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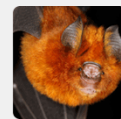
Evolutionary scenarios associated with the *Pteronotus parnellii* cryptic species-complex (Chiroptera: Mormoopidae)

Dear Dr RICARDO LOPEZ-WILCHIS,

I am pleased to inform you that your manuscript, entitled: Evolutionary scenarios associated with the *Pteronotus parnellii* cryptic species-complex (Chiroptera: Mormoopidae), has been finally accepted for publication in our journal. It is planned to be printed in the coming issue (April 2016).

Thank you for submitting your work to us.

Yours sincerely,
Wiesław Bogdanowicz
Editor-in-Chief
Acta Chiropterologica



Evolutionary scenarios associated with the *Pteronotus parnellii* cryptic species-complex (Chiroptera: Mormoopidae)

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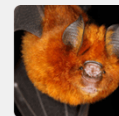
Original paper

Abstract:

One of the major challenges to understanding the evolution of Neotropical bats concerns our capacity to successfully scrutinize phylogenetic patterns associated with cases of cryptic species complexes. In this study *Pteronotus parnellii* is examined as a selected example of a known lineage of mormoopid bat that potentially contains several cryptic species. A samples of 452 individuals from 83 different localities, essentially covering its entire mainland distribution, was evaluated using two genetic markers: COI (mitochondrial) and DBY (nuclear) genes. The findings of this study strongly support the hypothesis of high genetic variability and identify at least six lineages within *P. parnellii*, some of which appear to be cryptic species.

Keywords:

Bats, COI, DBY, Neotropical America, Genetic diversity, Biogeography, Phylogeny, taxonomy



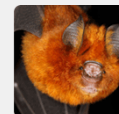
1 Evolutionary scenarios associated with the *Pteronotus parnellii* cryptic species-complex
2 (Chiroptera: Mormoopidae)

3 INTRODUCTION

4 The Neotropical region is home to the greatest diversity of bats in the world, and it is well
5 known that this fauna awaits a number of basic lines of research, among which cryptic
6 diversity stands as one of the most relevant. Thus, despite recent efforts to address the
7 issue, not only in the Neotropics (Clare, 2011; Clare *et al.*, 2011; Pavan *et al.*, 2011; Larsen
8 *et al.*, 2012; Hernández-Dávila *et al.*, 2012; Pavan *et al.*, 2013; Velazco and Patterson,
9 2013; Parlos *et al.*, 2014) but also in several other regions of the world (Mayer and von
10 Helversen, 2001; Ibáñez *et al.*, 2006; Furman *et al.*, 2010; Raghuram *et al.*, 2014;
11 Bogdanowicz *et al.*, 2015; Dammhahn *et al.*, 2015; and Hassanin *et al.*, 2015), cryptic
12 species complexes have become a top priority for a number of international agendas.
13 *Pteronotus parnellii* is investigated here as a special case in which to study cryptic species
14 among Neotropical bats, considering that due to its wide distribution and low
15 morphological differentiation, the family Mormoopidae is an excellent subject for this kind
16 of analysis.

17 The family has only two genera, *Mormoops* Leach, 1821 and *Pteronotus* Gray, 1838; both
18 comprising insectivorous, gregarious, and strict cave-dwelling bats, which are found in a
19 wide variety of habitats, ranging from tropical rainforests to arid regions, being particularly
20 abundant in low dry forests throughout the Neotropics.

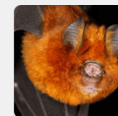
21 The genus *Mormoops* has two species, *Mormoops blainvillei* Leach, 1821 which is
22 distributed across the Greater Antilles and small adjacent islands, and *M. megalophylla*
23 (Peters, 1864), with an extant distribution extending from Mexico to northwestern South
24 America, and the West Indies. *Pteronotus* consists of a diverse group of six currently
25 recognized species: *P. davyi* Gray, 1838; *P. gymnotus* Naterer, 1843; *P. parnellii* (Gray,
26 1843) and *P. personatus* (Wagner, 1843), which are all distributed from Mexico to Brazil,
27 and *P. macleayi* (Gray, 1843) and *P. quadridens* (Gundlach, 1840), which are known from
28 the Antilles and the Bahamas (Simmons, 2005).



29 *Pteronotus parnellii*, as the most widely distributed species, is considered to be the basal
30 branch associated with the origin of the genus (Smith, 1972; Lewis-Oritt *et al.*, 2001; Van
31 Den Bussche and Weyandt, 2003). On the mainland the species ranges from northern
32 Mexico (Sonora and Tamaulipas), throughout Central America, to Peru, Ecuador, Bolivia,
33 Colombia, Brazil, Guyana, Suriname and Venezuela; and it is also found in the West Indies,
34 i.e. Cuba, Jamaica, Puerto Rico, Hispaniola, Saint Vincent, Trinidad and Tobago, Margarita
35 Island, and La Gonave Island (Simmons, 2005).

36 In a taxonomic revision of the family Mormoopidae, Smith (1972) recognized eight
37 subspecies within *P. parnellii*, namely: *P. p. fuscus*, *P. p. gonavensis*, *P. p. mesoamericanus*,
38 *P. p. mexicanus*, *P. p. parnellii*, *P. p. portoricensis*, *P. p. pusillus* and *P. p. rubiginosus*.
39 Later, a new subspecies, *P. p. paraguayensis*, was described (Linares and Ojasti, 1974); and
40 this taxonomic arrangement persisted until the year 2000. Since then, molecular studies
41 have questioned these *P. parnellii* subspecies arrangements. First, Lewis-Oritt *et al.* (2001)
42 relying on two genetic markers, Cyt b and RAG2, documented that *P. parnellii* is the most
43 divergent species within the genus, which presents higher genetic distances among island
44 forms than between these groups and mainland populations. On the basis of morphological
45 and molecular data Van Den Bussche *et al.* (2002) suggested that *P. parnellii* may represent
46 a complex of cryptic species. Clare *et al.* (2011) using COI as a genetic marker, not only
47 suggested the presence of four different genetic groups within *P. parnellii*, with one
48 restricted to Central America and three others from South America, but also proposed that
49 *P. p. mesoamericanus* should be considered a valid species. More recently, on the basis of
50 molecular (COI and Cyt b) and bioacoustical data, the groups proposed by Clare *et al.*
51 (2013) were confirmed by De Thoisy *et al.* (2014) who also indicated the existence of four
52 cryptic species within *P. parnellii*: *P. sp1* (Honduras and Mexico), *P. sp2* (Guyana), and *P.*
53 *sp3 and P sp4* (Guyana, Suriname, French Guiana and Brazil).

54 Although these publications contributed substantially to our understanding of the
55 systematics of this group, they were limited by the number of samples and the lack of
56 mainland representatives from Mexico and central Brazil. These are very important regions
57 in which *P. parnellii* populations are abundant and they represent the northern and southern
58 extremes of this species complex.



59 Here, we evaluate patterns of genetic variation based on the mitochondrial gene COI and
60 the nuclear gene DBY, using a total number of 452 individuals from 84 different localities,
61 distributed over 10 different countries and covering the majority of the known mainland
62 distribution of *P. parnellii*. The main goal is to evaluate phylogenetic relationships within *P.*
63 *parnellii*.

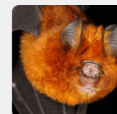
64 MATERIALS AND METHODS

65 *Sampling*

66 Twelve populations of *P. parnellii* distributed throughout Mexico were sampled (Fig. 1).
67 Specimens were captured using harp traps, measured, and then biopsied from wing
68 membranes using a 3mm biopsy puncher (Fray Products Corp., Buffalo, NY). Tissue
69 samples were stored in 70% ethanol. The captured bats were then immediately released,
70 except for a few specimens that were preserved as vouchers and deposited in the
71 Universidad Autónoma Metropolitana - Iztapalapa Mammal Collection (UAM-I). Tissue
72 samples from Goiás (Brazil) and Coiba Island (Panama) were made available by the
73 Brazilian National Museum of Natural History (Museu Nacional / UFRJ) and the Estación
74 Biológica de Doñana, respectively. In addition, COI and DBY sequences were obtained
75 from the GenBank database for populations from Belize, Mexico, Guatemala, El Salvador,
76 Panama, Jamaica, Guyana, Suriname, and Venezuela. Names and geographical coordinates
77 for the 84 studied localities are provided in Appendix 1. The sequences obtained in this
78 study were deposited in GenBank (Appendix 2).

79 All appropriate ethics and other approvals were obtained for the research (Anonymous,
80 2010; Sikes *et al.*, 2011). Specimens were collected under Mexican Government permits
81 SGPA/DGVS Nos. 09131/14; 05853/13; CC 08450/92.

82 *DNA Extraction, Amplification, Sequencing and Alignment*



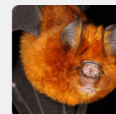
83 All DNA was extracted using standard salt extraction protocol, modified from Lopera-
84 Barrero *et al.* (2008). Primers, amplification conditions and sequencing for the
85 mitochondrial gene COI were performed on the basis of the methods described by Ivanova
86 *et al.* (2006); and for the nuclear gene intron7 DBY region those indicated by Lim *et al.*
87 (2008). The sequencing reaction was performed with BigDye Terminator (Applied
88 Biosystems) in Applied Biosystems 3130 Genetic Analyzer. All sequences were aligned and
89 edited using Geneious® Pro software v.5.6.4 (Biomatters Ltd., Auckland, New Zealand).

90 *Phylogenetic Analysis and Genetic Structure*

91 The software DNA SP v.5.10 (Librado and Rozas, 2009) was used to analyse the sequences
92 in order to obtain their corresponding haplotypes and building networks haplotype
93 networks were built using the Median-Joining algorithm (Bandelt *et al.*, 1999) in PopART
94 v.1.7 (<http://popart.otago.ac.nz>).

95 The phylogenetic relationship between haplotypes from COI and DBY genes was evaluated
96 using a Maximum Parsimony (MP) and Maximum likelihood (ML) criteria in PAUP*
97 v.4.0b10 (Swofford, 2002) with the heuristic search option and the Branch Exchange
98 algorithm Tree Bisection Reconnection (TBR). Branch support was calculated using
99 Bootstrap analysis (Felsenstein, 1985) with 1000 iterations. In addition we constructed a
100 Bayesian phylogeny (BP) in Mr. Bayes v.3.2 (Ronquist *et al.*, 2012) with four Markov
101 chains and 10 000 000 generations. For the ML and BP analyses we used the best fit model
102 of sequence evolution selected in Modeltest v.3.7 (Posada and Crandall, 1998) with the
103 Akaike information criteria (AIC). The HKY85 model (Hasegawa *et al.*, 1985) was
104 identified as the best nucleotide substitution model for COI, and GTR (Lanave *et al.*, 1984;
105 Rodriguez *et al.*, 1990) was selected for DBY. Additionally we conducted a combined
106 analysis with sequences from the studied regions, using the same parameters and software,
107 and using the GTR+I+G (Lanave *et al.*, 1984; Rodriguez *et al.*, 1990) model. The
108 Incongruence Length Difference (Farris *et al.*, 1994) score for the COI and DBY partitions
109 was 150 ($p = 0.089000$).

110 To perform all phylogenetic reconstructions we used *Saccopteryx bilineata* and *Uroderma*
111 *bilobatum* sequences as outgroups (Appendix 3). The choice of outgroup taxa was made



112 considering phylogenetic relationships (Agnarsson *et al.*, 2011; Clare *et al.*, 2013; Shi and
113 Radobsky, 2015) and the availability of sequences in the GenBank.

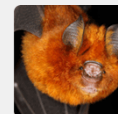
114 The genetic differentiations within and between groups were estimated with mean distance
115 analysis in MEGA v.6.0 (Tamura *et al.*, 2013) using the Kimura-2 parameter model
116 (Kimura, 1980) for COI data and the Tamura-Nei (Tamura and Nei, 1993) model for DBY.
117 The existence of genetic structure was assessed by an analysis of molecular variance
118 (AMOVA, Excoffier *et al.*, 1992) with genetic divergence values (FST) using Arlequin
119 v.3.5 (Excoffier and Lischer, 2009).

120 RESULTS

121 *Nucleotide Sequences*

122 For the 610 COI nucleotides, 394 (64.6%) were constant, 72 (11.8%) were variable non-
123 informative and 144 (23.6%) informative. The average base composition was A=25.2%,
124 C=28.3%, G=17.4%, and T=29.1%. For the 403 DBY nucleotides, 193 (47.9%) were
125 constant, 5 (1.2%) were variable non-informative and 205 (50.9%) informative. The
126 average base composition was A=29.1%, C=15.7%, G=15.7%, and T=39.5%. For
127 combined analysis 1013 nucleotides were recuperated, using only individuals with both
128 markers.

129 For the COI gene we recovered 52 different haplotypes (Hts) (Appendix 4). The haplotype
130 network contains six haplogroups (Hg) separated by 8 to 50 mutational steps. The first
131 group (Hg 1) is composed of Hts of populations located in the Mexican Pacific coastal
132 plains (PMex). The second (Hg 2) includes Hts of the Gulf of Mexico coastal plains
133 (GMex), southern Mexico and Central America, and also two Hts from the Mexican Pacific
134 Coast (gathered as a whole under Hg 1). The third group (Hg 3) has Hts from Venezuela
135 and northwestern Guyana. The fourth (Hg 4) has Hts from Brazil, Guyana and Suriname,
136 whereas Hg 5 has Hts only from Guyana and Suriname; these two groups shared seven Hts.
137 The final group (Hg 6) contains the sole island haplotype in this study (Jamaica). All
138 haplogroups present a marked pattern of genetic and geographic structure (Fig. 2)



139 For the DBY gene only five different Hts were recovered (Appendix 4). The haplotype
140 network contains two Hg separated by 10 mutational steps. The first group (Hg 1) includes
141 all Hts from Mexico, Guatemala, El Salvador, Panama, Venezuela, Brazil and northwestern
142 Guyana, whereas the second (Hg 2) included Hts from Guyana and Suriname (Fig. 3).

143 *Phylogenetic reconstruction*

144 For the mitochondrial COI gene, MP, ML and Bayesian topologies displayed a similar
145 hierarchical pattern, with quite high bootstrap and Bayesian values. The Bayesian tree
146 shows the Jamaican haplogroup placed basally with respect to two large sister lineages: one
147 encompasses terminals from PMex, GMex/Yucatan/Central America, and
148 Venezuela/Guyana; and the other branch embraces the terminals Guyana/Suriname and
149 Guyana/Suriname/Brazil (Fig. 4).

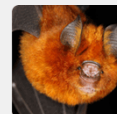
150 For the nuclear DBY gene, phylogenetic trees are also supported by high bootstrap values,
151 with the Bayesian tree displaying two monophyletic lineages, one encompassing haplotypes
152 from Mexico, Guatemala, El Salvador, Panama, Venezuela, Brazil and northwestern
153 Guyana; and the other containing haplotypes from Guyana and Suriname (Fig. 5). The
154 Jamaican haplogroup is not represented due to the absence of samples for the DBY gene.

155 The concatenated dataset provided low resolution for the detection of the lineages obtained
156 in the analyses of independent genes. However, the MP analysis supports the 5 lineages
157 obtained for with the COI gene (Fig. 6).

158 *Genetic distance and structure*

159 The average genetic distances between the six haplogroups fluctuated between 2.9 and 12%
160 (Table 1A) for the COI gene and was 2.6% for the DBY gene (Table 1B). The AMOVA
161 results reveal a high genetic variation among the groups for both the COI gene ($F_{ST} = 0.95$;
162 $P < 0.05$; Table 2A) and the DBY gene ($F_{ST} = 0.98$; $P < 0.05$; Table 2B), thereby supporting
163 the haplogroup schemes and their respective phylogenetic patterns and haplotype networks.

164 The COI results demonstrated that the Jamaican lineage exhibits the highest genetic
165 distances with respect to mainland populations (10.9 to 12.0%, Table 1A). This finding is in



166 agreement with genetic distances reported for Cyt b and RAG 2 genes (Lewis-Oritt *et al.*,
167 2001).

168 DISCUSSION

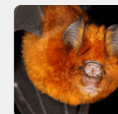
169 The results demonstrate high genetic variability within the *P. parnellii* species complex,
170 throughout its broad distribution across the continental Neotropics. The inclusion of
171 material deriving from both the northern and southern limits of its distribution in the
172 genetic analyses incorporating both mitochondrial and nuclear markers, as well as the
173 results previously obtained by other authors, allowed us to perform a more complete test
174 regarding the variation present in this species complex, thus producing a more
175 comprehensive phylogenetic arrangement for this mormoopid bat.

176 The four methods applied in the development of the phylogenetic reconstructions based on
177 the COI gene have resulted in the recognition of five mainland haplogroups plus an island
178 group from Jamaica. On the basis of these phylogenetic results we propose that five
179 independent monophyletic lineages should be considered under the taxonomic entity
180 *Pteronotus parnellii* encompasses five independent monophyletic lineages.

181 We identified a cryptic lineage that had not been previously detected corresponding to
182 populations from western Mexico, as well as the presence of four lineages
183 (GMex/Yucatan/Central America, Venezuela/Guyana, Guyana/Suriname,
184 Guyana/Suriname/Brazil), which had been previously recognized by other authors (Dávalos
185 2006; Clare *et al.*, 2013; De Thoisy *et al.*, 2014), although not with the exact same
186 geographic patterns.

187 The lineage from western Mexico (PMex) is distributed over the lowlands of the Mexican
188 Pacific coastal plains, from "Cueva del Tigre" in Carbo, Sonora, south to "Grutas de
189 Juxtlahuaca" in Colotlipa, Guerrero (Fig.1).

190 According to our results the Central America lineage, which was previously proposed by
191 Clare *et al.* (2013), also includes all the populations across the Gulf of Mexico coastal plain
192 and the southern Yucatan Peninsula. This lineage, which has been designated



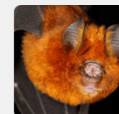
193 “GMex/Yucatan/Central America”, therefore has a broad geographic distribution that
194 extends from the north of Mexico (Tamaulipas) to Panama (Darien) (Fig. 1).

195 The results of this study reveal not only a confined lineage between the northwestern Andes
196 Mountains and the Guyana shield (Venezuela/Guyana), but also that the Guyana/Suriname
197 and the Guyana/Suriname/Brazil lineages are sympatric in an area between the Guyana
198 shield and the Amazonian Craton. This sympatric pattern was also documented in other
199 studies (Clare *et al.*, 2013; De Thoysi *et al.*, 2014). However, our results extend the
200 distribution of the “Guyana/Suriname/Brazil” lineage further south, reaching the central
201 portion of Brazil (State of Goiás), possibly via an arch-like biogeographical shape (Fig. 1),
202 as documented for other species of the genus (*Pteronotus personatus*, *P. gymnonotus* -
203 Patton and Gardner, 2008) as well as other Neotropical bats (Nunes *et al.*, 2005).

204 The Caribbean Sea and the large mountain chains located in the Neotropics seem to play an
205 important role in the process of allopatric speciation of these lineages by limiting the flow
206 of genes between them; the evidence provided by the COI gene also meets the criteria for
207 the genetic species concept (De Queiroz, 2005; Bradley and Baker, 2006) and allows us to
208 propose that five of these lineages must be considered cryptic species: *P. sp1* (Jamaica), *P.*
209 *sp2* (PMex/GMex /Yucatan/Central America), *P. sp3* (Venezuela/Guyana), *P. sp4*
210 (Guyana/Suriname), and *P. sp5* (Guyana/Suriname/Brazil).

211 Given the results reported in the present study, we were able to infer different scenarios for
212 the origin and diversification of the six lineages.

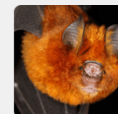
213 Based on the topology obtained for the COI gene the Jamaican lineage most likely was the
214 starting point for two different processes of invasion and diversification that gave rise to the
215 five continental lineages (Fig. 4). This include one in South America, in which the
216 Venezuela/Guyana lineage may have been the center of diversification. and another in the
217 South of Mexico, where populations expanded northward through both coastal plains of
218 Mexico as well as southward through Central America (Fig. 1). These results are in
219 agreement with the hypothesis of Morgan and Czaplewski (2012) wich states that the
220 ancestral area of mormoopids includes the Greater Antilles, and the divergences between
221 Antillean and continental lineages will be older than that between Central American and



222 northern South American mormoopid populations. Nuclear genes evolve too slowly for
223 intra-specific level analysis, but in this case the DBY intron appears to offer reliable
224 support for a split between Mexico-Central America and South American groups (Figure 3).
225 Both processes of diversification could be the result of geological, climatic and ecological
226 changes in the Pleistocene and thus strongly associated with the establishment of the
227 continental tropical dry forest (TDF), which is the shared habitat of these bats and where
228 they are currently most abundant.

229 Our results also allow us to suggest that formerly there was a continuous distribution
230 across South America and that lineages from Guyana/Suriname and
231 Guyana/Suriname/Brazil originated almost simultaneously following vicariant events in the
232 Guiana shield during the Pleistocene which allowed the establishment of refuge areas
233 associated with the TDF (Pennington *et al.*, 2000, 2004; Werneck *et al.*, 2011, 2012;
234 Collevatti *et al.*, 2013). These lineages have a common ancestor of both (Fig. 4) and share
235 seven haplotypes, and it is very likely that the same historical process is responsible for the
236 similar pattern described for them. Comparable processes have been described for other
237 vertebrates (Noonan and Gaucher 2005, 2006; Wuester *et al.*, 2005; Quijada-Mascareñas,
238 2007; Naka *et al.*, 2012; Capurucho *et al.*, 2013), including bats (Ditchfield, 2000;
239 Hoffmann and Baker, 2003; Pavan *et al.*, 2011).

240 Our results indicate that there are two cryptic lineages in Mexico which exhibit low genetic
241 variation between them (2.9%) and that the presence of shared haplotypes (H48, H49) in
242 southwest Mexico (Colotlipa, Guerrero) does not provide grounds for considering them two
243 separate cryptic species. If we consider the intraspecific phylogenies, the distribution of
244 lineages, and climatic and geomorphological events, it is possible to understand the
245 diversification process in Mexico. The Mexican Pacific lowland populations may have
246 diverged recently from those of the Gulf of Mexico coastal plain in association with the
247 latest geological and ecological events that occurred in the Pleistocene which shaped the
248 distribution of the TDF as they stand today (Barrera, 2005). Therefore, these events may be
249 related to an incipient speciation process such as the one indicated for the cryptic lineages
250 from Mexico. Similar biogeographical patterns seem to be shared by several other animal
251 species (ants, freshwater fishes, amphibians, reptiles, birds) and also by some native trees



252 (*Bursera* and *Spondias*) native to the TDFs of Mexico and Central America (Arbeláez-
253 Cortés *et al.*, 2014).

254 Given the type locality of *P. parnellii*, as well the result of this study the specific name *P.*
255 *parnellii* should be applied to *Pteronotus* populations living on the island of Jamaica and at
256 least until the taxonomic status of the West Indian population is established.

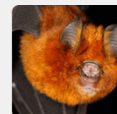
257 Clare *et al.* (2013) proposed that the Central America lineage should be recognized as *P.*
258 *mesoamericanus*, but in our analysis, all populations present in Mexico and Central
259 America belong to the same species, including the samples from the type locality of *P.*
260 *parnellii mexicanus*, as well as the western Mexico specimens contained within the PMex
261 lineage. Therefore, according to the "Principle of Priority", Article 23.1 in the International
262 Code of Zoological Nomenclature, the correct species name must be *Pteronotus mexicanus*
263 (Miller, 1902). In accordance with the Templeton criteria (1998), the two lineages can be
264 considered subspecies with the following taxonomic names: *P. mexicanus mexicanus*
265 (Miller, 1902) for samples in western Mexico, and *P. mexicanus mesoamericanus* Smith,
266 1972 for specimens from eastern Mexico to Central America.

267 Regarding the mainland cryptic species from South America, De Thoisy *et al.* (2014)
268 pointed out the problem of assigning species names to them.

269 CONCLUSIONS

270 The results of the present study, obtained with a large sample, allow us to recognize the
271 presence of different lineages within *P. parnellii* and its area of origin and diversification.
272 According to our phylogenetic data the continental lineages of *P. parnellii* could have been
273 diversified simultaneously from a Jamaican lineage, as a result of the same historical event
274 concurrent with climatic changes during Pleistocene that favored the establishment of
275 tropical dry forests in the Neotropics.

276 We postulate that the *P. parnellii* cryptic species-complex derived from a single speciation
277 event in Mexico, Central America and South America, and their common ancestor likely
278 originated from the island of Jamaica. If we consider the intraspecific phylogenies with



279 their history of origin and diversification, the distribution of lineages, as well as their close
280 relationship with the tropical dry forest, everything points to a recent settlement process.

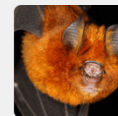
281 We propose that five of the lineages within *Pteronotus parnellii* should be recognized at
282 species level: *P. parnellii* (for the populations in Jamaica Island), *P. mexicanus* (populations
283 in Mexico and Central America), *P. sp3* (Venezuela/Guyana), *P. sp4* (Guyana/Suriname),
284 and *P. sp5* (Guyana/Suriname/Brazil).

285 The phylogenetic histories of these cryptic species of *Pteronotus parnellii* represent a
286 further contribution to the understanding of the diversity of bats in the Neotropics and
287 demonstrate the importance of vicariant events during the Pleistocene, such as cycles of
288 contraction and expansion of tropical dry forest areas in Mexico, Central America and
289 South America for the diversification and speciation of mormoopid bats in the continental
290 Neotropics.

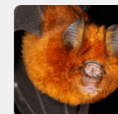
291 In addition to the results presented here a more complete evaluation of the phylogenetic
292 history of lineages within *P. parnellii* would benefit from the inclusion of additional
293 sequences and more variable markers. This next step will be necessary to obtain a better
294 understanding of the relationships among these cryptic species. Moreover, further studies
295 are needed to assess the variability and genetic divergence within the populations located in
296 the Brazilian Amazon arc and populations from Peru and Colombia, which due to their
297 geographical position could provide a great contribution to the knowledge of the patterns of
298 diversification in the mainland Neotropics.

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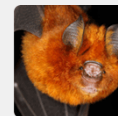
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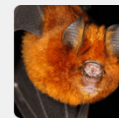
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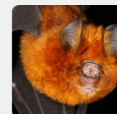
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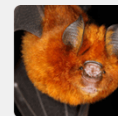
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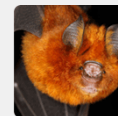
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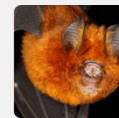
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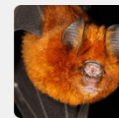
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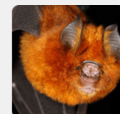


Table 1. Mean pairwise genetic distances for COI and DBY sequence divergences within and among the recognized haplogroups. For haplogroup names and respective geographic distributions see text and Fig. 4.

A. Percentage of divergence between COI gene sequences in the 6 haplogroups obtained in this study using the Kimura-2 parameter model.

	Hg 1	Hg 2	Hg 3	Hg 4	Hg 5	Hg 6
Hg 1						
Hg 2	2.9					
Hg 3	5.1	4.9				
Hg 4	11.1	9.9	11.2			
Hg 5	11.5	10.9	11.5	5.3		
Hg 6	11.7	10.9	10.8	11.3	12.0	

B. Percentage of divergence between DBY gene sequences in the 2 haplogroups obtained in this study using the Tamura-Nei model.

	Hg 1	Hg 2
Hg 1		
Hg 2	2.6	

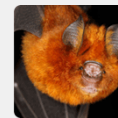


Table 2. Results of the analyses of molecular variance for COI and DBY genes with significance estimated from 1000 iterations

A. COI haplogroups.

Source of variation	Sum of squares	Variance components	Percentage of variation	FST
Among Hg	6731.63	21.72	95.11	0.95*
Within Hg	494.69	1.11	4.88	

* $P < 0.05$

B. DBY haplogroups.

Source of variation	Sum of squares	Variance components	Percentage of variation	FST
Among Hg	703.47	12.07	98.93	0.98*
Within Hg	14.94	0.12	1.06	

* $P < 0.05$

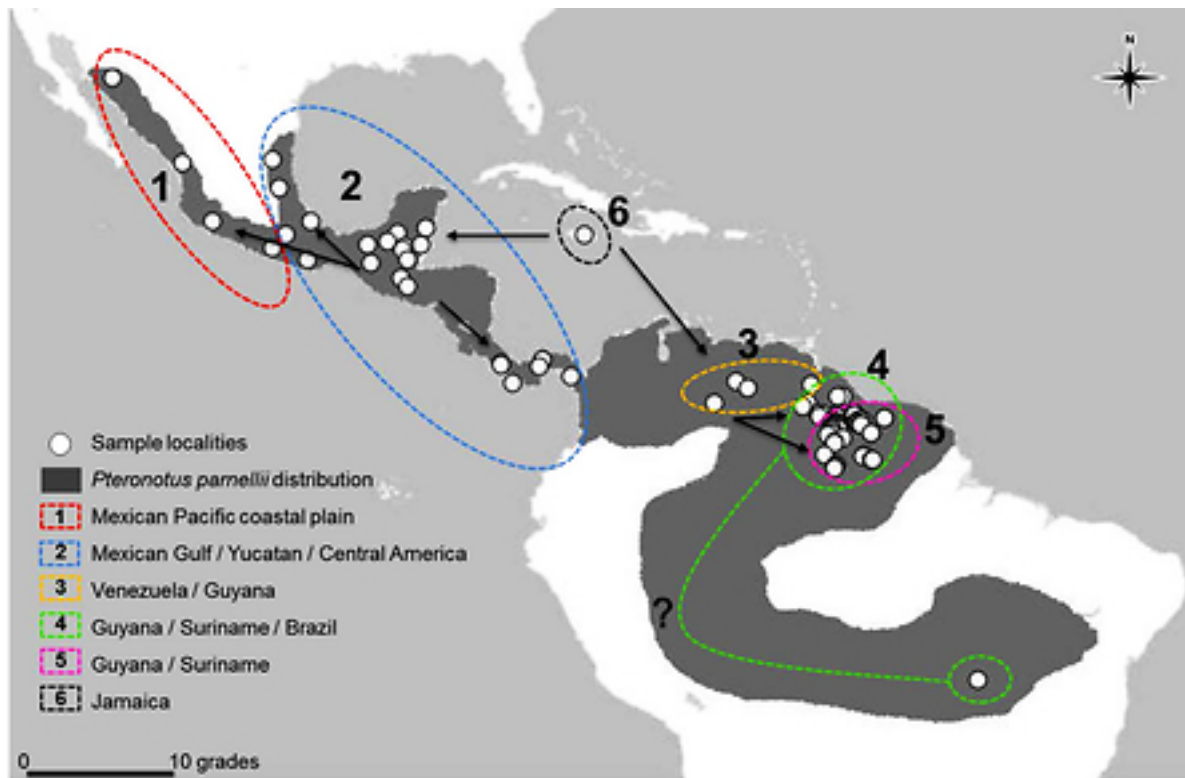
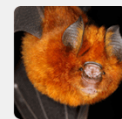


Fig. 1. Map depicting the original geographic distribution of *P. parnellii* (shaded area), over which the source locations of haplotypes (white dots), identified lineages (dashed squares), and proposed origin and diversification processes (black arrows) are displayed. All localities within Mexico were sampled for this study.

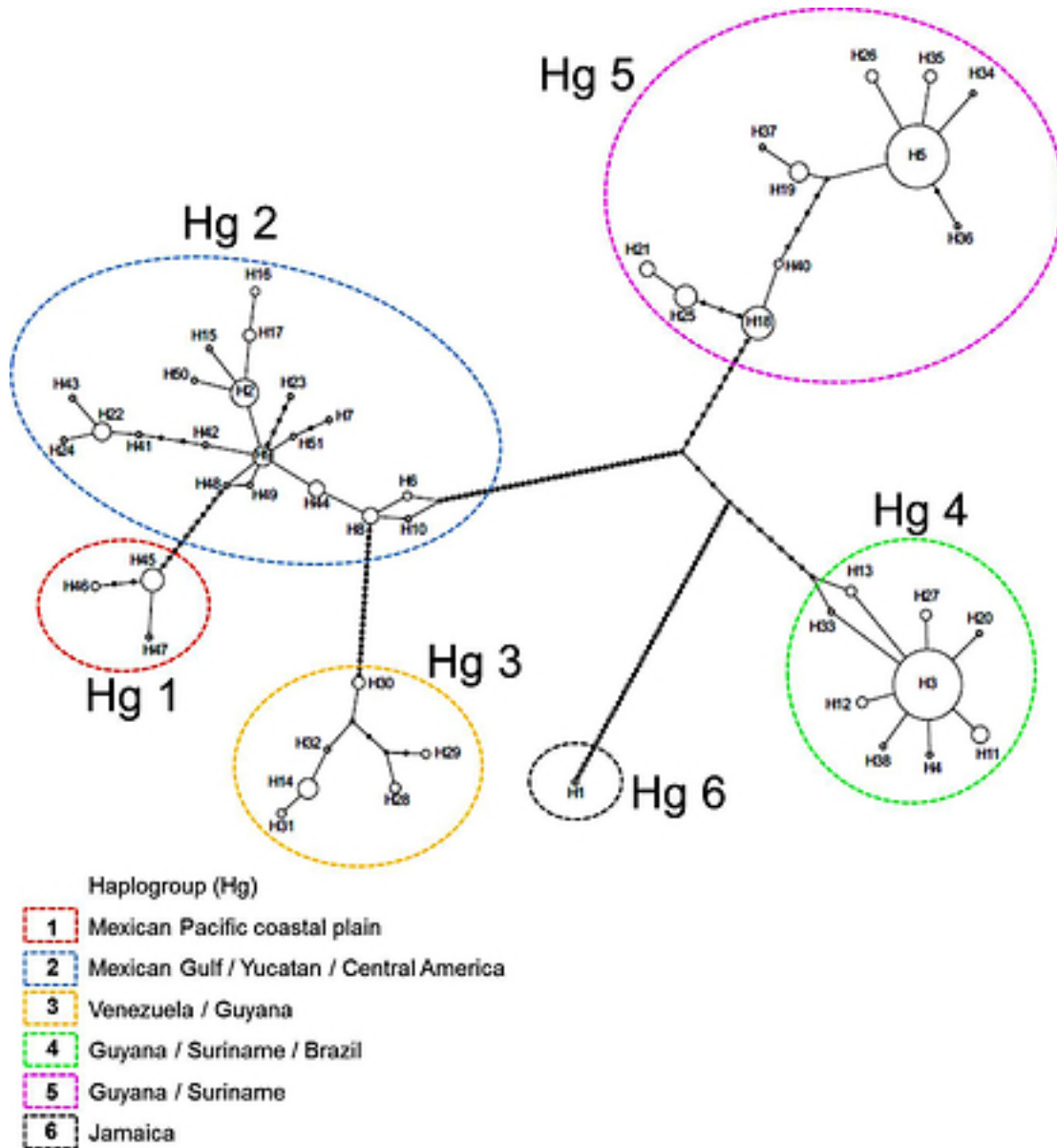
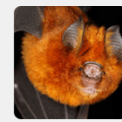


Fig. 2. Haplotype network for the COI gene of *Pteronotus parnellii*. The H-number indicates the haplotype identifier for the samples presented in Appendix 4. The size of circles denotes the relative number of samples represented in each haplotype. Lines between haplotypes indicate mutational steps between sequences, and the small black diamonds represent hypothetical haplotypes. Colours indicate the geographic correspondence of haplogroups.

Figure 3

[Download source file \(128.13 kB\)](#)

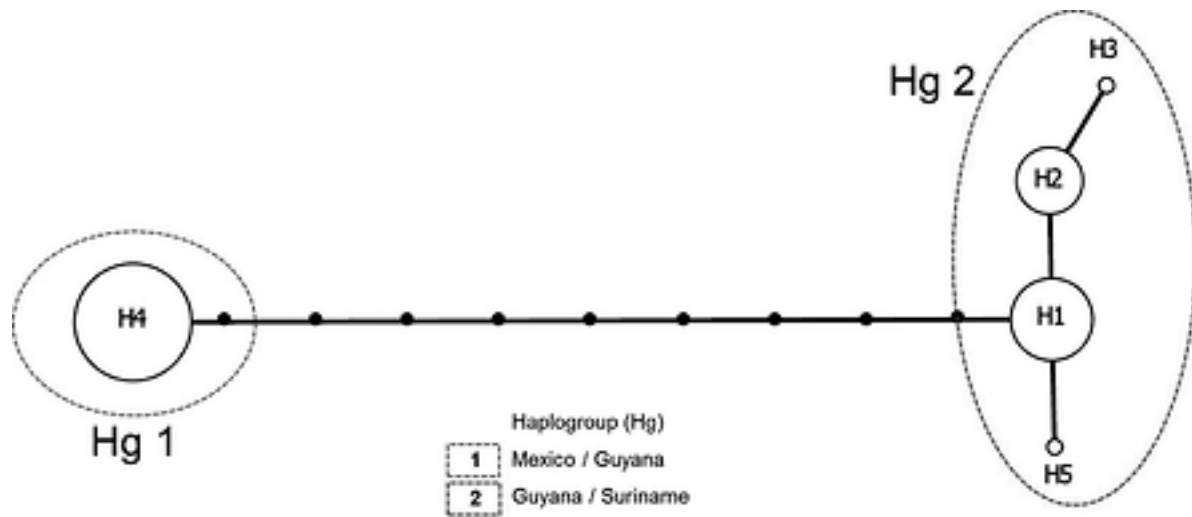
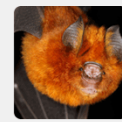


Fig. 3. Haplotype network for the DBY gene of *Pteronotus parnellii*. The H-number indicates the haplotype identifier for the samples presented in Appendix 4. The size of circles denotes the relative number of samples represented in each haplotype. Lines between haplotypes indicate mutational steps between sequences, and the small black diamonds represent hypothetical haplotypes. Colours indicate the geographic correspondence of haplogroups.

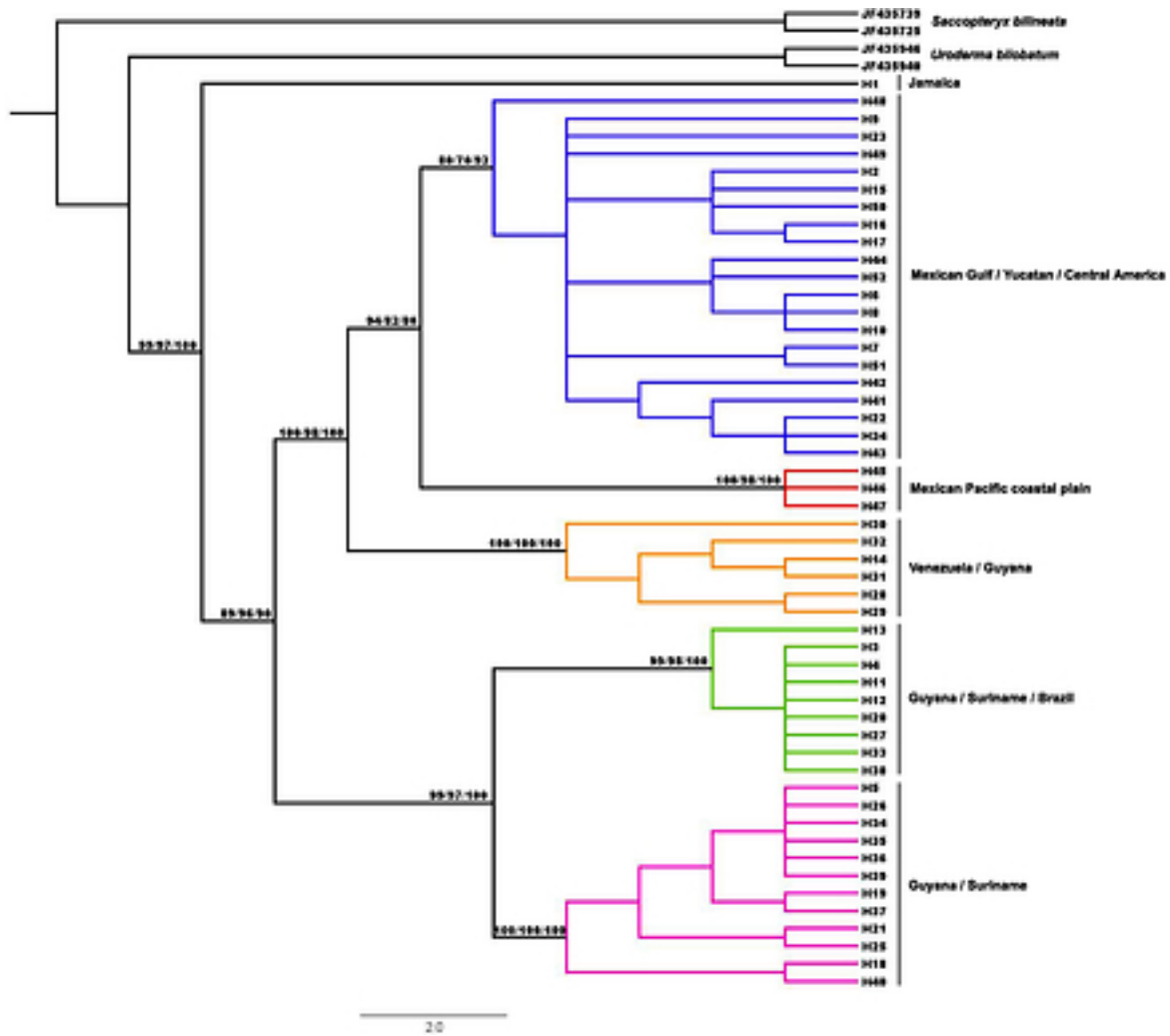
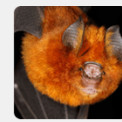


Fig. 4. Phylogenetic relationships of the studied groups within *Pteronotus parnellii* based on a fragment of the mitochondrial COI gene. The reconstruction presented is a Bayesian consensus tree with proportionally transformed branches. Branch support values indicate from left to right: Bayesian Posterior Probability, Bootstrap ML, and Bootstrap MP. Colours indicate the haplogroup and its respective geographic correspondence.

Figure 5

[Download source file \(417.27 kB\)](#)

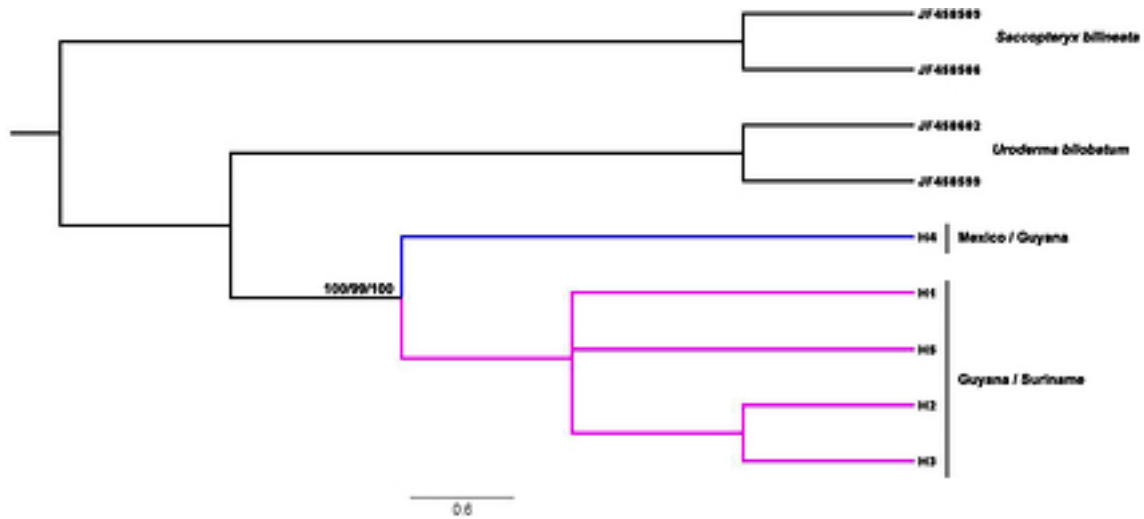
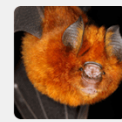


Fig. 5. Phylogenetic relationships of the studied groups within *Pteronotus parnellii* based on a fragment of the nuclear DBY gene. The reconstruction presented is a Bayesian consensus tree with proportionally transformed branches. Branch support values indicate from left to right: Bayesian Posterior Probability, Bootstrap ML, and Bootstrap MP. Colours indicate the haplogroup and its respective geographic correspondence.

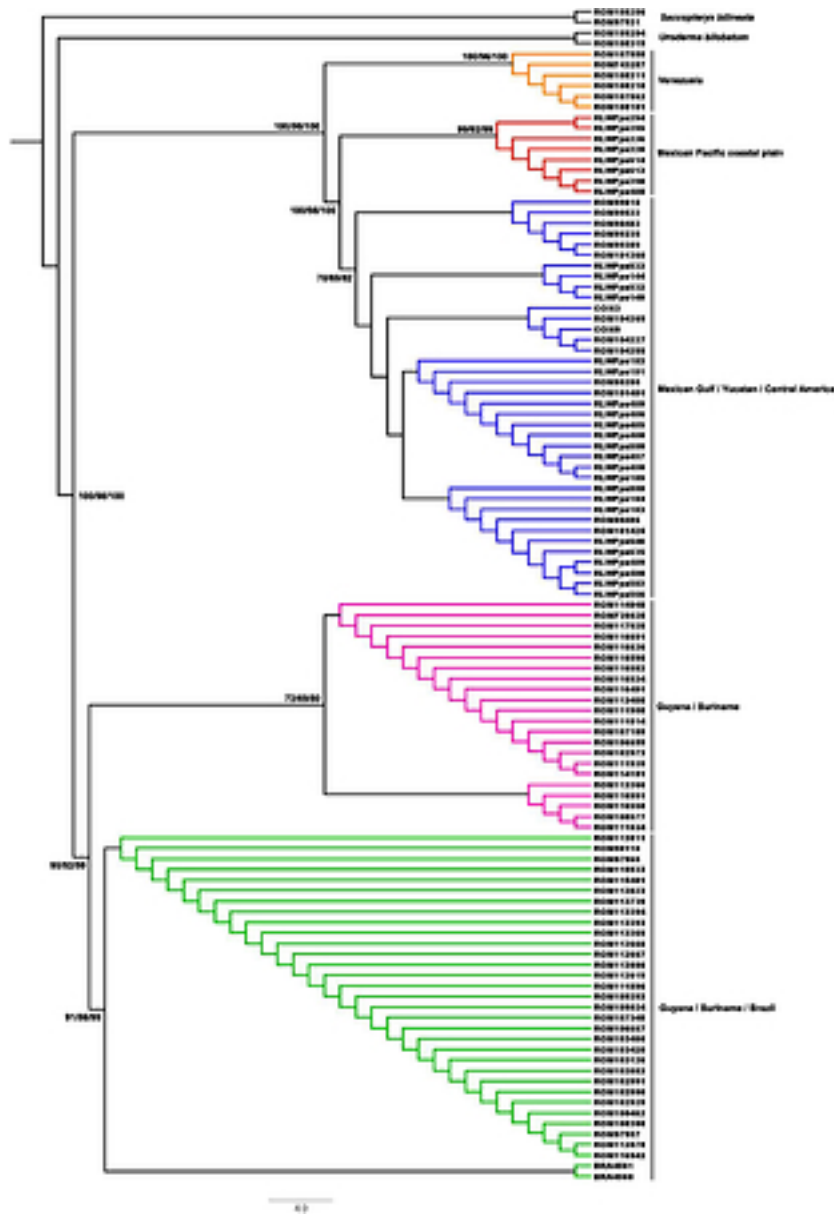
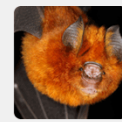
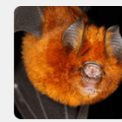


Fig. 6. Phylogenetic relationships of the studied groups within *Pteronotus parnellii* based on a concatenated analysis of mitochondrial COI and nuclear DBY genes. The reconstruction presented is a Bayesian consensus tree with proportionally transformed branches. Branch support values indicate from left to right: Bayesian Posterior Probability, Bootstrap ML, and Bootstrap MP. Colours indicate the haplogroup and its geographic correspondence.



Manuscript body

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Tables

Table 1 - [Download source file \(62.37 kB\)](#)

Table 1. Mean pairwise genetic distances for COI and DBY sequence divergences within and among the recognized haplogroups. For haplogroup names and respective geographic distributions see text and Fig. 4.

Table 2 - [Download source file \(52.8 kB\)](#)

Table 2. Results of the analyses of molecular variance for COI and DBY genes with significance estimated from 1000 iterations

Figures

Figure 1 - [Download source file \(6.52 MB\)](#)

Fig. 1. Map depicting the original geographic distribution of *P. parnellii* (shaded area), over which the source locations of haplotypes (white dots), identified lineages (dashed squares), and proposed origin and diversification processes (black arrows) are displayed. All localities within Mexico were sampled for this study.

Figure 2 - [Download source file \(3.04 MB\)](#)

Fig. 2. Haplotype network for the COI gene of *Pteronotus parnellii*. The H-number indicates the haplotype identifier for the samples presented in Appendix 4. The size of circles denotes the relative number of samples represented in each haplotype. Lines between haplotypes indicate mutational steps between sequences, and the small black diamonds represent hypothetical haplotypes. Colours indicate the geographic correspondence of haplogroups.

Figure 3 - [Download source file \(128.13 kB\)](#)

Fig. 3. Haplotype network for the DBY gene of *Pteronotus parnellii*. The H-number indicates the haplotype identifier for the samples presented in Appendix 4. The size of circles denotes the relative number of samples represented in each haplotype. Lines between haplotypes indicate mutational steps between sequences, and the small black diamonds represent hypothetical haplotypes. Colours indicate the geographic correspondence of haplogroups.

Figure 4 - [Download source file \(1.44 MB\)](#)

Fig. 4. Phylogenetic relationships of the studied groups within *Pteronotus parnellii* based on a fragment of the mitochondrial COI gene. The reconstruction presented is a Bayesian consensus tree with proportionally transformed branches. Branch support values indicate from left to right: Bayesian Posterior Probability, Bootstrap ML, and Bootstrap MP. Colours indicate the haplogroup and its respective geographic correspondence.

Figure 5 - [Download source file \(417.27 kB\)](#)

Fig. 5. Phylogenetic relationships of the studied groups within *Pteronotus parnellii* based on a fragment of the nuclear DBY gene. The reconstruction presented is a Bayesian consensus tree with proportionally transformed branches. Branch support values indicate from left to right: Bayesian Posterior Probability, Bootstrap ML, and Bootstrap MP. Colours indicate the haplogroup and its respective geographic correspondence.

Figure 6 - [Download source file \(3.14 MB\)](#)

Fig. 6. Phylogenetic relationships of the studied groups within *Pteronotus parnellii* based on a concatenated analysis of mitochondrial COI and nuclear DBY genes. The reconstruction presented is a Bayesian consensus tree with proportionally transformed branches. Branch support values indicate from left to right: Bayesian Posterior Probability, Bootstrap ML, and Bootstrap MP. Colours indicate the haplogroup and its geographic correspondence.

Supplementary Material

File 1 - [Download source file \(149.27 kB\)](#)

Appendix 1 to 4

