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Authors:
RICARDO LOPEZ-WILCHIS, MAYELA FLORES-ROMERO, LUIS GUEVARA-CHUMACERO, ALEJANDRA SERRATO-DÍAZ, JHOANA DÍAZ-LARREA, FERNANDO SALGADO-MEJIA, CARLOS IBAÑEZ, LEANDRO SALLES5 SALLES, JAVIER JUSTE

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

Type:

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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

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INTRODUCTION

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

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

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

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

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

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

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

MATERIALS AND METHODS

Sampling

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

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

DNA Extraction, Amplification, Sequencing and Alignment

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

Phylogenetic Analysis and Genetic Structure

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

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

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

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

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

RESULTS

Nucleotide Sequences

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

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

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

Phylogenetic reconstruction

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

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

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

Genetic distance and structure

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

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

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

DISCUSSION

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

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

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

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

According to our results the Central America lineage, which was previously proposed by Clare *et al.* (2013), also includes all the populations across the Gulf of Mexico coastal plain 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

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

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

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

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

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

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

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

CONCLUSIONS

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

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

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

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

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

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

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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**

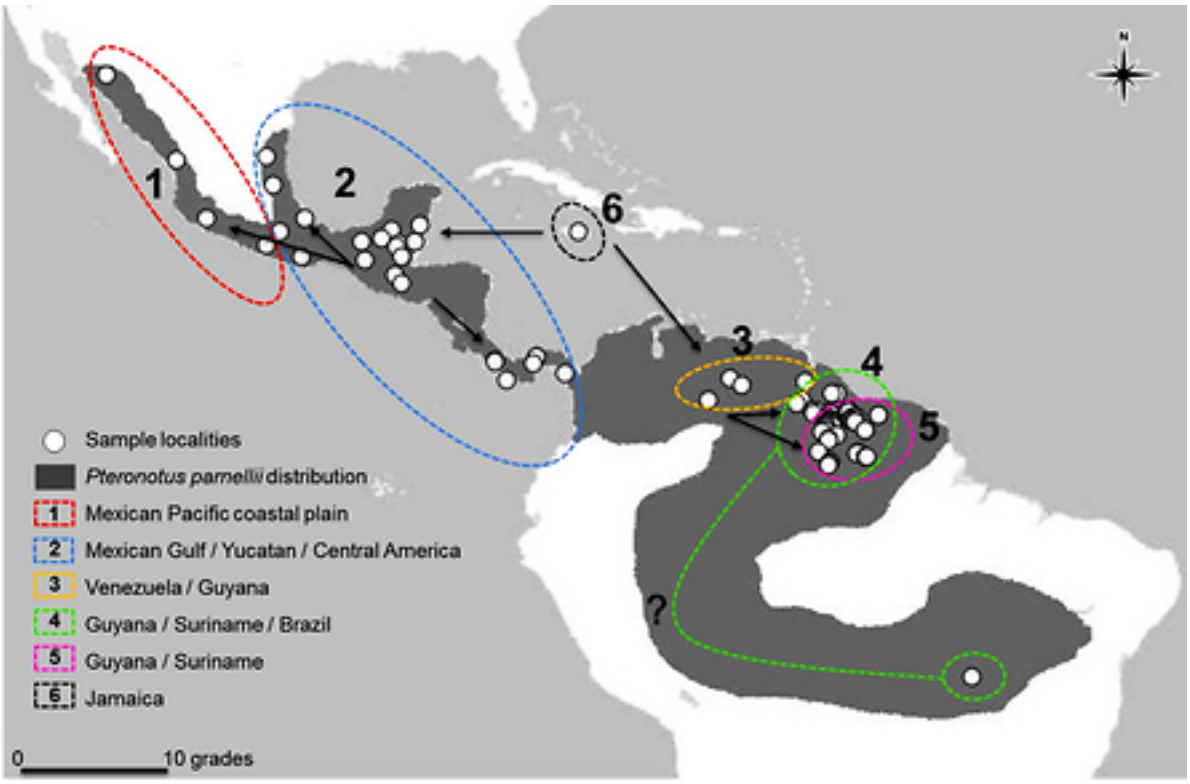

Fig. 1. Map depicting the original geographic distribution of P. parnellii (shaded area), over which the source locations of haplotypes (white dots), identified linages (dashed squares), and proposed origin and diversification processes (black arrows) are displayed. All localities within Mexico were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

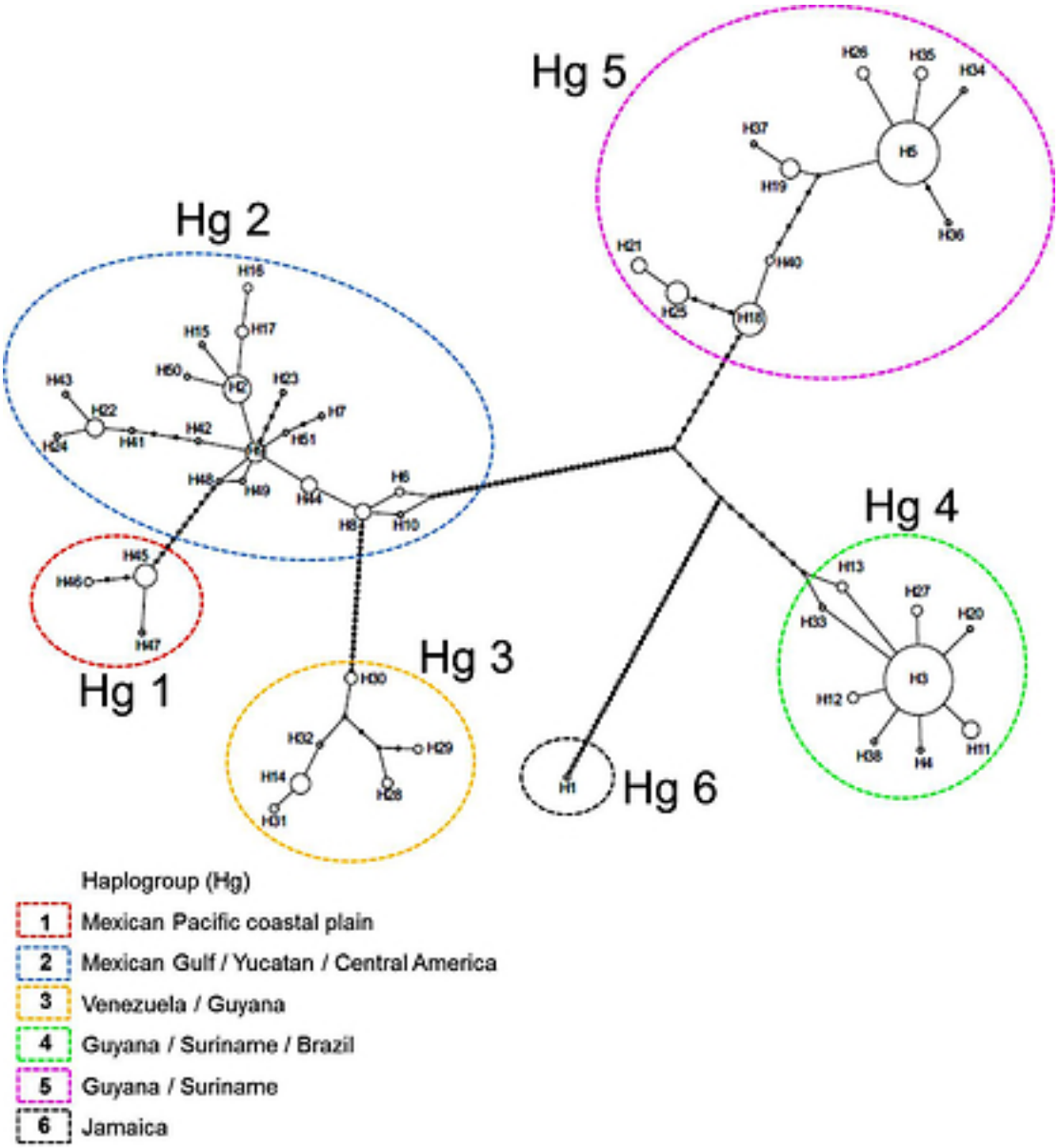

Fig. 2. Haplotype network for the COI gene of Pteronotus parnellii. The H-number indicates the haplotype identifier for the samples presented in Appendix 4. The size of circles denotes the relative number of samples represented in each haplotype. Lines between haplotypes indicate mutational steps between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

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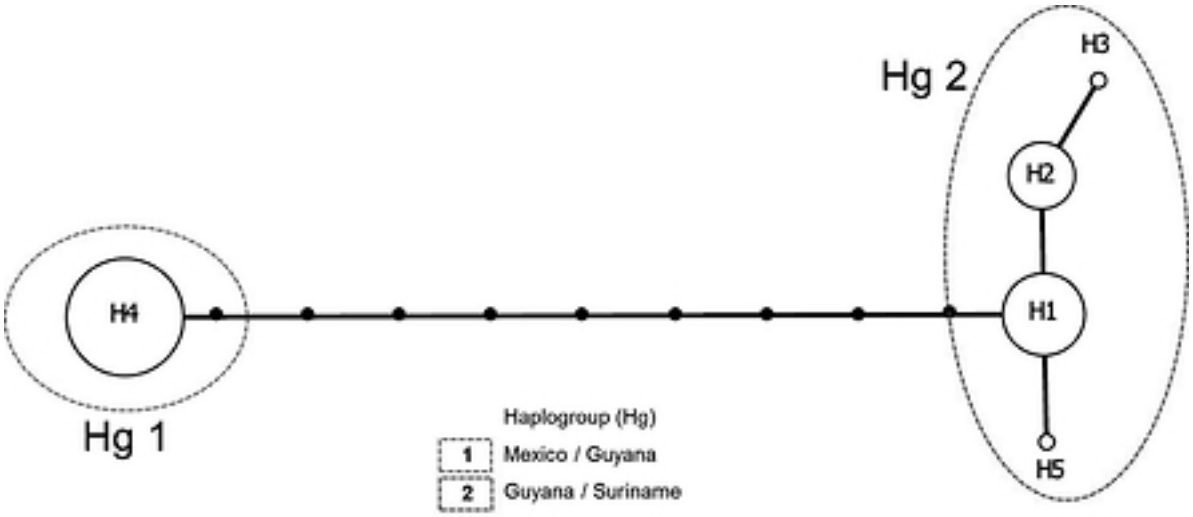

Fig. 3. Haplotype network for the DBY gene of Pteronotus parnellii. The H-number indicates the haplotype identifier for the samples presented in Appendix 4. The size of circles denotes the relative number of samples represented in each haplotype. Lines between haplotypes indicate mutational steps between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

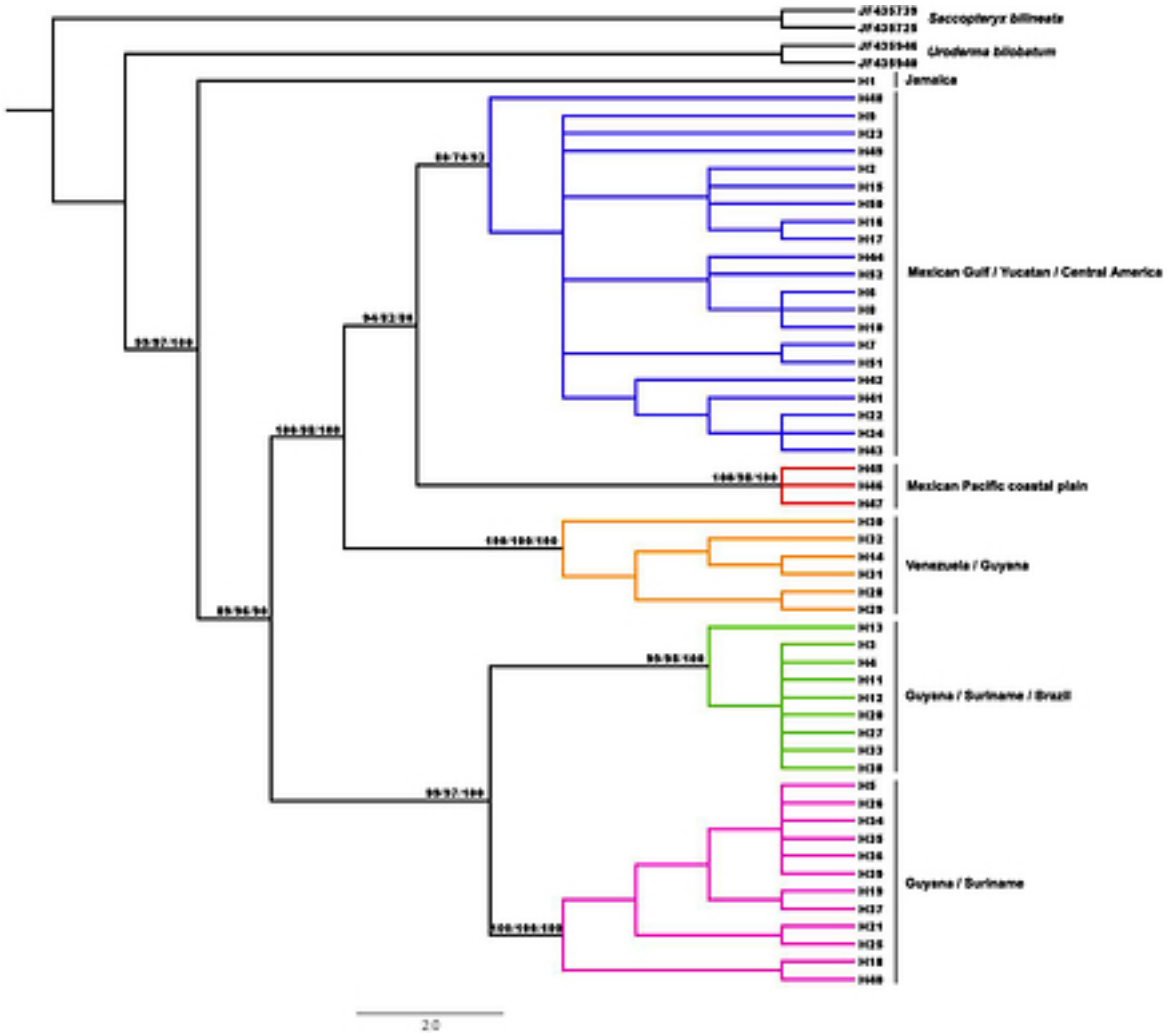

Fig. 4. Phylogenetic relationships of the studied groups within Pteronotus parnellii based on a fragment of the mitochondrial COI gene. The reconstruction presented is a Bayesian consensus tree with proportionally transformed branches. Branch support values indicate from left to right: Bayesian Posterior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

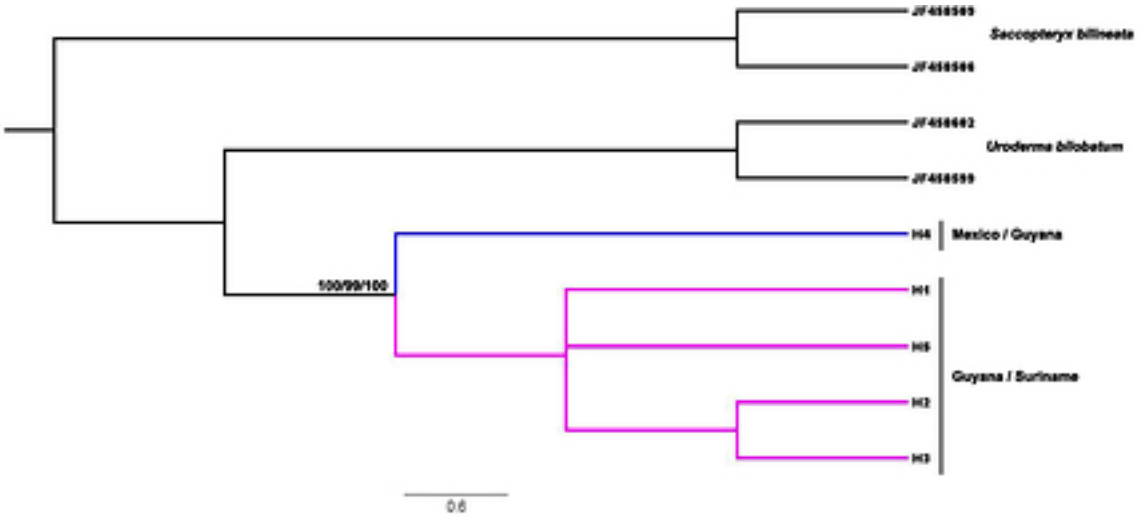

Fig. 5. Phylogenetic relationships of the studied groups within Pteronotus parnellii based on a fragment of the nuclear DBY gene. The reconstruction presented is a Bayesian consensus tree with proportionally transformed branches. Branch support values indicate from left to right: Bayesian Posterior Probability,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

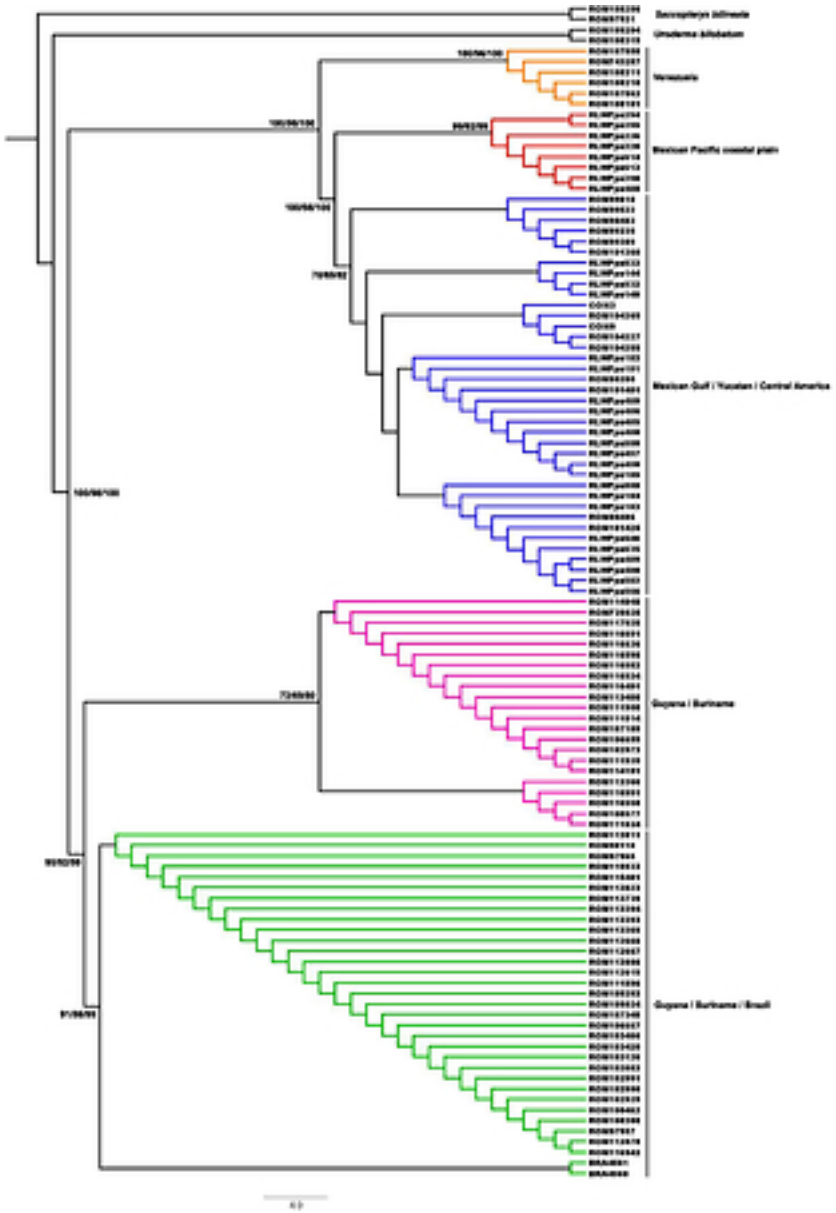

Fig. 6. Phylogenetic relationships of the studied groups within Pteronotus parnellii based on a concatenated analysis of mitochondrial COI and nuclear DBY genes. The reconstruction presented is a Bayesian consensus tree with proportionally transformed branches. Branch support values indicate from left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

Manuscript body

Manuscript body 1 - Download source file (73.76 kB)

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 linages (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

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Appendix 1 to 4

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