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Phylogenetics and biogeography of the broad-nosed bats, genus *Platyrrhinus* (Chiroptera: Phyllostomidae)

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ABSTRACT

The Neotropical broad-nosed bats, genus *Platyrrhinus,* represent a well-defined monophyletic group of 14 recognized species. A recent study of morphological characters confirmed *Platyrrhinus* monophyly and species diagnosis, but offered little support to their intra-specific relationships. We conducted phylogenetic analyses of the genus, using dense taxonomic sampling in combination with four gene sequences representing both mitochondrial and nuclear DNA transmission systems. Our aim was to elucidate the phylogenetic structure among species, using the resulting 3341 bp of DNA. Maximum parsimony, maximum likelihood, and Bayesian inference analyses produced similar topologies that confirm the monophyly of the genus *Platyrrhinus* and strongly support many previously unrecognized groups. Paraphyly of *Platyrrhinus helleri* and the unclear position of *P. brachycephalus* in the clades were also apparent in the data. Our biogeographical analysis suggests a Brazilian Shield origin for *Platyrrhinus*, followed by subsequent radiations of lineages in the Amazon Basin and Andes. Secondary dispersal from Amazonian and Andean centers is responsible for the *Platyrrhinus* inhabiting the Guianan Shield and the Pacific lowlands and Central America, respectively.

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1. Introduction

The Neotropics are one of the world's six major zoogeographical regions and one of the most diverse: about one quarter of all plant and animal species alive today occur in Central and South America. A variety of geophysical processes, including orogeny, marine transgressions, climate change, habitat expansion, and continental drift, have been implicated as important in the origin of this diversity. The sequence and spatial extent of these factors combine to produce diversification patterns that are shared among the organisms that experienced them, producing patterns of area relationships that are replicated in each group. Numerous other factors are also thought to have influenced diversification of Neotropical biotas, including Pleistocene forest refuges, riverine barriers, parapatric divergence on sharp ecological gradients, flood plain dynamics, chromosomal rearrangements, and ecological heterogeneity, among others (Bush, 1994; Da Silva and Sites, 1995; Haffer, 1997; Marroig and Cerqueira, 1997; Patton et al., 1997; Ron, 2000; Salo, 1987; Wallace et al., 1996). The importance of any one of these factors depends on the biology of the taxa in question, and often these factors may influence specific diversification patterns in concert. Only with careful phylogenetic reconstructions for multiple groups of organisms will it be possible to identify the common historical elements from those peculiar to any group (Cracraft, 1988; Patton and Da Silva, 1998).

The Neotropical bat fauna is rich and much of it is endemic—almost 40% of the mammal species in lowland rainforest sites are bats. Phylogenetic reconstructions are available for some Neotropical genera and species, including *Glossophaga* (Hoffmann and Baker, 2001), *Leptonycteris curasoae* (Wilkinson and Fleming, 1996), *Tadarida brasiliensis* (Russell and McCracken, 2006), *Artibeus* (Lim et al., 2004), *Uroderma bilobatum* (Hoffmann et al., 2003), *Vampyressa* (Hoofer and Baker, 2006; Porter and Baker, 2004), *Vampyressa pusilla* and *V. thyone* (Lim et al., 2003). However, most studies to date have inferred relationships on the basis of a single gene (but see Lim, 2007).

Platyrrhinus is one of the most speciose genera of the Neotropical family Phyllostomidae. It is diagnosed by a combination of characters: two accessory cusps on the posterior face of P4, presence of three upper molars, and the presence of a fringe of hair on the edge of the uropatagium (Velazco, 2005). Although other genera also have these characters (Albuja, 1999; Lim, 1993), no other possesses all three. *Platyrrhinus* ranges in varied habitats from southern Mexico to northern Argentina and Uruguay (Velazco, 2005). Currently, 14 species are recognized within the genus,

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most with restricted distributions (Fig. 1). Phylogenetic analyses of the taxonomic diversity and broad distribution of *Platyrrhinus* could therefore help to place Neotropical regions of endemism into a historical context.

Relationships among *Platyrrhinus* species have been evaluated phenetically and phylogenetically using morphological characters. Early phenetic examinations of the subfamily Stenodermatinae showed *Platyrrhinus* to be paraphyletic, the position of its species varying according to the phenetic method (Owen, 1988). Contemporaneously, phylogenetic relationships of *Platyrrhinus* species were evaluated using 22 discrete and 33 continuous external, cranial, and dental characters (Owen, 1987). That phylogenetic analysis supported *Platyrrhinus* monophyly, identified the monotypic genus *Vampyrodes* as its sister group, and identified two unresolved clades within the genus: *P. aurarius–P. lineatus–P. umbratus–P. vittatus* and *P. dorsalis–P. helleri–P. nigellus* (the remaining species —*P. brachycephalus–P. infuscus–P. recifinus*—were also unresolved). More recently, Velazco (2005) used 60 discrete characters representing external, cranial, dental, and post-cranial morphology, producing a well-resolved topology and robust diagnosis of species, but with low bootstrap and decay values on inter-specific relationships. His analysis supported *Platyrrhinus* monophyly and identified *Vampyrodes* as its sister genus (Fig. 2). It also uncovered four new forms deserving rank as species under a phylogenetic species concept (*P. albericoi*, *P. ismaeli*, *P. masu*, and *P. matapalensis*). Two main clades were apparent: a chiefly Andean grouping of *P. albericoi–P. aurarius–P. chocoensis–P. dorsalis–P. ismaeli–P. infuscus–P. masu–P. nigellus–P. vittatus* on one hand and a lowland forest assemblage of *P. brachycephalus–P. helleri–P. lineatus–P. matapalensis–P. recifinus* on the other.

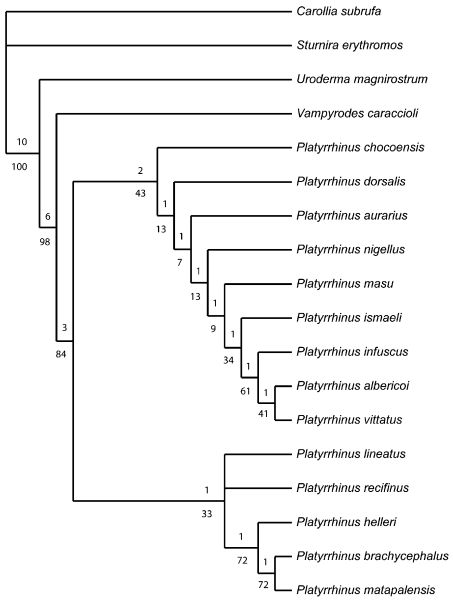

Fig. 2. Strict consensus tree from Velazco (2005), based on a parsimony analysis of morphological data of *Platyrrhinus* with *Carollia subrufa*, *Sturnira erythromos*, *Uroderma magnirostrum*, and *Vampyrodes caraccioli* as outgroups. Numbers above branches are decay values, and those below are bootstrap percentages from 10,000 iterations.

To uncover the phylogenetic relationships of *Platyrrhinus* we analyzed a total of 3341 bp, which included four genes, three mitochondrial and one nuclear. The analysis included complete

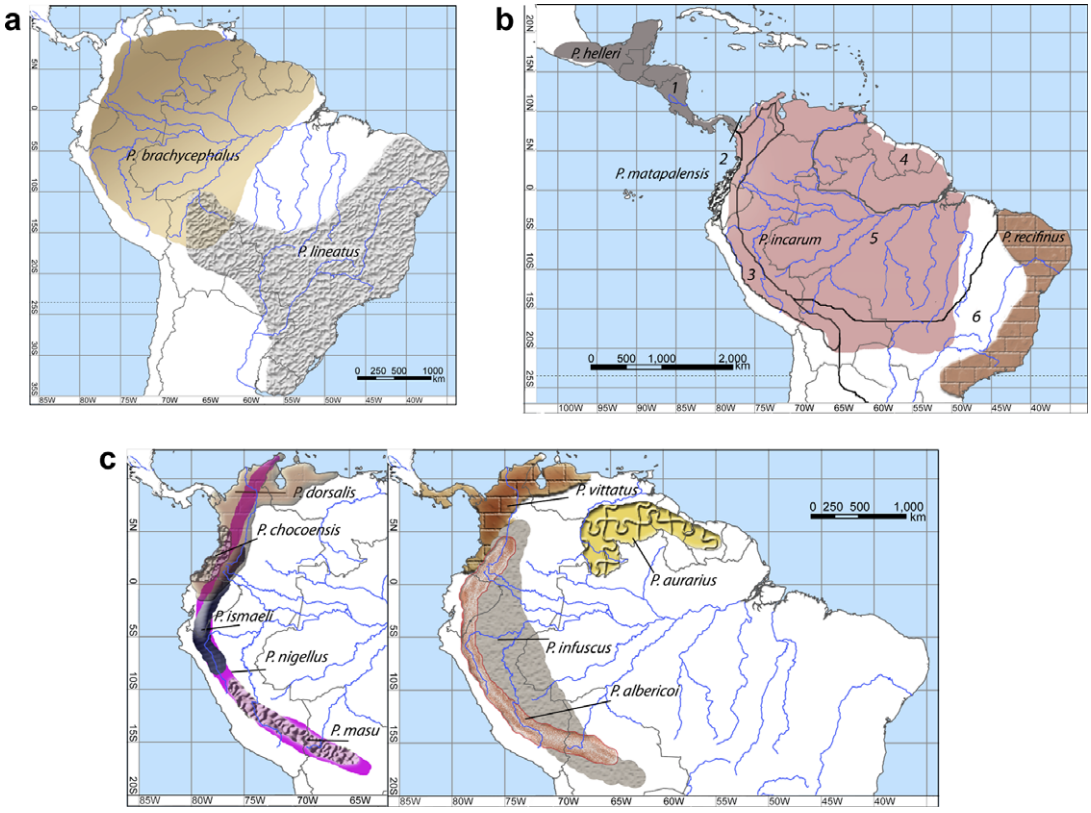

Fig. 1. Geographic distributions of *Platyrrhinus* species emphasizing patterns (no localities) from Velazco, 2005. (a) *P. lineatus and P. brachycephalus*, which present analyses show to represent clades A and D, respectively. (b) *P. helleri*, *P. incarum*, *P. matapalensis*, and *P. recifinus*, shown below to represent clade B. (c) *P. chocoensis*, *P. dorsalis*, *P. ismaeli*, *P. masu*, and *P. nigellus* (left map) and *P. albericoi*, *P. aurarius*, *P. infuscus*, and *P. vittatus* (right map), all shown below to represent clade C. Regions used in the biogeographical analyses are shown in Fig 1b: (1, Central America; 2, Chocó; 3, Andes; 4, Guianan Shield; 5, Amazon Basin; 6, Brazilian Shield).

Table 1
Species, tissue collection number, and GenBank Accession Numbers for the *Platyrrhinus* and outgroup samples used in the study

Taxon	Tissue/Collection Nos	GenBank Accession Nos			
		Cyt-*b*	ND2	D-loop	RAG2
Outgroups					
Carollia brevicauda	FMNH 174595	FJ154120	FJ154186	FJ154252	FJ154318
Chiroderma villosum	FMNH 174652	FJ154121	FJ154187	FJ154253	FJ154319
Mesophylla macconnelli	FMNH 174719	FJ154122	FJ154188	FJ154254	FJ154320
Sturnira erythromos	FMNH 174809	FJ154179	FJ154245	FJ154311	FJ154377
Uroderma magnirostrum	FMNH 174907	FJ154180	FJ154246	FJ154312	FJ154378
Vampyressa melissa	FMNH 174910	FJ154185	FJ154251	FJ154317	FJ154383
Vampyriscus bidens	ALG 14898	FJ154181	FJ154247	FJ154313	FJ154379
Vampyrodes caraccioli	FMNH 174912	FJ154182	FJ154248	FJ154314	FJ154380
Vampyrodes caraccioli	FMNH 174914	FJ154183	FJ154249	FJ154315	FJ154381
Vampyrodes caraccioli	FMNH 174915	FJ154184	FJ154250	FJ154316	FJ154382
Ingroup					
Platyrrhinus albericoi	FMNH 172107	FJ154123	FJ154189	FJ154255	FJ154321
Platyrrhinus albericoi	FMNH 172108	FJ154124	FJ154190	FJ154256	FJ154322
Platyrrhinus aurarius	ROM 108220	FJ154125	FJ154191	FJ154257	FJ154323
Platyrrhinus aurarius	ROM 114634	FJ154126	FJ154192	FJ154258	FJ154324
Platyrrhinus aurarius	ROM 114660	FJ154127	FJ154193	FJ154259	FJ154325
Platyrrhinus aurarius	ROM 114679	FJ154128	FJ154194	FJ154260	FJ154326
Platyrrhinus aurarius	ROM 114702	FJ154129	FJ154195	FJ154261	FJ154327
Platyrrhinus brachycephalus	FMNH 174745	FJ154130	FJ154196	FJ154262	FJ154328
Platyrrhinus brachycephalus	FMNH 174746	FJ154131	FJ154197	FJ154263	FJ154329
Platyrrhinus brachycephalus	FMNH 174748	FJ154132	FJ154198	FJ154264	FJ154330
Platyrrhinus dorsalis	TK 135521	FJ154133	FJ154199	FJ154265	FJ154331
Platyrrhinus dorsalis	TK 135522	FJ154134	FJ154200	FJ154266	FJ154332
Platyrrhinus dorsalis	TK 135530	FJ154135	FJ154201	FJ154267	FJ154333
Platyrrhinus dorsalis	TK 135531	FJ154136	FJ154202	FJ154268	FJ154334
Platyrrhinus dorsalis	TK 135629	FJ154137	FJ154203	FJ154269	FJ154335
Platyrrhinus dorsalis	TK 135668	FJ154138	FJ154204	FJ154270	FJ154336
Platyrrhinus dorsalis	TK 135940	FJ154139	FJ154205	FJ154271	FJ154337
Platyrrhinus helleri	LSU M536	FJ154140	FJ154206	FJ154272	FJ154338
Platyrrhinus helleri	LSU M537	FJ154141	FJ154207	FJ154273	FJ154339
Platyrrhinus incarum	FMNH 129150	FJ154142	FJ154208	FJ154274	FJ154340
Platyrrhinus incarum	FMNH 129154	FJ154143	FJ154209	FJ154275	FJ154341
Platyrrhinus incarum	FMNH 129156	FJ154144	FJ154210	FJ154276	FJ154342
Platyrrhinus incarum	FMNH 174765	FJ154145	FJ154211	FJ154277	FJ154343
Platyrrhinus incarum	FMNH 174766	FJ154146	FJ154212	FJ154278	FJ154344
Platyrrhinus incarum	AMNH/MUSM 13241	FJ154147	FJ154213	FJ154279	FJ154345
Platyrrhinus infuscus	FMNH 129160	FJ154148	FJ154214	FJ154280	FJ154346
Platyrrhinus infuscus	FMNH 129161	FJ154149	FJ154215	FJ154281	FJ154347
Platyrrhinus infuscus	FMNH 174767	FJ154150	FJ154216	FJ154282	FJ154348
Platyrrhinus infuscus	FMNH 174768	FJ154151	FJ154217	FJ154283	FJ154349
Platyrrhinus ismaeli	FMNH 129137	FJ154152	FJ154218	FJ154284	FJ154350
Platyrrhinus ismaeli	FMNH 129142	FJ154153	FJ154219	FJ154285	FJ154351
Platyrrhinus ismaeli	FMNH 129143	FJ154154	FJ154220	FJ154286	FJ154352
Platyrrhinus ismaeli	FMNH 129145	FJ154155	FJ154221	FJ154287	FJ154353
Platyrrhinus lineatus	MVZ 185596	FJ154156	FJ154222	FJ154288	FJ154354
Platyrrhinus lineatus	MVZ 185598	FJ154157	FJ154223	FJ154289	FJ154355
Platyrrhinus lineatus	MVZ 185601	FJ154158	FJ154224	FJ154290	FJ154356
Platyrrhinus lineatus	MVZ 185602	FJ154159	FJ154225	FJ154291	FJ154357
Platyrrhinus lineatus	MVZ 185603	FJ154160	FJ154226	FJ154292	FJ154358
Platyrrhinus masu	FMNH 172100	FJ154161	FJ154227	FJ154293	FJ154359
Platyrrhinus masu	FMNH 172101	FJ154162	FJ154228	FJ154294	FJ154360
Platyrrhinus masu	FMNH 172102	FJ154163	FJ154229	FJ154295	FJ154361
Platyrrhinus masu	MUSM 18265	FJ154164	FJ154230	FJ154296	FJ154362
Platyrrhinus matapalensis	TK 134723	FJ154165	FJ154231	FJ154297	FJ154363
Platyrrhinus matapalensis	TK 134726	FJ154166	FJ154232	FJ154298	FJ154364
Platyrrhinus matapalensis	TK 135094	FJ154167	FJ154233	FJ154299	FJ154365
Platyrrhinus matapalensis	TK 135295	FJ154168	FJ154234	FJ154300	FJ154366
Platyrrhinus nigellus	FMNH 172104	FJ154169	FJ154235	FJ154301	FJ154367
Platyrrhinus nigellus	FMNH 172106	FJ154170	FJ154236	FJ154302	FJ154368
Platyrrhinus nigellus	MUSM 18263	FJ154171	FJ154237	FJ154303	FJ154369
Platyrrhinus nigellus	MUSM 18264	FJ154172	FJ154238	FJ154304	FJ154370
Platyrrhinus nigellus	MUSM 18273	FJ154173	FJ154239	FJ154305	FJ154371
Platyrrhinus recifinus	MVZ 185605	FJ154174	FJ154240	FJ154306	FJ154372
Platyrrhinus recifinus	MVZ 185606	FJ154175	FJ154241	FJ154307	FJ154373
Platyrrhinus recifinus	MVZ 185610	FJ154176	FJ154242	FJ154308	FJ154374
Platyrrhinus vittatus	LSU M515	FJ154177	FJ154243	FJ154309	FJ154375
Platyrrhinus vittatus	ROM 97305	FJ154178	FJ154244	FJ154310	FJ154376

Localities are presented in Appendix A. ALG, Alfred L. Gardner; AMNH, American Museum of Natural History; FMNH, Field Museum of Natural History; LSU, Louisiana State University, Museum of Natural Science; MUSM, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru; MVZ, Museum of Vertebrate Zoology; TK, Museum of Texas Tech University; ROM, Royal Ontario Museum, Toronto, Canada.

sequences for the mtDNA genes cytochrome-*b* (cyt-*b*) and NADH dehydrogenase subunit 2 (ND2) and control region (D-loop), and a fragment of the nuclear recombination activating gene 2 (RAG2).

2. Materials and methods

2.1. Taxon sampling

To examine relationships among *Platyrrhinus* species, all currently recognized species were included (Table 1, Appendix A), except for *P. chocoensis*. Two or more individuals per species were sequenced to assess intra-specific variation. All sequences used, both ingroup and outgroup, were obtained for this study (Table 1) and have accompanying voucher specimens in museum collections. *Carollia brevicauda*, *Chiroderma villosum*, *Mesophylla macconnelli*, *Sturnira erythromos*, *Uroderma magnirostrum*, *Vampyressa melissa*, *Vampyriscus bidens*, and *Vampyrodes caraccioli*, all thought to be close relatives of *Platyrrhinus* (Baker et al., 2003; Hoofer and Baker, 2006; Jones et al., 2002; Owen, 1987; Velazco, 2005; Wetterer et al., 2000), were included as outgroups.

Voucher specimens for the samples are deposited in the following mammal collections: American Museum of Natural History, New York (AMNH); Field Museum of Natural History, Chicago (FMNH); Louisiana State University, Museum of Natural Science, Baton Rouge (LSU); Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM); Museum of Vertebrate Zoology, University of California, Berkeley (MVZ); Museum of Texas Tech University, Lubbock, Texas (TTU; TK); and Royal Ontario Museum, Toronto, Canada (ROM).

2.2. DNA sequencing

Total DNA was isolated from a small (~0.05 g, wet weight) portion of liver or muscle samples that had been frozen or preserved in lysis buffer or ethanol. DNA was extracted using a Puregene DNA isolation kit (Gentra System, Minneapolis, Minnesota). Briefly, tissue was homogenized in a low-molarity salt buffer and digested overnight with proteinase K (Davis et al., 1986). DNA was precipitated in 100% isopropanol with 20 mgml^{-1} glycogen at room temperature. We pelleted the DNA by high-speed centrifugation followed by washing in 70% ethanol. DNA was then stored at 4 °C in Puregene DNA hydration solution.

Aliquots of genomic DNA isolates were used as templates for polymerase chain reaction (PCR) to amplify double-stranded DNA products from two mitochondrial genes, cyt-*b* and ND2, one regulatory region, D-loop, and one nuclear gene, RAG2. Each PCR had a reaction volume of 25 μl and contained 1 μl of DNA stock, 2.5 μl 10× reaction buffer, 2.5 μl of 8 mM premixed deoxynucleotide triphosphates, 15 μl of ddH_2O, 2.0 μl of FMNH Taq, and 1 μl of each oligonucleotide, each at 10 μM concentration. Primers used for amplification and sequencing are listed in Table 2.

PCR profiles included an initial denaturation step at 94–95 °C for 2–3 min, followed by 30–35 cycles of PCR. The cycles involved denaturation at 94–95 °C for 30 s, annealing at 50–65° C for 30–90 s, polymerization at 68–72 °C at 2 min, and a final extension at 72 °C at 5–8 min. The PCR bands were cut, and intact DNA was melted at 70 °C for 10 min, and then 1.5 μl of gelase was added and incubated for at least 2 h at 45 °C. The PCR products were cycle-sequenced using ABI PRISM Big Dye v. 3.1 (Applied Biosystems, Foster City, CA). The cycling protocol used involved an initial denaturation step at 96 °C for 60 s, followed by 25 cycles of denaturation at 96 °C for 10 s, annealing at 50 °C for 5 min, and extension at 60 °C for 4 min. Cycle-sequencing products were purified through an EtOH–EDTA precipitation protocol and run on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA) using the amplification primers. Sequences were edited and compiled using Sequencer™ 4.1.2 software (Gene Codes). Base-calling ambiguities between strands were resolved by choosing the call on the cleanest strand. All the sequences presented in this study have been deposited in GenBank with the Accession Nos. FJ154120-FJ154383 (Table 1).

2.3. Data analysis

2.3.1. Phylogeny reconstruction

DNA sequences were aligned by eye using Sequencer™, 4.1.2 (Gene Codes Corporation). Apparent heterozygosities in the nuclear sequences were coded using the IUPAC ambiguity codes. After exclusions and trimming, the cyt-*b* gene data set contained 1140 characters, ND2 1044 characters, D-loop 426 characters, and RAG2 731 characters, totaling 3341 nucleotide characters. To explore the degree of saturation present, we plotted raw sequence divergence (uncorrected *p* distance) vs. substitution number for all pair-wise comparisons among individuals (Fig. 3). If sequences are saturated (i.e., multiple substitutions occur at a given nucleotide position), the plots should reach an asymptote at higher sequence divergence values. Because no such plateaus are seen, we conclude that saturation has not yet occurred and so did not exclude characters or employ a weighting scheme in the parsimony analyses.

Analyses were conducted on two data partitions: (1) mitochondrial sequences (cyt-*b*, ND2, and D-loop); (2) concatenated mitochondrial and nuclear data set.

2.3.2. Maximum parsimony

Maximum parsimony analyses (MP) were conducted using PAUP* v. 4.0b10 (Swofford, 1998). Heuristic searches to find the most parsimonious tree(s) were performed using tree bisection-reconnection (TBR) branch-swapping. One thousand random sequence addition replicates were used to minimize the chance of finding only locally optimal trees (Maddison, 1991). All sites were equally weighted and gaps treated as missing characters. Nonparametric bootstrapping (Felsenstein, 1985) probed support of clades, with 100 total pseudoreplicates and TBR branch swapping with ten random sequence addition replicates per pseudoreplicate.

Table 2
Primers and primer sequences used for amplification and sequencing in this study

Gene	Primer name	Primer sequence	Source
Cyt-*b*	L14724	5′-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3′	Irwin et al. (1991)
	H15915	5′-AAC TGC AGT CAT CTC CGG TTT ACA AGA C-3′	Irwin et al. (1991)
ND2	L-Met3841	5′-GGT CAG CTA AAT AAG CTA TCG GG-3′	Lloyd (2003)
	H-Asn5149	5′-GGA GAA GTA GAT TGA AGC CAG TTG T-3′	Lloyd (2003)
D-loop	L0	5′-CCC AAA GCT GAA ATT CTA CTT AAA CTA-3′	Douzery and Randi (1997)
	E3	5′-ATG ACC CTG AAG AAA SAA CCA G-3′	Huchon et al. (1999)
RAG2	RAG2F220	5′-GAT TCC TGC TAY CTY CCT CCT CT-3′	Teeling et al. (2000)
	RAG2R995	5′-CCC ATG TTG CTT CCA AAC CAT A-3′	Teeling et al. (2000)

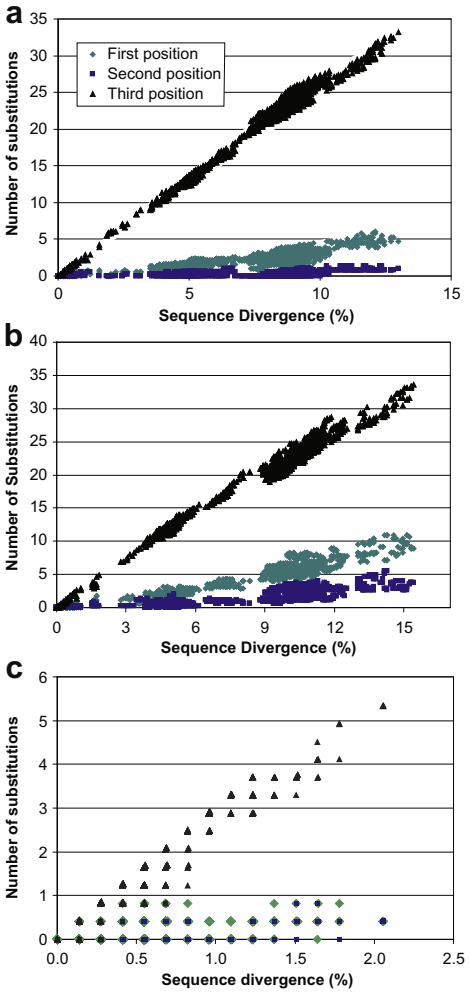

Fig. 3. Saturation plots of mitochondrial (a) cyt-*b*, (b) ND2, and nuclear, (c) RAG2 genes.

2.3.3. Maximum likelihood

The best-fit model of molecular evolution was selected by the Akaike Information Criterion (AIC) as implemented in Modeltest v. 3.7 (Posada and Crandall, 1998). Maximum likelihood analyses (ML) were conducted using Garli v. 0.951 (Zwickl, 2006). ML searches were performed using a GTR + I + Γ model. The run was repeated five times from random starting trees using the auto-terminate setting and default parameters. Garli was used to generate 1000 ML nonparametric bootstrap replicates with the generation threshold (without an improvement in topology) halved to 5000 as suggested by the program; the replicates were used to calculate a majority rule consensus tree in PAUP* to assess clade support.

Although the ILD test is frequently used to evaluate combinability of data partitions, we did not use this test because it has been shown to be misleading in a range of empirical and simulation studies (Barker and Lutzoni, 2002; Cunningham, 1997; Yoder et al., 2001). We statistically evaluated the combinability of the four genes using the one-tailed Shimodaira–Hasegawa test (SH test; Shimodaira and Hasegawa, 1999, 2001; Goldman et al., 2000) implemented in the CONSEL package (Shimodaira and Hasegawa, 2001). We performed ML analyses for each gene using its respective model: cyt-*b* (GTR + I + Γ), ND2 (TVM + I + Γ), D-loop (TrN + I + Γ), and RAG2 (HKY + I). For each partition, four sets of likelihood values were obtained: the likelihood values of the cyt-*b*, ND2, D-loop, and RAG2 trees using the cyt-*b* data set, the likelihood values of those trees using the ND2 data set, etc. Then we evaluated if the likelihood values from the different trees obtained under one data set were significantly different.

2.3.4. Bayesian inference

Bayesian inference analyses (BI) were used to estimate a phylogeny employing different models of molecular evolution for each data set. Each gene was run under different models of nucleotide substitution and parameters as suggested by the AIC as implemented in Modeltest: cyt-*b* (GTR + I + Γ), ND2 (TVM + I + Γ), D-loop (TrN + I + Γ), and RAG2 (HKY + I). Bayesian inference analysis was conducted using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001), with random starting trees without constraints, four simultaneous Markov chains were run for 1×10^7 generations, trees were sampled every 100 generations, stationarity was assessed by examining the standard deviation of split frequencies and by plotting the –ln *L* per generation using Tracer v. 1.3 (Rambaut and Drummond, 2003), and trees generated before stationarity were discarded as "burn-in."

2.3.5. Biogeography of Platyrrhinus

We used two approaches to infer ancestral areas. The first approach, dispersal-vicariance analysis as implemented in DIVA v. 1.1 (Ronquist, 1996, 1997), involved parsimony reconstruction. DIVA reconstructs the ancestral distribution at each of the internal nodes of a given phylogeny. This is accomplished by means of optimization rules and set costs for extinction (cost of 1 per area lost) and dispersal (cost of 1 per area added). Vicariance and sympatric speciation carry no cost. The second approach was a ML method, dispersal-extinction-cladogenesis (DEC) as implemented in Lagrange (Ree et al., 2005; Ree and Smith, 2008). This method provides likelihood values for the different biogeographic scenarios, enabling reconstruction of ancestral ranges and inferring the directionality of dispersal events. To conduct these analyses, the following parameters are required: (a) a phylogenetic tree in the case of DIVA and an ultrametric phylogenetic tree (tree where terminal nodes are all equally distant from the root) with branch lengths for Lagrange; and (b) a set of areas assigned to each taxon. Species were coded geographically by six regions in which *Platyrrhinus* species occur: Central America, Chocó, Andes, Amazon Basin, Guianan Shield, and Brazilian Shield (Fig. 1b). We used a reduced tree using one individual for each taxon, we also constrained the ancestral area estimate to contain a maximum of 3 areas.

We tested the combined dataset for clock-like evolution using a likelihood ratio (LR) test. Likelihood scores for the ML topology, with and without enforcing a molecular clock, were determined in PAUP. Significance was assessed by comparing two times the difference in log likelihood values to a χ^2 distribution with n–2 degrees of freedom, where n was the number of taxa. When the LR test determined that data did not adhere to a molecular clock, we transformed the ML tree into an ultrametric topology using nonparametric rate smoothing (NPRS; Sanderson, 1997) implemented in the APE package (Paradis et al., 2004) in R (R-Development-Core-Team, 2008).

Fossil records of *Platyrrhinus* are scarce, reflecting the poor taphonomic conditions of the wet moist forests that most of these bat species occupy. Only a handful of Pleistocene records of *P. incarum* and *P. lineatus* are reported from eastern Brazil (Czaplewski and Cartelle, 1998; Fracasso and Salles, 2005). For this reason the ultrametric tree obtained through NPRS was used to compare relative divergence among clades of *Platyrrhinus*.

3. Results

3.1. Sequence variation and saturation analysis

3.1.1. Cytochrome-b, ND2, and D-loop

Complete cyt-*b* (1140 bp), ND2 (1044 bp), and D-loop (426 bp) sequences were obtained for all taxa (Table 1). Base composition

Table 3
Shimodaira-Hasegawa test results

Gene	Tree	–ln *L*	SH test	
			P	Significantly different[a]
Cyt-*b*	Cyt-*b*	7142.05	1.000	No
	ND2	7310.97	0.371	No
	D-loop	3474.66	0.051	No
	RAG2	1551.70	0.000	Yes
ND2	Cyt-*b*	7142.05	0.363	No
	ND2	7310.97	1.000	No
	D-loop	3474.66	0.105	No
	RAG2	1551.70	0.000	Yes
D-loop	Cyt-*b*	7142.05	0.233	No
	ND2	7310.97	0.276	No
	D-loop	3474.66	1.000	No
	RAG2	1551.70	0.053	No
RAG2	Cyt-*b*	7142.05	0.139	No
	ND2	7310.97	0.120	No
	D-loop	3474.66	0.057	No
	RAG2	1551.70	1.000	No

[a] The tree is considered to be significantly different if *p*-value is lower that 0.05.

across this data set was homogeneous, except for low values of guanine (32% A, 30% C, 11% G, and 27% T), and no evidence of saturation was found among the sequences used (Fig. 3).

Within cyt-*b*, 379 (33%) of sites were variable and 305 (30%) were parsimony informative. The proportion of parsimony informative sites depended on position in the codon: 13.8% in first, 2.6% in second, and 83.6% in third positions. In the case of ND2, 421 (40%) of sites were variable and 319 (27%) were parsimony informative. Parsimony informative sites also varied by codon position: 19.7% in first, 8.5% in second, and 71.8% in third positions.

3.1.2. RAG2

Fragments of the nuclear RAG2 (731 bp) gene were obtained for all taxa (Table 1). Base composition was homogeneous (30% A, 21% C, 23% G, and 26% T), and there was no evidence of saturation (Fig. 3).

In RAG2, 56 (8%) of sites were variable and 20 (3%) were parsimony informative. The distribution of the parsimony informative sites was dependent on codon position: 15% in first, 0% in second, and 85% in third.

3.2. Phylogenetic analyses

The Shimodaira and Hasegawa (1999) tests indicated there were significant differences in the fit of the likelihood values obtained from the RAG2 tree using the cyt-*b* and ND2 data sets. However, when assessing the likelihood values of the cyt-*b* and ND2 trees using the RAG2 data set, there were no significant differences

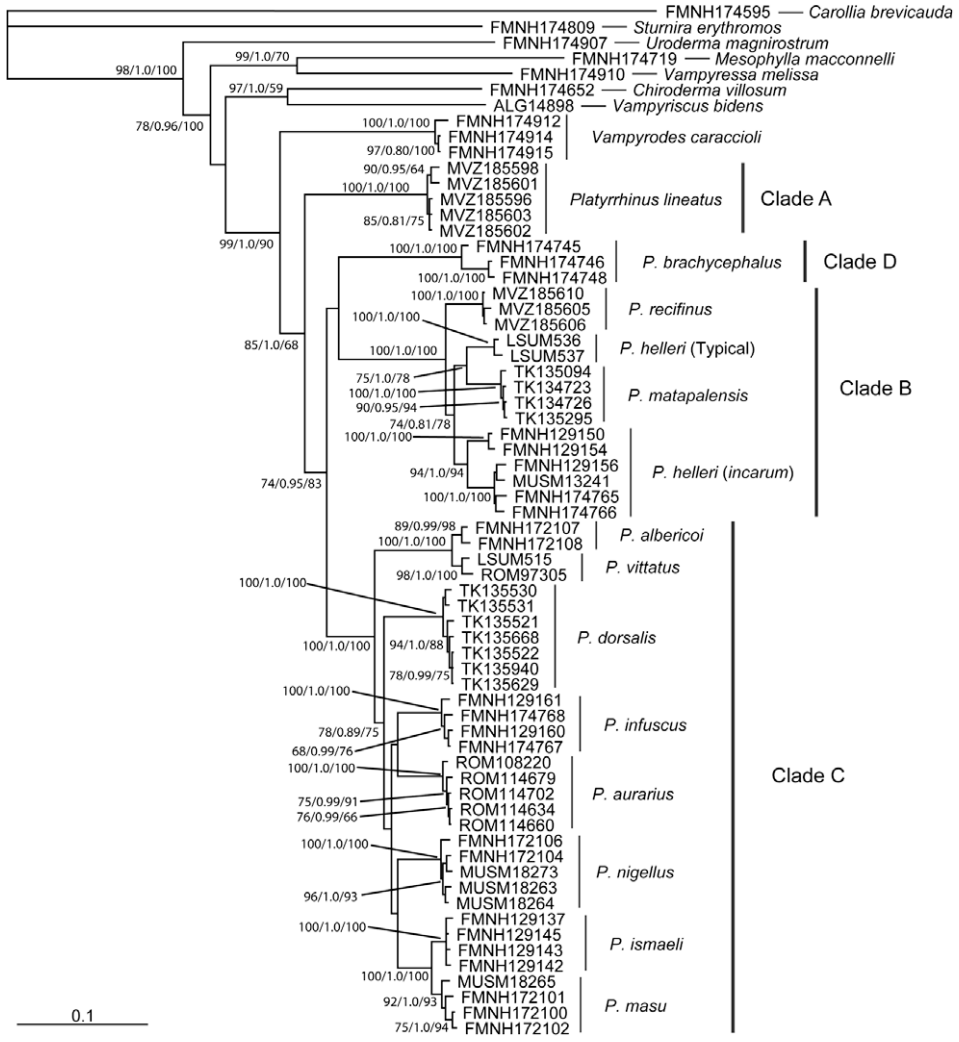

Fig. 4. Combined mtDNA and nDNA maximum-likelihood phylogram for *Platyrrhinus*. Clades of interest labeled A–D. Support for nodes are shown as ML bootstrap/Bayesian posterior probabilities/MP bootstrap (values greater than 50% are presented).

(Table 3). Conflict between RAG2 and cyt-*b* or ND2 does not appear to undermine phylogenetic resolution when combining data (Fig. 4). The molecular data from the different genes were concatenated from the same individual for combined analyses.

3.2.1. Combined cyt-b, ND2, and D-loop

We combined all the mitochondrial data sets as suggested by the SH tests (Table 3). The combined dataset (2610 bp) includes the 66 specimens shared between data sets. Unweighted MP analysis resulted in 90 most parsimonious trees (CI = 0.42 and 30% parsimony informative characters). MP, ML, and BI analyses of the mitochondrial cyt-*b*, ND2, and D-loop all recover *Platyrrhinus* monophyly, confirming other taxonomically extensive studies (Baker et al., 2000, 2003; Jones et al., 2002; Owen, 1987, 1988; Velazco, 2005; Wetterer et al., 2000). The analyses all produced similar topologies, and each was strongly supported: (1) the placement of *Vampyrodes* as the sister genus of *Platyrrhinus*; (2) most of the inter-specific relationships; and (3) the paraphyly of *P. helleri*, where the South American individuals group together and are sister to the clade conformed by Central American individuals of *P. helleri* and *P. matapalensis*. Four clades were recovered: (A) one comprised solely by *P. lineatus* that is sister to all other *Platyrrhinus* species; (B) the second *P. helleri*, *P. matapalensis*, and *P. recifinus*; (C) the third *P. albericoi*, *P. aurarius*, *P. dorsalis*, *P. infuscus*, *P. ismaeli*, *P. masu*, *P. nigellus*, and *P. vittatus*; and (D) the fourth *P. brachycephalus*. The four clades are strongly supported. Clade A is sister to clades B–D, while relationships among B–D are only weakly supported.

3.2.2. Combined mitochondrial and nuclear partitions

We combined cyt-*b*, ND2, D-loop, and RAG2 sequences as suggested by the SH tests (Table 3). Analyses of the combined mitochondrial and nuclear partitions (3341 bp) included the 66 specimens shared between data sets. Unweighted MP analysis resulted in 580 most parsimonious trees (CI = 0.43 and 24.2% parsimony informative characters). MP, ML, and BI analyses resulted in similar topologies, *Platyrrhinus* monophyly (68% bootstrap under MP, 85% bootstrap under ML, and 1.0 posterior probabilities under BI, Fig. 4) was strongly supported. As in the mitochondrial analyses, *Vampyrodes caraccioli* is recovered as the sister genus of *Platyrrhinus*, and *P. brachycephalus* was inconsistently placed among clades. Four clades resembling those in the mitochondrial analyses were recovered: (A) comprised by *P. lineatus* and sister to all other *Platyrrhinus* species; (B) the second *P. helleri*, *P. matapalensis*, and *P. recifinus*; (C) the third *P. albericoi*, *P. aurarius*, *P. dorsalis*, *P. infuscus*, *P. ismaeli*, *P. masu*, *P. nigellus*, and *P. vittatus*; and (D) the fourth *P. brachycephalus* (Fig. 4). The four clades are strongly supported, with slightly higher support values than the mitochondrial clades. Clade A is sister to clades B–D, while relationships among B–D are only weakly supported.

Maximum likelihood was used to test for a clock-like sequence evolution. The molecular clock hypothesis was rejected by the likelihood ratio test (LR = 129.60; $df = 64$; $p < 0.001$).

3.3. Biogeographical analysis

The ancestral range inheritance scenarios inferred by the parsimony-based DIVA and the likelihood-based DEC analyses of the 17 terminal taxa distribution matrix were congruent (Fig. 5). The optimal reconstruction in DIVA required 19 dispersal events. These analyses indicated that the ancestor of *Platyrrhinus* was historically distributed over the Brazilian Shield, and subsequently gave rise to radiating lineages in the Andes and Amazon Basin. *Platyrrhinus* species endemic to the Guianan Shield, Chocó, and Central America resulted from secondary dispersal from Amazonian and Andean centers, respectively (Fig. 5).

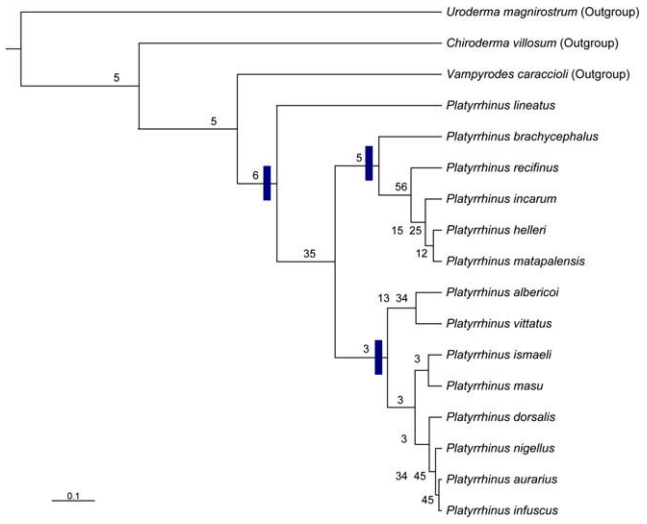

Fig. 5. Ultrametric NPRS Tree showing the biogeographical scenario of *Platyrrhinus* based on dispersal-vicariance and on dispersal-extinction-cladogenesis (DEC) analyses, using six geographical regions (1, Central America; 2, Chocó; 3, Andes; 4, Guianan Shield; 5, Amazon Basin, and 6, Brazilian Shield) and imposing a constraint of three maximal ancestral areas. Letters correspond to the DIVA and DEC analyses and indicate the ancestral area for that particular clade.

4. Discussion

We consider the topology of the combined mitochondrial and nuclear partitions to offer the best estimate of this group's phylogeny. Each data partition contributed to improving the resolution of the resulting tree, and there was a lack of apparent conflict between partitions. Therefore, we base our taxonomic conclusions on this tree (Fig. 4).

4.1. Phylogeny of Platyrrhinus

The molecular analyses extend support for *Platyrrhinus* monophyly, resolve four clades within the genus, and identify one species as paraphyletic. Clade A comprises solely *P. lineatus*. Clade B includes *P. helleri* (which is paraphyletic), *P. matapalensis*, and *P. recifinus*. Clade C comprises *P. albericoi*, *P. aurarius*, *P. dorsalis*, *P. infuscus*, *P. ismaeli*, *P. masu*, *P.nigellus*, and *P. vittatus*. Finally, clade D includes only *P. brachycephalus* (Fig. 4). Relationships among clades B–D were weakly supported, and this whole group (clades B–D) was sister to clade A (Fig. 4).

The present analyses support Ferreira et al. (2005) and Velazco (2005) in recognizing *P. lineatus* and *P. recifinus* as valid species. The position of *Platyrrhinus lineatus* as sister to the remaining *Platyrrhinus* species was not suggested by Owen (1987) or Velazco (2005). Owen (1987) recovered *P. lineatus* in a polytomy together with *P. aurarius*, *P. umbratus*, and *P. vittatus*, whereas Velazco (2005) found it in the lowland clade that also included *P. brachycephalus*, *P. helleri*, *P. matapalensis*, and *P. recifinus* (Fig. 2).

Within clade B, specimens identified as *P. helleri* were shown to be paraphyletic (Fig. 4), comprised of a South American group that was sister to the clade conformed by *P. helleri* (Central America) and *P. matapalensis*. The cyt-*b* divergence between these two groups of *P. helleri* ranges from 3.60% to 4.30% (Table 4), suggesting that two different species might be involved. Koopman (1994) recognized two subspecies in *P. helleri* [under the generic synonym *Vampyrops*]: *P. h. helleri,* distributed from Mexico to northwestern Peru and Trinidad; and *P. h. incarum,* distributed east of the Andes from southern Colombia, southern Venezuela, and the Guianas to Bolivia and Amazonia Brazil (Fig. 1). The nominate subspecies

Table 4
Pair-wise uncorrected percentage of cyt-*b* sequence divergence ($\bar{x}$± SE) among *Platyrrhinus*

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Platyrrhinus albericoi*	0.88													
2	*P. aurarius*	6.05 ± 0.15	0.39 ± 0.20												
3	*P. brachycephalus*	8.51 ± 0.43	7.32 ± 0.40	1.11 ± 0.81											
4	*P. dorsalis*	6.35 ± 0.22	5.28 ± 0.18	9.47 ± 0.48	0.76 ± 0.18										
5	*P. helleri*	8.68 ± 0.10	8.14 ± 0.12	9.24 ± 0.31	8.33 ± 0.20	0.35									
6	*P. incarum*	9.61 ± 0.22	8.54 ± 0.19	9.64 ± 0.40	8.83 ± 0.15	3.99 ± 0.26	1.71 ± 0.93								
7	*P. infuscus*	6.03 ± 0.25	4.36 ± 0.16	8.33 ± 0.42	5.10 ± 0.14	8.55 ± 0.17	9.01 ± 0.17	0.48 ± 0.18							
8	*P. ismaeli*	6.43 ± 0.28	5.00 ± 0.10	8.22 ± 0.40	5.67 ± 0.12	7.74 ± 0.04	8.99 ± 0.18	5.09 ± 0.14	0.22 ± 0.09						
9	*P. lineatus*	9.34 ± 0.26	8.91 ± 0.23	9.19 ± 0.26	8.96 ± 0.20	8.11 ± 0.16	9.47 ± 0.46	8.74 ± 0.16	7.14 ± 0.16	0.42 ± 0.20					
10	*P. masu*	5.48 ± 0.31	4.97 ± 0.10	7.97 ± 0.44	4.94 ± 0.21	7.68 ± 0.05	8.82 ± 0.18	4.63 ± 0.20	2.00 ± 0.10	7.57 ± 0.18	0.41 ± 0.23				
11	*P. matapalensis*	8.40 ± 0.12	7.91 ± 0.15	9.38 ± 0.31	8.54 ± 0.11	3.07 ± 0.09	3.90 ± 0.17	8.33 ± 0.18	8.36 ± 0.10	7.93 ± 0.21	7.94 ± 0.10	0.18 ± 0.11			
12	*P. nigellus*	6.05 ± 0.30	5.40 ± 0.17	8.61 ± 0.39	5.18 ± 0.17	7.54 ± 0.13	8.92 ± 0.22	4.98 ± 0.14	4.55 ± 0.18	7.65 ± 0.17	4.17 ± 0.11	7.95 ± 0.13	0.37 ± 0.18		
13	*P. recifinus*	9.05 ± 0.17	8.15 ± 0.20	9.73 ± 0.27	8.26 ± 0.13	4.24 ± 0.19	4.01 ± 0.31	8.92 ± 0.22	8.60 ± 0.06	8.81 ± 0.27	8.69 ± 0.14	3.74 ± 0.18	8.25 ± 0.20	0.29 ± 0.18	
14	*P. vittatus*	1.18 ± 0.09	6.07 ± 0.04	8.22 ± 0.51	6.65 ± 0.08	8.42 ± 0.00	9.52 ± 0.21	6.21 ± 0.18	6.29 ± 0.11	9.12 ± 0.12	5.48 ± 0.05	8.31 ± 0.08	6.32 ± 0.11	9.09 ± 0.18	0.53

P. h. helleri is based on a Mexican specimen and applies to Central American populations. South American records from west of the Andes formerly identified as *P. helleri* were recently assigned to the newly described *P. matapalensis* (Figs. 1 and 4).

P. matapalensis was recently described from the Pacific lowlands of Ecuador, Colombia, and Peru (Fig. 1). Formerly considered part of *P. helleri*, *P. matapalensis* is sister to the Central American component of *P. helleri* (Fig. 4). This arrangement was not proposed by Owen (1987) or Velazco (2005). *P. matapalensis* and the two groups of *P. helleri* show divergences of 2.98% to 4.21% from one another in cyt-*b* (Table 4). Sequence divergence between these groupings and differences in size and morphology leads us to believe that *incarum* should be recognized as a distinct species. South America populations east of the Andes formerly called *P. helleri* should be distinguished as *P. incarum* (Figs. 1 and 4).

The position of *P. brachycephalus* (clade D) remains uncertain.

Within clade C, three Andean species group together: *P. ismaeli* and *P. masu* as sister taxa, with *P. nigellus* basal to them. *P. ismaeli* and *P. masu* were formerly recognized as "*P. dorsalis*" and have parapatric distributions (Velazco and Solari, 2003). Although more than 5% divergent in cyt-*b* sequences from *P. dorsalis*; *P. ismaeli* and *P. masu* are weakly divergent (1.84–2.19%, Table 4), suggesting a recent split between these two species. An endemic of the *tepuis* and the Guianan Shield, *P. aurarius* and a circum-Amazonian species *P. infuscus* are sister to this strictly Andean group (Fig. 1 and 4).

Platyrrhinus albericoi and *P. vittatus* are found sympatrically in Colombia, are sister taxa, and show low cyt-*b* divergence (1.05–1.23%, Table 4). Morphologically, Velazco (2005) found that these species can be distinguished by their size and presence or absence of an accessory cuspulid on the anterolingual cristid of the last lower premolar. Their low cyt-*b* divergence could be the effect of a recent split (Table 4). These two species are sister to the remaining species of clade C (Fig. 4).

Although Andean *P. nigellus* was long treated as a subspecies of *P. lineatus* (Koopman, 1978, 1994; Willig and Hollander, 1987), Velazco and Solari (2003) and Velazco (2005) emphasized their separation as distinct species. Cyt-*b* sequence divergence between these two species is quite high (>7%, Table 4), and the two species belong to different clades (*P. lineatus* to clade A and *P. nigellus* to clade C, Fig. 4).

4.2. Diversification and biogeography of *Platyrrhinus*

Species-level phylogenies can permit the identification of biogeographic events that have affected the distributions and origins of extant species (Willis et al., 2007). The broad Neotropical distribution of *Platyrrhinus* and its radiation into most areas of endemism makes it an excellent group for exploring phylogeography (Fig. 1).

Cracraft and Prum (1988) presented phylogenetic analyses for several groups of Neotropical birds, and identified a general pattern of area relationships common to all taxa in their study. They hypothesized that, because these relationships were historical, that they should apply to other, not-yet-studied organisms distributed in the same region. Their work suggested that some of the oldest divergences in Neotropical clades separate Atlantic Forest habitats from the remainder; trans-Andean biotas were the next isolated (presumably by Andean orogeny), and diversification of lowland *cis*-Andean faunas (those on the east side of the cordilleras) took place most recently, sometimes with secondary dispersal to Atlantic Forest habitats. A subsequent analysis of endemicity confirmed the general application of this model to the entire avifauna (Bates et al., 1998). This pattern of relationships should apply to other sympatric and contemporaneous groups.

The *Platyrrhinus* phylogeny only partially reflects these area relationships. The earliest divergence event in Cracraft and Prum

(1988) involved the Atlantic Forest clade and the remaining clades. Others suggest that this first split occurred between a Central America—Chocó clade and the remaining clades (Eberhard and Bermingham, 2005; Prum, 1988). In *Platyrrhinus*, the oldest divergence separates *P. lineatus* (clade A, Figs. 4 and 5) in Atlantic Forest and adjacent ecoregions from the remaining species (Fig. 1). There is no obvious trans-Andean component. The remaining species of *Platyrrhinus* fall into Amazon (clades B and D) and Andean (Clade C) radiations (Figs. 1, 4 and 5). Derived elements of each group extend into Central America, and the Andean radiations are unquestionably more profuse (Fig. 5).

Weir (2006) found that speciation rates in the highlands increased during late Pliocene and late Pleistocene, especially during the last one million years. This may explain why the Andean clade (clade C in Figs. 1, 4 and 5) is the most speciose, with eight species, and why some pairs of species (*P. albericoi* + *P. vittatus*, *P. ismaeli* + *P. masu*) show very low cyt-*b* divergence (1–2%, Table 4). Elevational zonation of forested habitats and their interruption by major river valleys along Andean slopes offers another explanation for the rapid proliferation of Clade C taxa and their somewhat parapatric distributions (Fig. 1; Patton et al., 1990). Two sister taxa nested within this clade, *P. infuscus* and *P. aurarius,* occur in Amazonia and the Guianan Shield, respectively (Fig. 1). Their most recent common ancestor may have expanded from Andean stock during climatic oscillations of the Pleistocene, thought to carry other taxa to similar distributions (Gardner, 1989), or they resulted from separate dispersal and speciation events.

The topology and distributions of Clade B (Figs. 4 and 5) also suggest relatively recent colonization. In many groups, including other fruit bats (Van Den Bussche et al., 1998), birds (Cracraft and Prum, 1988), and snakes (Zamudio and Greene, 1997), Pacific coastal forests and Central America are occupied by basally divergent taxa; the divergence event itself may be old enough to reflect Andean orogeny and vicariance (Patterson et al., 1992). In contrast, *Platyrrhinus matapalensis* of western Ecuador is clearly allied with Central American *P. helleri*. Koopman (1982) identified the Pacific coastal region as far south as southern Ecuador as a distinct zoogeographic region, harboring many species with broader distributions in Central America but found nowhere else in South America. In like manner, *Orthogeomys thaeleri*, a pocket gopher (Geomyidae) otherwise restricted to North and Central America, is found only in this part of South America (Alberico, 1990).

The Chocó forests of Colombia's Andean slopes, occupied by *P. chocoensis*, are another matter, and may constitute a different region of endemism. Additional analyses are needed that place *P. chocoensis* into this historical framework. Morphological features suggest that it was derived from the Andean radiation. Additional research will also be needed to resolve the placement of *P. brachycephalus*, to elucidate the species present in the *P. helleri* complex, and to resolve the relationships between the major clades of *Platyrrhinus*.

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Appendix A

A.1. of specimens sequenced. For abbreviations, see Section 2

Platyrrhinus albericoi: PERU: Cusco, Paucartambo, San Pedro, 1480 m. 13°3'16"S, 71°32'46"W (FMNH 172107 - FMNH 172108).

Platyrrhinus aurarius: GUYANA: Cuyuni-Mazaruni, Namai Creek, 5 km W of Parmina, 800 m. (ROM 108220); Potaro-Siparuni, Mount Ayanganna, Toe Slope Camp, 700 m. (ROM 114634, 114660, 114679); Potaro-Siparuni, Mount Ayanganna, First Plateau Camp, 1100 m. (ROM 114702).

Platyrrhinus brachycephalus: PERU: Madre de Dios, Manu, Maskoitania, 13.4 km NNW Atalaya, left bank Rio Alto Madre de Dios, 480 m. 12°46'18"S, 71°23'7"W (FMNH 174745–174746, 174748).

Platyrrhinus dorsalis: ECUADOR: Esmeraldas, Comuna San Francisco de Bogota (TK 135521–135522, 135530–135531, 135668); Esmeraldas, Estacion Experimental "La Chiquita" (TK 135629); Esmeraldas, Comuna San Francisco de Bogota, Culvert Pipe 2 (0.61 km N & 0.59 km E of the Town) (TK 135940).

Platyrrhinus helleri: PANAMA: Darién, Cana (LSU M536–M537).

Platyrrhinus incarum: PERU: Amazonas, Bongara, Rio Utcubamba, entre Churuja y Pedro Ruiz, 4250 ft. 5°57'59"S, 77°54'59"W (FMNH 129150); Amazonas, Luya, Rio Utcubamba, 11 km by rd NW Pedro Ruiz, 3600 ft. 5°55'59"S, 78°6'W (FMNH 129154, 129156); Loreto, Rio Galvez, Nuevo San Juan (MUSM 13241); Madre de Dios, Manu, Maskoitania, 13.4 km NNW Atalaya, left bank Rio Alto Madre de Dios, 480 m. 12°46'18"S, 71°23'7"W (FMNH 174765); Cusco, Paucartambo, Consuelo, 15.9 km SW Pilcopata, 1000 m. 13°1'25"S, 71°29'30"W (FMNH 174766).

Platyrrhinus infuscus: PERU: Amazonas, Luya, Rio Utcubamba, 15 km by rd NW Pedro Ruiz, 3600 ft. 5°55'59"S, 78°6'W (FMNH 129160–129161); Cusco, Paucartambo, Consuelo, 15.9 km SW Pilcopata, 1000 m. 13°1'25"S, 71°29'30"W (FMNH 174767–174768).

Platyrrhinus ismaeli: PERU: Amazonas, Chachapoyas, Balsas, 19 km by rd E. 6380 ft. 6°49'40"S, 77°54'46"W (FMNH 129137); Cajamarca, Celendin, Hacienda Limon. 6720 ft. 6°49'59"S, 78°4'59"W (FMNH 129142–129143, 129145).

Platyrrhinus lineatus: BRAZIL: Paraíba, Mata do Buraquinho, Jopo Pessoa (MVZ 185596); Pernambuco, Coqueiral, coconut tree grove outside S border Ibama headquarters at Tamandare, Municipio de Tamandare (MVZ 185598); Bahia, Municipio Valenca, Guaibim (MVZ 185601); Espírito Santo, Estacão Biológica de Santa Lúcia, 650 m. (MVZ 185602); Rio de Janeiro, Municipio Duas Barras, Duas Barras, Garden at house of Mario and Tereza Habib (MVZ 185603).

Platyrrhinus masu: PERU: Cusco, Paucartambo, San Pedro, 1480 m. 13°3'16"S, 71°32'46"W (FMNH 172100–172101); Cusco, Paucartambo, Pillahuata, 2460 m. 13°9'43"S, 71°35'51"W (FMNH 172102); Huánuco, Huánuco, Chinchao, Caserio San Pedro de Carpish, Cordillera de Carpish, 2757 m. (MUSM 18265).

Platyrrhinus matapalensis: ECUADOR: Guayas, Reserva Ecologica Manglares-Churute, Cerro Cimalon (TK 134723, 134726); El Oro, Quebrada Seca, Fuerte Militar Arenillas (7.1 Km W and 12.5 Km S of the Militar Base) (TK 135094); Loja, Puyango, Bosque Petrificado-Sector Las Pailas, Quebrada Los Chirimoyos (TK 135295).

Platyrrhinus nigellus: PERU: Cusco, Paucartambo, San Pedro, 1480 m. 13°3'16"S, 71°32'46"W (FMNH 172104, 172106); Huánuco, Huánuco, Chinchao, Caserio San Pedro de Carpish, Cordillera de Carpish, 2757 m. (MUSM 18263–18264, 18273).

Platyrrhinus recifinus: BRAZIL: São Paulo, Ilha de São Sebastião, Parque Estadual Ilhabela, 200 m. (MVZ 185605–185606, 185610).

Platyrrhinus vittatus: COSTA RICA: Puntarenas, Monteverde (ROM 97305); PANAMA: Darién, ca. 6 km. NW Cana, E Slope Cerro Pirre (LSU M515).

Carollia brevicauda: PERU: Cusco, Paucartambo, Consuelo, 15.9 km SW Pilcopata, 1000 m. 13°1'25"S, 71°29'30"W (FMNH 174595).

Chiroderma villosum: PERU: Madre de Dios, Manu, Maskoitania, 13.4 km NNW Atalaya, left bank Rio Alto Madre de Dios, 480 m. 12°46'18"S, 71°23'7"W (FMNH 174652).

Mesophylla macconnelli: PERU: Cusco, Paucartambo, Consuelo, 15.9 km SW Pilcopata, 1000 m. 13°1'25"S, 71°29'30"W (FMNH 174719).

Sturnira erythromos: PERU: Cusco,Paucartambo, La Esperanza, 2880 m. 13°10'39"S, 71°36'16"W (FMNH 174809).

Uroderma magnirostrum: PERU: Madre de Dios, Manu, Maskoitania, 13.4 km NNW Atalaya, left bank Rio Alto Madre de Dios, 480 m. 12°46'18"S, 71°23'7"W (FMNH 174907).

Vampyressa melissa: PERU: Cusco, Paucartambo, Consuelo, 15.9 km SW Pilcopata, 1000 m. 13°1'25"S, 71°29'30"W (FMNH 174910).

Vampyriscus bidens: BRAZIL: Rondônia, Cachoeira Nazaré, Rio Ji-Paraná (ALG 14898).

Vampyrodes caraccioli: PERU: Madre de Dios, Manu, Maskoitania, 13.4 km NNW Atalaya, left bank Rio Alto Madre de Dios, 480 m. 12°46'18"S, 71°23'7"W (FMNH 174912, 174915); Cusco, Paucartambo, Consuelo, 15.9 km SW Pilcopata, 1000 m. 13°1'25"S, 71°29'30"W (FMNH 174914).

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