



RESEARCH ARTICLE

Notes on the distribution, morphology, and phylogenetics of *Platyrrhinus incarum* (Chiroptera: Phyllostomidae) in Brazil, and confirmation that *Platyrrhinus helleri* does not occur in the country

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ABSTRACT. Changes in Neotropical bat taxonomy in the past two decades have caused significant shifts in our understanding of the diversity and distribution of the group. Taxonomic revisions have shown that the once widespread *Platyrrhinus helleri* (Peters, 1866) represented a species complex, which resulted in the elevation of *Platyrrhinus helleri incarum* (Thomas, 1912) to the species level for cis-Andean South America and the restriction of *P. helleri* to Central America and northern South America west of the Andes. Nevertheless, some studies still mention *P. helleri* for Brazil. Aiming to investigate if *P. helleri* occurs in Brazil, we collected specimens of small-sized *Platyrrhinus* in the Cerrado and Pantanal ecosystems, and revised vouchers in museum collections. Based on DNA sequence data and analysis of the pelage and craniodental morphology, we confirm that the only small *Platyrrhinus* species occurring in Cerrado and Pantanal of central Brazil corresponds to *P. incarum*. This is the first study to provide sequence data for Brazilian *P. incarum*. We also provide new records of *P. incarum*, extending its range by 390 km to southeast and 480 km to the east in Brazil.

KEY WORDS. Atlantic Forest, broad-nosed bat, Cerrado, Cytochrome b, Pantanal, phylogeny, Stenodermatinae.

INTRODUCTION

Over the last two decades, the taxonomy of bats has been in constant flux (Tsang et al. 2016), as revisionary studies have unveiled that many widely distributed bats were species complexes – e.g., *Cynomops* (Moras et al. 2016, 2018), *Sturnira lilium* (É. Geoffroy, 1810) (Velazco and Patterson 2013, 2014). Given these taxonomic reorganizations, it is essential to regularly review local species inventories and compilations of endangered taxa (Thomson et al. 2018).

Members of the broad-nosed bat genus *Platyrrhinus* can be arranged into two species groups, the "large species" and the "small species" (Velazco and Patterson 2008, Velazco and Gardner 2009). One small-sized species, *Platyrrhinus*

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helleri (Peters, 1866), was initially considered widespread, occurring from southern Mexico to southeastern Brazil (Gardner 2008). Two subspecies were recognized in *P. helleri*: *P. h. incarum* (Thomas, 1912) with a cis-Andean distribution in South America and the nominal *P. h. helleri* (Peters, 1866) with a trans-Andean distribution from Mexico to northern South America (Simmons 2005, Gardner 2008).

A revision of the "small species" clade showed that *P. helleri* represented a species complex, which led to the elevation of *P. h. incarum* to species level and description of *Platyrrhinus angustirostris* Velazco, Gardner & Patterson, 2010 and *Platyrrhinus fusciventris* Velazco, Gardner & Patterson, 2010 (Velazco and Patterson 2008, Velazco et al. 2010). The new taxonomic concept of *P. helleri* restricted the occurrence of the species to Central America and northern South America west of the Andes (Velazco et al. 2010, Solari et al. 2019). Previous records of *P. helleri* from Brazil represent either *P. angustirostris*, *P. fusciventris* or *P. incarum* (Nogueira et al. 2014).

Even though the occurrence of *P. helleri* has been redefined to areas west of the Andes, recent publications still mention its occurrence in the Brazilian states of Mato Grosso do Sul (Fischer et al. 2015, 2022, Acero-Murcia et al. 2023) and Pará (Franco Filho et al. 2023). Fischer et al. (2015, 2022) argue that the specimens they examined lacked the typical external characteristics of *P. incarum* and displayed the diagnostic characteristics of *P. helleri*. Some of the purported records of "*P. helleri*" from eastern Brazil were found to be misidentifications, such as the one from eastern Minas Gerais (Nascimento et al. 2013), which was reidentified as *Platyrrhinus recifinus* (Thomas, 1901) (Garbino et al. 2021).

Our goal in this paper is to review the distribution of *P. incarum*, report on new DNA sequences for Brazil, and describe morphologically specimens from the Pantanal, Cerrado, and Atlantic Forest. We have examined specimens from the same broad geographical area that Fischer et al. (2015) considered to be within the occurrence area of *P. helleri* and examine one of the specimens identified as such by the latter authors. Based on phylogenetic analyses of the mitochondrial cytochrome b gene and morphological characters, we confirm that the *Platyrrhinus* populations previously assigned to *P. helleri* correspond to *P. incarum*. Our results suggest that *P. helleri* does not occur in Brazil and extend southeast the distribution of *P. incarum*.

MATERIAL AND METHODS

Specimen collection and examined specimens

Five small-sized *Platyrrhinus* were collected in the

Cerrado and Pantanal ecosystems (Mato Grosso state, central Brazil), one specimen was collected in São Paulo state, southeastern Brazil, and another new record is based on a museum specimen from Minas Gerais state, southeastern Brazil. We analyzed the genetics and morphology of these and other specimens from the same geographical area (Supplementary Materials Tables S1, S2). Specimen handling and collection followed current guidelines for using wild mammal species in research (Sikes and The Animal Care and Use Committee 2016). The Instituto Chico Mendes de Conservação (SISBIO numbers 76825, 77787), Ministry of the Environment, provided permits to collect the specimens. Specimens were collected and either preserved in ethanol 70% or taxidermied and are deposited in the collections of the Museu de Zoologia João Moojen (MZUFV), Viçosa, and Coleção de Mamíferos da Universidade Federal de Mato Grosso (UFMT), Cuiabá. These specimens were compared with other small-sized Platyrrhinus taxa from the following Institutions: American Museum of Natural History (AMNH), New York; Universidade Federal de Lavras (CMUFLA), Lavras; Departamento de Zoologia da Universidade Estadual Paulista (DZSJRP), São José do Rio Preto; Museu de Zoologia da Universidade Estadual de Campinas (ZUEC), Campinas; and Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo. We have also received photographs of specimen ZUFMS 158, which is housed in the Universidade Federal de Mato Grosso do Sul, Campo Grande, and was identified by Fischer et al. (2015) as P. helleri. A detailed list of the examined material is in Appendix 1.

Fieldwork was carried out in three localities in the Brazilian state of Mato Grosso. Two of the localities are in the Brazilian Pantanal: Comunidade Baía São João, Barão de Melgaço (16°44.38'S; 55°33.30'W), and Fazenda Jofre Velho, Poconé Mato Grosso (17°20.40'S; 56°46.20'W). The third locality is in the Cerrado, the Loteamento Ecoville, Cuiabá (15°11.37'S; 55°56.28'W). Sampling in these areas was carried out as part of the PREVIR network, a virus surveillance project focused on wild animals (Rede Nacional de Vigilância de Vírus em Animais Silvestres – https://sites.usp.br/previr/).

On June 6, 2022, officers of the DVZ – SP (Divisão de Vigilância de Zoonoses) were contacted to remove a dead bat specimen hanging from the license plate of a bus in São Paulo city (23°34.66'S; 46°30.60'W). The bus owner commonly organizes day trips to a nearby religious sanctuary in Aparecida, state of São Paulo, (ca. 22°50.00'S; 45°13.00'W). The specimen (MZUFV 5132; Fig. 1A) was preserved in ethanol 70% and had the cranium removed, which was severely damaged.





Figure 1. External morphology of *Platyrrhinus incarum*: (A) adult female *P. incarum* found hanging from a bus license plate in São Paulo, Brazil (MZUFV 5132) and (B) adult female *P. incarum* from Poconé, Brazil (MZUFV 5177).

Distribution review

To assess the entire distribution of the species, we compiled exact or approximated geographical coordinates after a bibliographic review using the term "*Platyrrhinus incarum*" on Google Scholar. We did not use the term "*Platyrrhinus helleri incarum*" in our searches because the pre-2010 records could refer to *P. angustirostris* and *P. fusciventris*. We avoided using records available only as "gray literature" (e.g., theses, conference abstracts). Localities, coordinates, and references can be found in Supplementary Material Table S1.

Molecular analyses

Genomic DNA of *P. incarum* was extracted from three specimens (MZUFV 5178, UFMT 4928, 4929) according to the protocol described by Aljanabi and Martinez (1997), with modifications. Polymerase chain reactions (PCR) were conducted with MVZ05 and MVZ16 primers (Smith and Patton 1993) for amplification of the mitochondrial Cytochrome b gene (cyt-b), following the protocol of Saldanha et al. (2019). The PCR purification, preparation and sequencing were outsourced at the "Biotecnologia, Pesquisa e Inovação – BPI, São Paulo, Brazil". Sequences of both directions were aligned to assemble the consensus in the software Geneious v. 7.1.3 (Kearse et al. 2012) and deposited in GenBank under the accession numbers OR371476, OR371477, and OR371478. We also gathered every available GenBank cyt-b sequence of small-sized species of *Platyrrhinus* and included the large species *P. infuscus* as an outgroup (Velazco and Patterson 2008). The new sequences had a length of 792 base pairs for the three individuals, and the final matrix was composed of 45 taxa (Supplementary Material Table S2).

Sequences were aligned in MUSCLE (Edgar 2004) using the program's default settings. We carried out phylogenetic analyses using Bayesian inference and maximum likelihood in the aligned dataset. Bayesian inference phylogenetic analysis was carried out in Mr. Bayes v.3.2.6 (Ronquist et al. 2012). We selected a HKY + I nucleotide substitution model based on the AIC values in jModelTest 2 (Darriba et al. 2012). We ran two independent Markov Chain Monte Carlo (MCMC) analyses for 20 million generations, sampling at every 2,000 generations in four independent chains. A burn-in fraction of 25% was applied. The phylogenetic analysis using maximum likelihood was conducted in the online platform W-IQ-TREE using the default parameters (Nguyen et al. 2015, Trifinopoulos et al. 2016). We calculated the ultrafast bootstrap (1000 samples) as a relative measure of branch support (Hoang et al. 2017)

Morphological analyses

We took 13 craniodental and one external (forearm length) measurements with a digital caliper (precision 0.01 mm). Measurement limits followed Velazco et al. (2010). We compared our specimens with the external (e.g., number



and position of vibrissae, pelage banding pattern, uropatagium shape) and craniodental traits (e.g., M1 protocone morphology, presence of hypoconid on m2) that are used to distinguish among the small-sized species of *Platyrrhinus* (Velazco et al. 2010).

RESULTS

Field observations

Bats were sampled in Baía São João between February 07 and 11, 2021. Seven 9x3 m mist nests were set at ground level close to forest fragments, ponds, and abandoned buildings. Mist nets were kept open for four hours daily, for a total effort of 3,780 m².h. Besides two adults *Platyrrhinus incarum* (UFMT 4927, 4928), we captured individuals of *Carollia perspicillata* (Linnaeus, 1758), *Glossophaga soricina* (Pallas, 1766), *Macrophyllum macrophyllum* (Schinz, 1821), *Molossops temminckii* (Burmeister, 1854), *Molossus molossus* (Pallas, 1766), *Myotis albescens* (É. Geoffroy, 1806), *Noctilio albiventris* Desmarest, 1818, *Noctilio leporinus* (Linnaeus, 1758), and *Phyllostomus hastatus* (Pallas, 1767).

In Loteamento Ecoville, Cuiabá, sampling occurred on December 05, 2022. Seven 9x3 m mist nests were set at ground level close to a secondary growth Cerrado vegetation and ponds. Mist nets were kept open for four hours for a total effort of 756 m².h. We captured at this site the following species, besides *P. incarum* (UFMT 4929): *Artibeus lituratus* (Olfers, 1818), *C. perspicillata*, *G. soricina*, *M. macrophyllum*, *Myotis* sp., *Rhinophylla pumilio* Peters, 1865, *Tonatia bidens* (Spix, 1823), and *Trachops cirrhosus* (Spix, 1823).

Bats were sampled in Fazenda Jofre Velho, Poconé, between November 14 and 15, 2022. Five 9x3 m mist nests were set at ground level on both days, along secondary forest surrounded by pasture and near ponds. Mist nets were kept open for three hours on both days for a total effort of 810 m².h. In the same site, two *P. incarum* (MZUFV 5177, 5178; Fig. 1B) were captured along with specimens of *C. perspicillata*, *Desmodus rotundus* (É. Geoffroy, 1810), *Glossophaga soricina* (Pallas, 1766), *Molossus rufus* É. Geoffroy, 1805, *Myotis* sp., *N. albiventris*, *Platyrrhinus lineatus* (É. Geoffroy, 1810), *Rhynchoncyteris naso* (Wied, 1820), and *T. cirrhosus*.

In Fazenda Jofre Velho (Poconé), *P. incarum* accounted for two captures out of 27 (7.4% of the sample); in Ecoville (Cuiabá), there were two captures in a total of 14 (14.3%), and in Baía São João, two specimens out of 159 (1.25%) represented the species. A pregnant female was recorded in February (UFMT 4928), and a lactating specimen was captured in December (UFMT 4929). Measurements of the collected *P. incarum* specimens are shown in Table 1.

Our analysis of museum material yielded an additional record of small *Platyrrhinus* for Minas Gerais state, south-eastern Brazil (CMUFLA 2138).

Table 1. Craniodental measurements and forearm length of *Platyrrhinus incarum* from Brazil in the Museu de Zoologia João Moojen (MZUFV), Universidade Federal de Mato Grosso (UFMT) and Universidade Federal de Lavras (CMUFLA) collections. For comparison, measurements (mean and ± standard deviation) of other *P. incarum* and of *Platyrrhinus helleri* were obtained from Velazco et al. (2010).

Character	MZUFV 5132	MZUFV 5177	MZUFV 5178	UFMT 4927	UFMT 4928	UFMT 4929	CMUFLA 2138	Mean	± SD	P. incarum	P. helleri
Greatest length of the skull	-	20.79	20.67	20.99	20.70	21.08	21.47	20.95	0.3	20.9 ± 0.5	21.6 ± 0.4
Condylocanine length	-	18.69	18.69	18.89	18.74	19.93	18.87	18.97	0.5	18.6 ± 0.4	19.6 ± 0.5
Condyloincisive length	-	19.15	19.11	19.41	19.74	20.30	19.43	19.52	0.4	19 ± 0.4	20.1 ± 0.5
Braincase breadth	-	9.01	9.45	9.7	8.98	9.81	9.38	9.39	0.3	9.2 ± 0.3	9.3 ± 0.2
Zygomatic breadth	-	12.25	12.44	11.27	11.38	10.98	12.06	11.73	0.6	12.1 ± 0.8	12.4 ± 0.3
Postorbital constriction	5.43	5.08	5.61	5.35	5.29	5.40	5.40	5.37	0.1	5.3 ± 0.1	5.4 ± 0.2
Mastoid breadth	-	9.58	10.08	9.6	9.25	9.26	9.62	9.57	0.3	9.8 ± 0.2	10 ± 0.2
Palatal length	10.27	9.94	10.16	9.51	9.87	9.98	10.09	9.97	0.2	9.7 ± 0.3	10.2 ± 0.3
Maxillary toothrow length	7.41	7.25	7.32	7.58	7.59	7.40	7.45	7.43	0.1	7.4 ± 0.2	8 ± 0.2
Width at M1	8.64	8.55	8.86	9.11	8.44	8.71	8.66	8.71	0.2	8.4 ± 0.3	8.9 ± 0.3
Width at M2	8.58	8.48	8.59	9.03	8.4	8.6	8.62	8.61	0.2	8.6 ± 0.3	9 ± 0.3
Dentary length	13.98	14.28	13.95	12.98	12.77	13.1	13.91	13.57	0.6	13.8 ± 0.4	14.8 ± 0.4
Mandibular toothtrow length	8.00	8.18	8.10	8.60	8.40	8.35	8.28	8.27	0.2	8 ± 0.3	8.7 ± 0.2
Forearm length	38.04	37.50	40.10	38.76	37.66	37.78	37.39	38.18	0.9	37.2 ± 1.0	38.5 ± 1.1



Distribution review

Our review resulted in 241 records of *P. incarum* distributed along center-north South America, of which five are new records from Brazil, covering the Atlantic Forest, Cerrado, and Pantanal ecosystems (Fig. 2, Supplementary Material Table S2). The new record from São Paulo city (specimen MZUFV 5132) is approximately 391 km southeast of the closest previous species record, in Olímpia, São Paulo (Garbino 2016). The new record from Conceição do Mato Dentro, Minas Gerais (CMFULA 2138) extends the species range in Minas Gerais by approximately 480 km east of the closest previous species record, in Uberaba, Minas Gerais (Taddei and Vicente-Tranjan 1998).

Molecular analyses

Both Bayesian Inference (IB) and Maximum Likelihood (ML) analyses of the cyt-b gene sequences recovered identical topologies for *P. incarum*. As the ML tree had a better overall resolution, we discussed our results based on this tree (Fig. 3) but the IB tree can be found in Supplementary Material Fig. S1. The three new sequences from Mato Grosso state, Brazil, were recovered nested within the *P. incarum*



Figure 2. *Platyrrhinus incarum* occurrence map: known occurrence records in South America (circles), and new localities reported here (filled stars), including newly sequenced specimens (white stars). The species distribution polygon (light green) is based on Marsh et al. (2022). 1 = Aricanduva, São Paulo, São Paulo (23°34.66'S; 46°30.60'W); 2 = Conceição do Mato Dentro, Minas Gerais (19°02.00'S; 43°25.00'W); 3 = Fazenda Jofre Velho, Poconé Mato Grosso (17°20.40'S; 56°46.20'W; 4 = Comunidade Baía São João, Barão de Melgaço, Mato Grosso (16°44.38'S; 55°33.30'W); 5 = Loteamento Ecoville, Cuiabá (15°11.37'S; 55°56.28'W). The red square refers to the record from "Corguinho" (19°46'S; 55°14'W), represented by specimen ZUFMS 158 that we examined by photos and was identified by Fischer et al. (2015) as *P. helleri*. The question mark denotes the unconfirmed record of Damásio et al. (2021).



clade in a subclade with two individuals from the Cerrado of Bolivia (Parque Nacional Noel Kempff Mercado – USNM 584494, AVE12). The other clade of *P. incarum* is composed by specimens from Peruvian Amazonia (Fig. 3). Phylogenetic analyses using both algorithms also recovered *P. incarum* as a sister taxon to the *angustirostris* + *fusciventris* clade.

Morphological analyses

Measurements of the *P. incarum* specimens examined fell in the known variation of the species (Table 1). Externally, the specimens had four bright white facial stripes (Fig. 1) and a dorsal stripe extending from the crown to the most posterior part of the rump. The general color of the dorsum is light brown. Interramal vibrissae were not observed. Metacarpal III is subequal in length to metacarpal V. Dorsal surface of feet is densely haired. The number of major vibrissae around the noseleaf varied individually from 6 to 8 on each side.

The skulls were small (GLS: 20.67 - 21.47 mm) and delicate, with paraoccipital processes poorly developed (Fig. 4). The dental characters (Fig. 5) used here to separate our *P. incarum* from *P. helleri* are (following Velazco et al. 2010):



Figure 3. Maximum-likelihood tree estimated in IQ-TREE based on mitochondrial cytochrome b gene sequences of *Platyrrhi-nus*: the three new sequences of *P incarum* reported here are in blue (UFMT 4928-29, MZUFV 5178). Support values shown on the branches of the clades of interest denote the posterior probabilities (0–1) from the Bayesian inference analysis and the UltraFast Bootstrap (0–100) from the Maximum likelihood analysis.





Figure 4. Skull plate of adult female *Platyrrhinus incarum* (MZUFV 5177) from Poconé, Mato Grosso, Brazil: the plate shows the cranium in (A) dorsal, (B) ventral, and (C) lateral views, and the mandible in (D) dorsal and (E) lateral views. Scale bar: 10 mm.





Figure 5. Comparisons between upper and lower tooth row characters of *Platyrrhinus incarum* and *P. helleri*: (A, B) *P. incarum* (MZUFV 5177) from Mato Grosso (Brazil); (C, D) *P. incarum* (ZUFMS 158) from Mato Grosso do Sul (Brazil); and (E, F) *P. helleri* (AMNH 254646) from Veracruz (Mexico). The arrows indicate the main differences between the two taxa in M1 protocone (black arrow) and m2 hypoconid (white arrow), as outlined by Velazco et al. (2010). Scale bar = 1 mm.

first upper molar (M1) with well-developed protocone and second lower molar (m2) with well-developed hypoconid. However, the specimen from São Paulo city (MZUFV 5132), has three stylar cuspules on the posterior cristid on the second upper premolar (P4) instead of two, as would be expected for *P. incarum* (Velazco et al. 2010).

DISCUSSION

We provide new records of *P. incarum* from Brazil, extending its known distribution, and present original sequence data for the species. Our results show that it is highly unlikely that *P. helleri* occurs in Brazil, as every small

Platyrrhinus specimens we examined from the Pantanal and Cerrado of Mato Grosso and Mato Grosso do Sul had the diagnostic characters of *P. incarum*. In this context, a specimen from Mato Grosso do Sul (ZUFMS 158) that Fischer et al. (2015) identified as *P. helleri* has the dental characters of *P. incarum* (Fig. 5). Mitochondrial DNA sequence data also show that the small *Platyrrhinus* from the Pantanal and Cerrado belong to the *P. incarum* clade (Fig. 3).

The new records and some of the literature records from central Brazil indicate that the distribution polygon of *P. incarum* needs to be updated and expanded eastwards (Marsh et al. 2022). Our new record from São Paulo extends the distribution of *P. incarum* to an area where only *P. re*-



cifinus and *P. lineatus* were known to occur (Garbino 2016, Cláudio et al. 2020). The pattern found in *Platyrrhinus* species that occur in sympatry evidences that usually a small-sized species co-occurs with a larger one. For example, *P. infuscus* (larger) occurs in sympatry with *P. incarum* (smaller) in the Amazonia and *P. lineatus* (larger) occurs with *P. recifinus* (smaller) in the Atlantic Forest (Gardner 2008, Velazco et al. 2010, Solari et al. 2019). Although small-sized *Platyrrhinus* may occur in sympatry in some sites, it is rare and restricted to the distribution edges of the species (Velazco et al. 2010). Moreover, *P. helleri* seems to be restricted, in South America, to trans-Andean lowlands and the northern part of the continent (Velazco et al. 2010, Solari et al. 2019, Marsh et al. 2022).

Our record from São Paulo highlights the importance to document and collect roadkilled bat individuals (Ramalho and Aguiar 2020). The absence of previous records of the species from this intensively sampled region in São Paulo indicates that *P. incarum* may be locally rare there, perhaps because it represents one of the limits of its distribution (Brown 1984). The presence of the similar-sized *P. recifinus* in eastern São Paulo may also negatively affect the occurrence of *P. incarum* in that region.

Addressing earlier uncertainties regarding the existence of *P. incarum* in Minas Gerais, as documented by Tavares et al. (2010), we offer morphological evidence that validates its presence in that region of Brazil. However, we chose not to include the record for Espírito Santo, Brazil, in our map due to the lack of available vouchers for taxonomic identification. This record, based on roadkilled individuals from Sooretama Biological Reserve, Espírito Santo (Damásio et al. 2021), was not verifiable. We also do not include the isolated record from "Bahia, Brazil" reported by Gardner (2008), which is based on specimen BMNH 1849.10.15.42. This specimen, according to the Natural History Museum (London) database, was collected in Bolivia during the Castelnau expedition.

A previous study that sampled *P. incarum* (named as *P. helleri*) in Mato Grosso do Sul, northern São Paulo, and western Minas Gerais found a high capture rate of the species using ground-level mist nets (Taddei and Vincente-Tranjan 1998). According to these authors, *P. incarum* was the third most frequent species sampled (14.47% capture frequency), after *P. lineatus* and *A. lituratus*. Like Taddei and Vincente-Tranjan (1998), *P. incarum* was commonly captured in one of the localities sampled in this study (Baía São João). However, this relatively high capture rate, is not consistent among studies and was not consistent in the three localities we sampled, suggesting that *P. incarum* may be locally abun-

dant. For example, after intensive sampling in the Pantanal of Mato Grosso do Sul (98,841 m².h, 1,143 captures), Munin et al. (2012) captured just a single *P. incarum*.

Reproductive data obtained by us conform with the supposition of Taddei and Vincente-Tranjan (1998) that *P. incarum* has a bimodal polyestrous pattern. We found a pregnant female in February and a lactating one in December, matching the proposed reproductive period from June to October and February to March (Taddei and Vincente-Tranjan 1998).

Some of the characters we observed in *P. incarum* from Brazil do not correspond to what has been described for the species. For example, the metacarpal III of the examined specimens is subequal to metacarpal V, instead of V shorter than III (Velazco et al. 2010). The number of vibrissae around the noseleaf also is variable and, therefore, less useful to separate *P. incarum* from *P. helleri*. Dental characters, particularly of M1 and m2 are more helpful in separating both taxa (Fig. 5). This challenges the accuracy of field identifications and highlights the necessity to collect representative specimens to obtain genetic data and to observe diagnostic craniodental characteristics to identify some species of Neotropical bats.

Final remarks

Specimens of small-sized Platyrrhinus from Cerrado and Pantanal analyzed by us were genetically and morphologically identifiable as *P. incarum*. Based on our findings, we conclude that it is highly unlikely that *P. helleri* occurs in central Brazil, as every specimen analyzed from this region corresponded to P. incarum. Therefore, we agree with previous authors that P. helleri does not occur in Brazil (Velazco et al. 2010, Solari et al. 2019, Garbino et al. 2020). We also have shown that P. incarum occurs along a wider area than previously assumed. We cannot rule out, however, that the phenotypically similar P. angustirostris and P. fusciventris may occur in the same areas we captured P. incarum in central Brazil. This study highlights the importance of integrating data from museum collections, road-kill, and pathogen surveys, as this gave us a clearer picture of the distribution of P. incarum in South America.

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LITERATURE CITED

- Acero-Murcia AC, Severgnini MR, Provete DB (2023) An evolutionary ecomorphological perspective on the assembly of a Neotropical bat metacommunity. Journal of Mammalian Evolution (preprint). https://doi.org/10.21203/ rs.3.rs-2302646/v1
- Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high-quality genomic DNA for PCR-based techniques. Nucleic Acids Research 25: 4692–4693. https:// doi.org/10.1093/nar/25.22.4692
- Brown JH (1984) On the relationship between abundance and distribution of species. The American Naturalist 124: 255–279.
- Cláudio VC, Barbosa GP, Rassy FB, Rocha VJ, Moratelli R (2020) The bat fauna (Mammalia: Chiroptera) of Carlos Botelho State Park, Atlantic Forest of southeastern Brazil, including new distribution records for the state of São Paulo. Zoologia 37: e36514. https://doi.org/10.3897/ zoologia.37.e36514
- Damásio L, Ferreira LA, Pimenta VT, Paneto GG, Santos AR, Ditchfield AD, Bergallo HG, Banhos A (2021) Diversity and abundance of roadkilled bats in the brazilian Atlantic Forest. Diversity 13(7): 335. https://doi.org/10.3390/d13070335
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel com-

puting. Nature Methods 9: 772. https://doi.org/10.1038/ nmeth.2109

- Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughout. Nucleic Acids Research 32: 1792–1797. https://doi.org/10.1093/nar/ gkh340
- Fischer E, Santos CF, Carvalho LFAC, Camargo G, Cunha NL da, Silveira M, et al. (2015) Bat fauna of Mato Grosso do Sul, southwestern Brazil. Biota Neotropica 15: 1–17. https://doi.org/10.1590/1676-06032015006614
- Fischer E, Eriksson A, Francisco AL, Pulchério-Leite A, Santos CF, Gonçalves F, et al. (2022) Morcegos da Bacia do Alto Paraguai: Revisão da fauna e distribuição de registros. Boletim do Museu Paraense Emílio Goeldi – Ciências Naturais 17: 585–687. https://doi.org/10.46357/bcnaturais.v17i3.817
- Franco Filho LCF, Barata RR, Coelho MS, Cardoso JF, Lemos PS, dos Reis HS, et al. (2023) Genome sequencing of dengue virus serotype 4 in a bat brain sample (*Platyrrhinus helleri*) from the Brazilian Amazon. Infection, Genetics and Evolution 109: 105407. https://doi.org/10.1016/j. meegid.2023.105407
- Garbino GST (2016) Research on bats (Chiroptera) from the state of São Paulo, southeastern Brazil: Annotated species list and bibliographic review. Arquivos de Zoologia, Museu de Zoologia da Universidade de São Paulo 47: 43–128. https://doi.org/10.11606/issn.2176-7793. v47i3p43-128
- Garbino GST, Gregorin R, Lima IP, Loureiro L, Moras LM, Moratelli R, et al. (2020) Updated checklist of Brazilian bats: versão 2020. Comitê da Lista de Morcegos do Brasil – CLMB. Sociedade Brasileira para o Estudo de Quirópteros (Sbeq). Available from: https://www.sbeq.net/lista--de-especies (04/07/2023).
- Garbino GST, Silva FP, Silva LG (2021) Distribution, habitat suitability, and revised morphological diagnosis confirm that the fruit bat *Platyrrhinus recifinus* is an Atlantic Forest endemic. Studies on Neotropical Fauna and Environment 58: 344–355. https://doi.org/10.1080/01650521.2 021.1962678
- Gardner A (2008) Genus *Platyrrhinus* Saussure, 1860. In: Gardner AL (Ed.) Mammals of South America, Volume 1: Marsupials, xenarthrans, shrews, and bats. The University of Chicago Press, Chicago, 329–342.
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2017) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35: 518–522. https://doi.org/10.5281/zenodo.854445



- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/ bts199
- Marsh CJ, Sica YV, Burgin CJ, Dorman WA, Anderson RC, Mijares IT, et al. (2022) Expert range maps of global mammal distributions harmonised to three taxonomic authorities. Journal of Biogeography 49: 979–992. https://doi.org/10.1111/jbi.14330
- Moras LM, Tavares VC, Pepato AR, Santos FR, Gregorin R (2016) Reassessment of the evolutionary relationships within the dog-faced bats, genus *Cynomops* (Chiroptera: Molossidae). Zoologica Scripta 45: 465–480. https://doi. org/10.1111/zsc.12169
- Moras LM, Gregorin R, Sattler T, Tavares VC (2018) Uncovering the diversity of dog-faced bats of the genus *Cynomops* (Chiroptera: Molossidae), with the redescription of *C. milleri* and the description of two new species. Mammalian Biology 89: 37–51. https://doi.org/10.1016/j. mambio.2017.12.005
- Munin RL, Fischer F, Gonçalves F (2012) Food habits and dietary overlap in a phyllostomid bat assemblage in the Pantanal of Brazil. Acta Chiropterologica 14: 195–204. https://doi.org/10.3161/150811012X654871
- Nascimento MC, Stumpp R, Lessa G (2013) Bats (Mammalia: Chiroptera) of Mata do Paraíso research station, Viçosa, Minas Gerais, Brazil. Check List 9: 1406–1409. https://doi.org/10.15560/9.6.1406
- Nguyen LT, Schmidt HA, Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. https://doi. org/10.1093/molbev/msu300
- Nogueira MR, Lima IP, Moratelli R, Tavares VC, Gregorin R, Peracchi AL (2014) Checklist of Brazilian bats, with comments on original records. Check List 10: 808–821. https://doi.org/10.15560/10.4.808
- Ramalho DF, Aguiar LMS (2020) Bats on the road a review of the impacts of roads and highways on bats. Acta Chiropterologica 22: 417–433. https://doi.org/10.3161/15 081109ACC2020.22.2.015
- Ronquist F, Teslenko M, van Der MP, Ayres DL, Darling A, Höhna S, et al. (2012) MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi. org/10.1093/sysbio/sys029

- Saldanha J, Ferreira DC, Silva VF, Santos-Filho M, Mendes-Oliveira AC, Rossi RV (2019) Genetic diversity of *Oecomys* (Rodentia, Sigmodontinae) from the Tapajós River basin and the role of rivers as barriers for the genus in the region. Mammalian Biology 97: 41–49. https://doi.org/10.1016/j.mambio.2019.04.009
- Sikes RS, The Animal Care and Use Committee (2016) 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97(3): 663–688. https://doi. org/10.1093/jmammal/gyw078
- Simmons NB (2005) Order Chiroptera. In: Mammal species of the world: a taxonomic and geographic reference. Johns Hopkins University Press, Baltimore, 312–529.
- Smith MF, Patton JL (1993) The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biological Journal of the Linnean Society 50: 149–177. https://doi. org/10.1111/j.1095-8312.1993.tb00924.x
- Solari S, Medellín R, Rodríguez-Herrera B, Tavares VC, Garbino G, Camacho MA, et al. (2019) Family Phyllostomidae (New World leaf-nosed bats). In: Wilson DE, Mittermeier RA (Eds) Handbook of the Mammals of the World. Lynx Edicions, Barcelona, Vol. 9, 444–583.
- Taddei VA, Vincente-Tranjan EC (1998) Biological and distributional notes on *Platyrrhinus helleri* (Chiroptera: Phyllostomidae) in Brazil. Mammalia 62: 113–117.
- Tavares VC, Aguiar LMS, Perini FA, Falcão FC, Gregorin R (2010) Bats of the state of Minas Gerais, southeastern Brasil. Chiroptera Neotropical 16: 675–705.
- Thomson SA, Pyle RL, Ahyong ST, Alonso-Zarazaga M, Ammirati J, Araya JF, et al. (2018) Taxonomy based on science is necessary for global conservation. Plos Biology 16(3): e2005075. https://doi.org/10.1371/journal.pbio.2005075
- Trifinopoulos J, Nguyen L, Haeseler AV, Minh BQ (2016) W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44: 232–235. https://doi.org/10.1093/nar/gkw256
- Tsang SM, Cirranello AL, Bates PJJ, Simmons NB (2016) The roles of taxonomy and systematics in bat conservation. In: Voigt CC, Kingston T (Eds) Bats in the Anthropocene: Conservation of bats in a changing world. Springer International Publishing, Cham, 503–538. https://doi. org/10.1007/978-3-319-25220-9_16
- Velazco PM, Gardner AL (2009) A new species of *Platyrrhinus* (Chiroptera: Phyllostomidae) from western Colombia and Ecuador, with emended diagnoses of *P. aquilus*, *P. dorsalis*, and *P. umbratus*. Proceedings of the



Biological Society of Washington 122: 249–281. https:// doi.org/10.2988/08-40.1

- Velazco PM, Gardner AL, Patterson BD (2010) Systematics of the *Platyrrhinus helleri* species complex (Chiroptera: Phyllostomidae), with descriptions of two new species. Zoological Journal of the Linnean Society 159: 785–812. https://doi.org/10.1111/j.1096-3642.2009.00610.x
- Velazco PM, Patterson BD (2008) Phylogenetics and biogeography of the broad-nosed bats, genus *Platyrrhinus* (Chiroptera: Phyllostomidae). Molecular Phylogenetics and Evolution 49: 749–759. https://doi.org/10.1016/j.ympev.2008.09.015
- Velazco PM, Patterson BD (2013) Diversification of the yellow-shouldered bats, genus *Sturnira* (Chiroptera, Phyllostomidae), in the New World tropics. Molecular Phylogenetics and Evolution 68: 683–698. https://doi.org/10.1016/j.ympev.2013.04.016
- Velazco PM, Patterson BD (2014) Two new species of yellow-shouldered bats, genus *Sturnira* Gray, 1842 (Chiroptera, Phyllostomidae) from Costa Rica, Panama and western Ecuador. ZooKeys 402: 43–66. https://doi.org/10.3897/zookeys.402.7228

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Author Contributions

GSTG, JASVP, JS, TBFS, and PMV: Writing – original draft, Writing – review & editing; GSTG: Conceptualization, Supervision; GSTG, JASVP, TBFS, JS: Formal Analysis, Resources; GSTG, JASVP, JS, TBFS, TSA, ARR and PMV collected the data in the field and scientific collections.

Competing Interests

The authors have declared that no competing interests exist.

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Appendix 1. List of examined specimens. Acronyms refer to the following collections: Museu de Zoologia João Moojen (MZU-FV), Viçosa; Coleção de Mamíferos da Universidade Federal de Mato Grosso (UFMT), Cuiabá; American Museum of Natural History (AMNH), New York; Universidade Federal de Lavras (CMUFLA), Lavras; Departamento de Zoologia da Universidade Estadual Paulista (DZSJRP), São José do Rio Preto; Museu de Zoologia da Universidade Estadual de Campinas (ZUEC); Museu de Zoologia da Universidade de São Paulo (MZUSP).

Platyrrhinus helleri (N = 1): MEXICO: Veracruz (AMNH 254646).

- Platyrrhinus incarum (N = 8): BRAZIL: Mato Grosso, Baía São João (UFMT 4927-28), Loteamento Ecoville (UFMT 4929), Pousada Jofre Velho, Poconé (MZUFV 5177-78); Minas Gerais, Conceição do Mato Dentro (CMUFLA 2138); São Paulo, Icém (DZSJRP 3030), São Paulo, Aricanduva (MZUFV 5132).
- Platyrrhinus recifinus (N = 5): BRAZIL: Rio de Janeiro, Piraí (ZUEC 2513); São Paulo, Bertioga (MZUSP 26422), Campinas, Sousas (ZUEC 1143), Lavrinhas (ZUEC 2532), Ubatuba, P.E. Ilha Anchieta (MZUSP 29443).

Supplementary material 1

- Table S1. Localities of *Platyrrhinus incarum* gleaned from the literature and based on museum specimens examined by us. Localities in bold refer to new localities. (*) Type locality of *Vampyrops zarhinus incarum* Thomas, 1912.
- Authors: GST Garbino, JASV Paes, J Saldanha, TS Alves, TBF Semedo, AR da Rosa, PM Velazco

Data type: distribution data.

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Link: https://doi.org/10.1590/S1984-4689.v41.e23044

Supplementary material 2

- Table S2. Genbank accession numbers, voucher numbers, and locality information of the *Platyrrhinus* cytochrome b sequences used in this study. Sequences in bold were generated in this study.
- Authors: GST Garbino, JASV Paes, J Saldanha, TS Alves, TBF Semedo, AR da Rosa, PM Velazco



Data type: Genbank accession numbers.

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Supplementary material 3

Figure S1. Bayesian inference tree estimated in MrBayes based on mitochondrial cytochrome b gene sequences

of *Platyrrhinus*: the three new sequences of *P. incarum* reported here are UFMT 4928, 4829 and MZUFV 5178.

Authors: GST Garbino, JASV Paes, J Saldanha, TS Alves, TBF Semedo, AR da Rosa, PM Velazco

Data type: phylogenetic tree.

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