strong genetic structure has been found in different rodent
368 species that are highly fragmented for which, consequently, their populations behave as islands
369 (see Loew et al. 2005; Castañeda-Rico et al. 2009) and are comparable to island rodents, like
370 the case of *Ctenomys magellanicus* of Isla Grande de Tierra del Fuego (Argentina) (Fasanella et
371 al. 2013) and *Nesoryzomys narboroughi* of the Galapagos Islands (Johnson 2005).

372 Behavior also has effects on genetic structure in animals. *R. spectabilis* shows lunar
373 phobia, a strategy to evade predation that has been associated with its lower abundance and
374 less activity near edges (Fuentes-Montemayor et al. 2009). The latter limits long distance
375 movements of individuals, again favoring differentiation between populations. Different studies
376 have related behavior, life history and social characteristics with population structure; for
377 instance Vázquez-Domínguez et al. (2002) showed that female philopatry and sex-biased
378 dispersal influence genetic structure in the rodent *Liomys pictus*. Kraaijeveld-Smith et al. (2007),
379 in a comparative study with three sympatric species showed that genetic structure is defined by
380 male dispersal in the marsupials *Antechinus agilis* and *A. swainsonii*, while for the third, the
381 rodent *Rattus fuscipes*, is related with its small size and habitat requirements that result in very
382 little dispersal (see also Paetkau et al. 2009). It is interesting to notice the high differentiation
383 shown by the two central sampling localities, CAPA4.4 and CAPA4.8 (Fig. 1), considering that
384 they are separated by the shortest distance (3.02 km) between all localities; such structuring is
385 in agreement with Fuentes-Montemayor et al. (2009), who found that *R. spectabilis* in CAPA is
386 distributed within vegetation patches in accordance with sex and age differences. CAPA4.8, the
387 most differentiated locality ($F_{ST} = 0.124$), showed a significant deficit of heterozygotes in one
388 locus (mexr19), which can be an indicator of inbreeding. CAPA is the most conserved area on
389 the island, with extensive semi-evergreen tropical forest and good vegetation cover, providing
390 resources like refuge and food, likely contributing to the low dispersal of this species.

391 The effective population size (N_e) observed for each genetic group was between 11 and
392 179 individuals, and although this estimate is sensitive to sample size and could be under-
393 estimated (England et al. 2005), it gives a good approximation as to the low population numbers
394 on the island for this species (Sunny et al. 2014). N_e is a measure directly related with the rate
395 of genetic diversity loss and the increase of inbreeding in a population, and the rule of thumb
396 suggests that a N_e higher than 50 is needed to minimize consanguinity effects, while higher than
397 500 to retain adaptive genetic variation (Allendorf and Ryman 2002). If we add the fact that it is
398 affected by population size fluctuations, a pattern observed for *R. spectabilis* in Cozumel due to
399 the island's strong seasonality, the estimated effective population size could be even lower.
400 Multiple negative consequences can be associated with a small population size, both at the
401 demographic and genetic levels (Frankham 1998; Eldridge et al. 1999). Regarding relatedness,
402 our results show that the highest percentage is of unrelated individuals, which suggests that
403 there is low chance of inbreeding. Given the fact that there is little exchange of individuals
404 among populations and that most of the genetic variability resides within individuals, the low
405 relatedness observed could be explained by mating behavior, often associated with differential

406 male dispersal, although in the present case it could not account for the low relatedness
407 observed. A plausible explanation in addition to mating behavior includes juvenile mortality,
408 which will result in few relatives coexisting within a colony (Dixon 2011). In accordance, adult
409 and juvenile mortality are probably high in Cozumel, directly related to the edge effect detected
410 for the species (Fuentes-Montemayor et al. 2009), and also to the strong seasonality and natural
411 disturbances like hurricanes and tropical storms that affect the island on a regular basis. Our
412 results indicated no recent population changes, without significant bottleneck or expansion
413 events, however we need to consider it may be due to a lack of power, thus this needs to be
414 further explored.

415

416 **Conservation implications**

417

418 *Reithrodontomys spectabilis*, despite being an island endemic and a critically endangered
419 species, maintains high levels of genetic and allelic diversity, a result that adds significance to its
420 conservation value and confers an advantage for its potential future. Also, while genetic
421 evidence suggests a degree of differentiation between sampling localities, they are not
422 completely isolated. Urbanization, road construction and deforestation, which have increased in
423 recent years on the island, will exacerbate the isolation of groups and may potentially lead to the
424 extinction of the species (Neuwald 2010), specially considering the low effective population size
425 suggested by our results. Hence, conservation actions should aim to maintain enough suitable
426 habitat and connectivity between populations that sustain the natural dynamics of this rodent, in
427 order to preserve its genetic diversity and survival on the long term.

428 The present genetic information can serve as a basis for future research and
429 conservation of *R. spectabilis*, particularly considering the current conservation problems the
430 island is facing from habitat perturbation, urbanization and introduction of exotic species (for
431 examples see Vega et al. 2007; Cuarón et al. 2009; Fuentes-Montemayor et al. 2009; Vázquez-
432 Domínguez et al. 2012). Indeed, factors like exotic species, hurricanes and anthropogenic
433 activities affect population numbers, with the consequent loss of genetic diversity. In Cozumel,
434 feral dogs and cats and boas (*Boa constrictor*) are abundant and widely distributed, and it has
435 been demonstrated they have devastating effects on the native biota of the island, including the
436 rodent fauna, which have become part of these exotics' pray (Bautista-Denis 2006; Romero-
437 Nájera et al. 2007; Vázquez-Domínguez et al. 2012). The removal of introduced competitors
438 (*Mus musculus* and *Rattus rattus*; Sotomayor-Bonilla 2009) and predators is therefore a
439 conservation priority. The present genetic information is also crucial for a potential conservation

440 strategy, which could involve captive breeding of wild individuals for later reintroduction to
441 protected areas within the island. Natural disturbances can also have catastrophic
442 consequences. We have documented significant population declines and genetic diversity loss
443 for the rodent species after two hurricanes, Emily and Wilma (Level 4) that severely affected the
444 island in 2005 (Vázquez-Domínguez et al. in prep.). For all the above, it is essential to establish
445 and reinforce protected areas and management programs for the conservation of this critically
446 endangered endemic rodent, and which takes into consideration the structure and genetic
447 variability we have documented.

448

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463

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637

638

639 **Figure legends**

640

641 **Fig. 1** Genetic structure of *Reithrodontomys spectabilis* from Cozumel Island. **a)** Map depicting
642 sampling localities (black points) in Cozumel: **1** = CAPA4.4, **2** = CAPA4.8, **3** = MANGLAR, **4** =
643 MEZCALITOS, **5** = PALMAS, **6** = POTABILIZADORA. Grey lines represent main and secondary
644 roads; main transversal paved road is marked with a blue line. Pie charts on the map show
645 proportion of membership of each sampling locality in each of the genetic groups ($K = 4$)
646 identified with STRUCTURE. **b)** STRUCTURE results for the genetic groups obtained are indicated
647 as we named them (see Results) and with a different color: yellow = CAPA4.4, blue = CAPA4.8,
648 red = NORTHWEST, green = NORTH. Sampling locality (x-axis) and inferred ancestry percent
649 (y-axis) are shown

650

651 **Fig. 2** Posterior probabilities of K estimated with STRUCTURE. Mean (\pm SD) of log probability of
652 the data is shown for each estimated K value.

Figure 1
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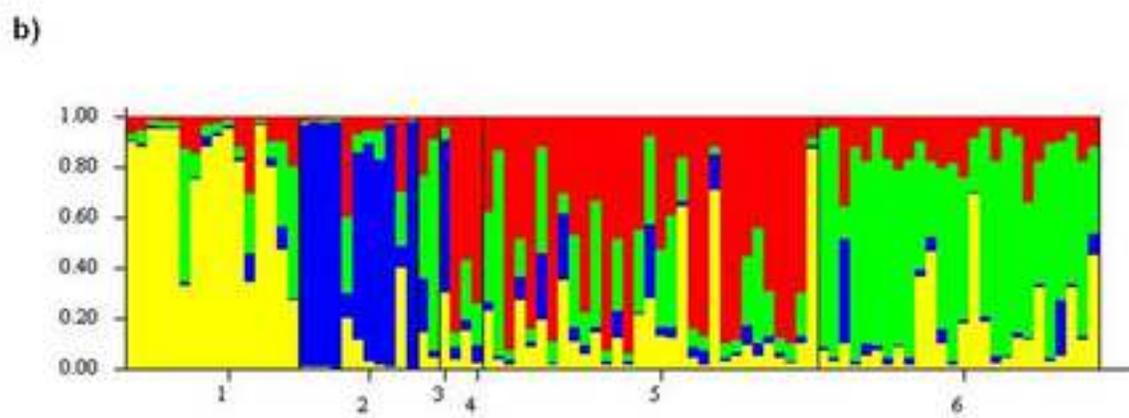
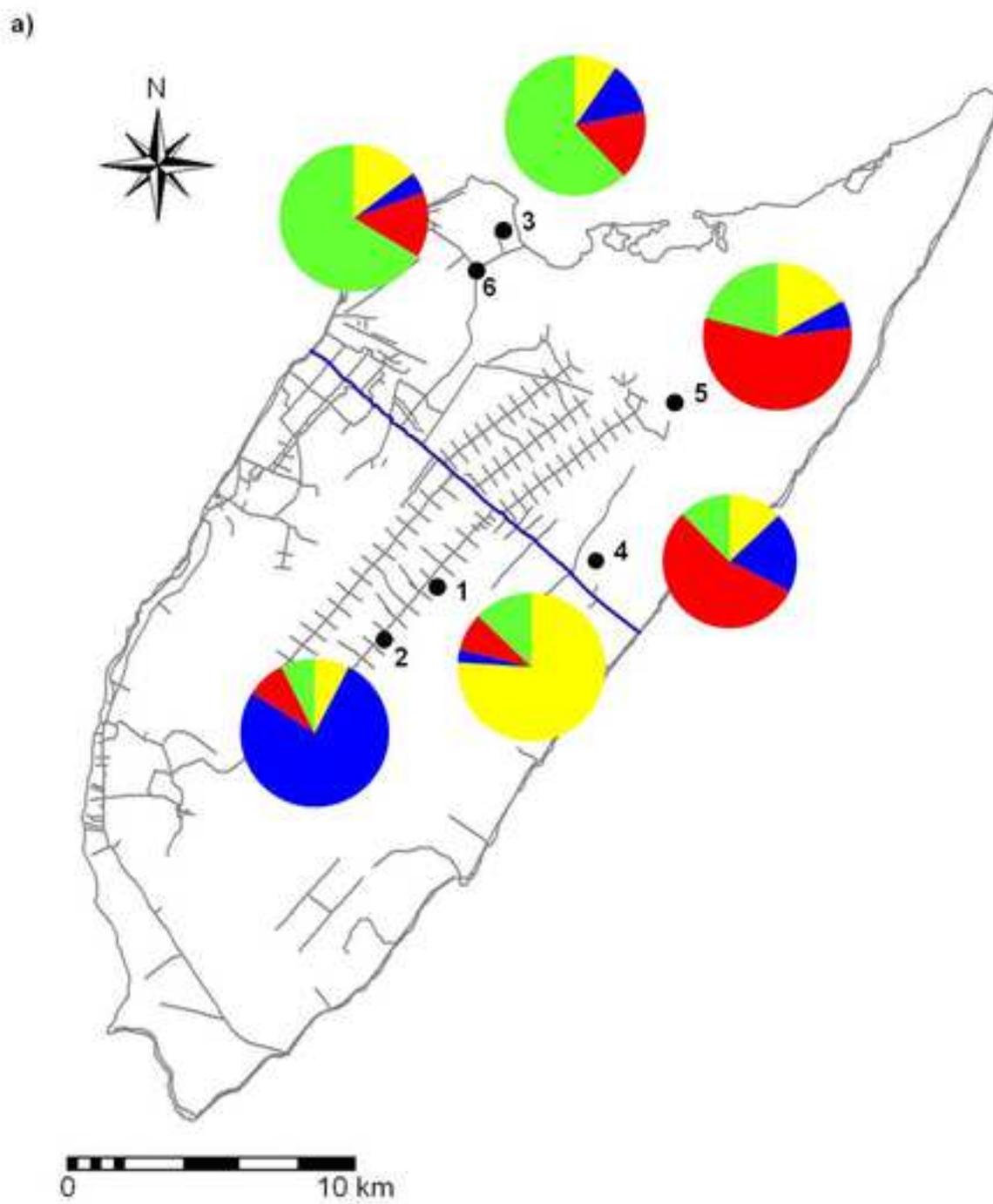


Figure 2
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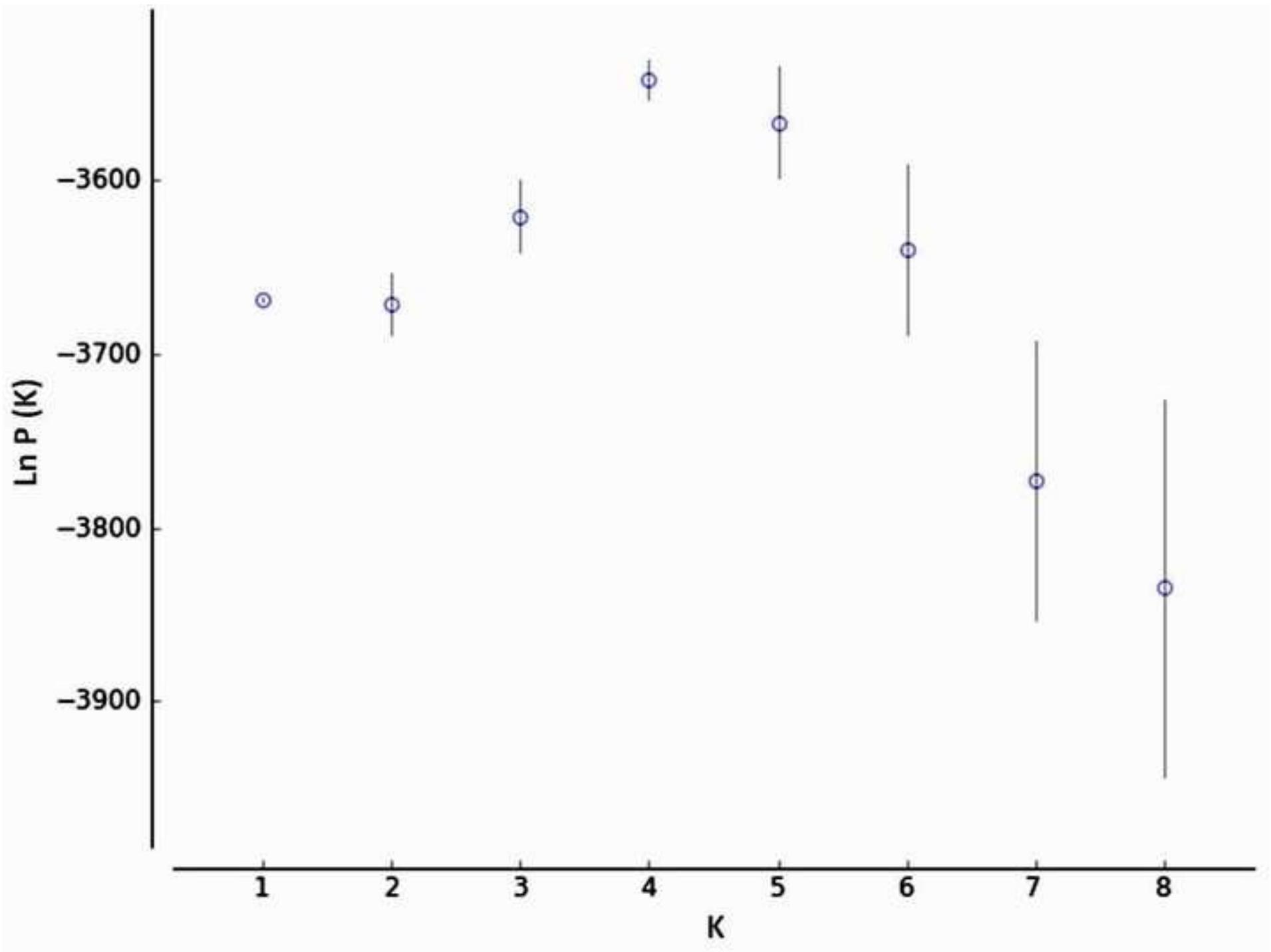


Table 1 Genetic diversity values for *Reithrodontomys spectabilis* for each sampling locality and the entire Cozumel Island. N = sample size, n_a = number of alleles, n_e = number of effective alleles, n_p = number of private alleles, H_o = observed heterozygosity, H_E = expected heterozygosity, H_{Nei} = Nei's unbiased expected heterozygosity, F_{IS} = Fixation index.

Locality	N	n_a	n_e	n_p	H_o	H_E	H_{Nei}	F_{IS}
CAPA 4.4	16	7.556	5.373	0.444	0.799	0.759	0.784	-0.039
CAPA 4.8	11	5.889	4.147	0.111	0.838	0.732	0.767	-0.111
MANGLAR	2	2.889	2.548	0.222	0.833	0.597	0.796	-0.125
MEZCALITOS	4	4.222	3.422	0	0.778	0.659	0.754	-0.063
PALMAS	31	9.667	5.716	0.666	0.774	0.765	0.778	-0.005
POTABILIZADORA	25	9.667	5.536	0.889	0.773	0.755	0.770	-0.010
COZUMEL	90	12.56	7.096	--	0.789	0.804	0.808	-0.021

Table 2 Nei's genetic distance between sampling localities for *Reithrodontomys spectabilis* from Cozumel Island. Codes refer to C4.4 = CAPA4.4, C4.8 = CAPA 4.8, MA = MANGLAR, ME = MEZCALITOS, PA = PALMAS, PO = POTABILIZADORA.

	C4.4	C4.8	MA	ME	PA
C4.4	--				
C4.8	0.687	--			
MA	0.934	0.823	--		
ME	0.590	0.521	0.845	--	
PA	0.361	0.559	0.680	0.457	--
PO	0.408	0.594	0.504	0.650	0.288

Table 3 Marginal posterior density of ancestral N_e , current N_e and time since populations started to decline/expand, estimated per genetic group for *Reithrodontomys spectabilis* from Cozumel Island.

Genetic group		Mean	SD
CAPA 4.4	Ancestral N_e	4.4599	1.1905
	Current N_e	3.8427	0.9083
	Time	4.3228	2.3278
CAPA 4.8	Ancestral N_e	4.4827	1.5589
	Current N_e	4.0408	1.0831
	Time	5.3609	2.3135
NORTHWEST	Ancestral N_e	4.2736	1.6034
	Current N_e	4.3647	0.9590
	Time	5.5113	2.2558
NORTH	Ancestral N_e	4.3034	1.4818
	Current N_e	4.6349	1.1711
	Time	5.1129	2.4414