Analyses of plumage coloration and genetic variation confirm the hybridization of *Pipra fasciicauda* and *Pipra aureola* in the Brazilian Amazon basin

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Abstract

The present study aimed to confirm the occurrence of a hybridization event between the Band-tailed Manakin (*Pipra fasciicauda*) and the Crimson-hooded Manakin (*Pipra aureola*), based on the existence of a specimen that presents morphological traits of both taxa. We analyzed 297 taxidermized skins of adult males of the two species, including the potential hybrid. We also analyzed the mitochondrial (ND2, ND3 e COI) and nuclear (FGB-I5, MB-I2 e GAPDH-I3) genes of 12 adult specimens of the two taxa, diagnosed phenotypically, in addition to the potential hybrid. The analyses of the plumage indicated that the potential hybrid has an intermediate pattern of white banding on the tail that is less extensive than that found in *Pipra fasciicauda*, but that its other phenotypic traits are characteristic of *Pipra aureola*. The molecular topologies revealed two clades, one that groups *P. aureola* together with the potential hybrid, and the other, that corresponds to *P. fasciicauda*. These findings allowed us to confirm the occurrence of a process of hybridization and potential introgression through secondary events in the *P. aureola* lineage.

Key words: Hybrids; intrageneric hybridization; introgression; manakins; Pipridae; secondary contact

Introduction

Hybridization and introgression events are common in birds, especially in closely-related species (De Kort et al. 2002). These events have been interpreted as the result of many different processes, including the influence of natural stimuli, the lack of potential mates of the same species or errors in conspecific recognition. Grant and Grant (1992) concluded that hybridization events are extremely common in birds, occurring in approximately one in every ten species, a process that may support the formation of new species (Toews et al. 2018). In a recent study, Weir et al. (2015) concluded that hybridization in Amazonian birds may be a consequence of the slow evolution of reproductive isolation, given that even taxa known to have diverged at least four million years ago may hybridize when they come into contact.

The species of the family Pipridae, known as manakins, are a group of small Neotropical birds with an ample distribution in the Amazon basin. There is a number of reported cases of hybridization in piprids, including intra- and inter-generic matings, confirmed by both morphological traits and genetic analyses (Brumfield et al. 2001). Both types of hybridization have been recorded in the genus *Pipra*, with intergeneric matings between the Wire-tailed Manakin (*Pipra filicauda*) and White-crowned Manakin (*Pseudopipra pipra*) (Graves 1993), Crimson-hooded Manakin (*Pipra aureola*) and Flame-crested Manakin (*Heterocercus linteatus*) (Parkes 1961), and an intrageneric case between *P. aureola* and *P. filicauda*, a hybrid that was initially identified as *Pipra heterocerca* (Haffer 1970).

Although no evidence of hybridization involving the Band-tailed Manakin (*Pipra fasciicauda*) has been reported previously, an enigmatic specimen from the municipality of Acará, in the northern Brazilian state of Pará, raised the possibility of hybridization in this species. In the present study, we investigated the probable process of intrageneric hybridization/introgression between the sister species *P. fasciicauda* and *P. aureola*, based on the analysis of plumage traits and molecular data.

Material and Methods

During ann expedition to survey *Pipra fasciicauda* in the area of Acará, a known locality for the occurrence of this species (Novaes and Lima 2009), PVC detected several individuals with plumage slightly different from that of a typical *P. fasciicauda* and more similar to *P. aureola*. The plumage of these individuals was more reddish in general than that of *P. fasciicauda*, and lacked the white band on the tail. Given these characters, one specimen was collected for a more systematic evaluation, based on plumage patterns and molecular data, in addition to a specimen with typical *P. fasciicauda* plumage. Specimen collection was authorized by the Chico Mendes Institute of Biodiversity-ICMBio, through protocol number 44560-3. The presence of *P. aureola* in this region was never well documented, but there are some recent reports from this region, and the habitat in the study area appears to be favorable to the contact of the two species, given that it represents a transition between '*terra firme*' forest and '*várzea*' (swamp) forests with *Heliconia* sp.

We analyzed the plumage patterns in 297 taxidermized skins of male manakins deposited in the ornithological collection of the Emílio Goeldi Museum (MPEG: Museu Paraense Emílio Goeldi), in Belém. These specimens included 190 *Pipra fasciicauda*, 106 *Pipra aureola*, and one individual with an intermediate phenotype (the putative hybrid) between *P. fasciicauda* and *P. aureola*. We classified the plumage of the head, throat, breast, and flanks, using Smithe's (1975) color catalog, and determined the presence and configuration of the white band in the tail to summarize the phenotypic differences between the study species.

We complemented this analysis of the plumage with genetic analyses of 13 samples of muscle tissue, including nine *P. fasciicauda*, three *P. aureola*, and the putative hybrid. These specimens were collected from six localities within the geographic ranges of the respective species (Fig 1), and were provided by the Emílio Goeldi Museum (Online Resource 1). We extracted total genomic DNA using the Wizard Genomic DNA Purification kit (Promega Corporation), following the protocol provided by the manufacturer. We used Polymerase Chain Reaction (PCR) to amplify three mitochondrial markers (NADH dehydrogenase subunit 2 (ND2), NADH dehydrogenase subunit 3 (ND3), Cytochrome oxidase subunit I, COI), and three nuclear regions, intron 5 of the Beta-fibrinogen gene (FGB-I5), intron 2 of the Myoglobin (MB-I2), and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH-I3). We determined the nucleotide sequences in an ABI 3500 automatic sequencer (Applied Biosystems). We provide information on the primers and amplification conditions of each gene or region analyzed in the present study in the Electronic Supplementary Material (Online Resource 2).

We edited the sequences in the FASTA format, corrected them in BioEdit version 7.1.3 (Hall 1999), and aligned them automatically in CLUSTAL-W (Thompson et al 1994), an option from the above-mentioned software . We codified the double peaks observed in the nuclear markers, which were confirmed as heterozygous sites, based on the IUPAC nucleotide nomenclature. We concatenated the genes in Sequence Matrix version 1.7.8 (Vaidya et al. 2011). We further selected the optimum nucleotide substitution model in PartitionFinder, version 1.1.1 (Lanfear et al. 2012), and identified the best partition of these data for the construction of the Bayesian Inference topology, based on the Akaike Information Criterion, in MrBayes 3.2.0 (Huelsenbeck and

Ronquist 2001). We rooted the phylogenetic tree (multilocus and mitochondrial) at *P. filicauda* (N = 4), using samples provided by the National Institute for Amazonian Research (INPA).

Results

Our morphological data indicate that the specimen collected in the region of Acará shares some plumage traits with the males of both *Pipra fasciicauda* and *Pipra aureola*, but shows more similarities to the latter species (Table 1). In the ventral view of the tail of the potential hybrid, the specimen presents the continuous white band typical of *Pipra fasciicauda*, while in the dorsal view, only the three outer rectrices have white marks, and no white coloration is exhibited when the bird is perched in a natural position. The flanks of the potential hybrid have more black than *P. fasciicauda*, and the undertail coverts are almost entirely black, as in *P. aureola*, also masking the white band of the rectrices when the bird is perched naturally, while in *Pipra fasciicauda*, only a small distal portion is black, displaying the diagnostic white band of this species. More subtle traits, such as the plumage of the head, throat, breast, the rudimentary blackish flanks, and the presence of a partial white band indicate that *Pipra aureola* and *Pipra fasciicauda* are the parental species (Fig 2, Table 1).

The mitochondrial markers had a total of 2085 base pairs (bps), 901 bps for the ND2 gene (17 variable sites), 385 bps for ND3 (13 variable sites), and 799 bps for COI (07 variable sites). We found no insertion/deletions (indels), stop codons or ambiguous peaks in the electropherograms of these markers, confirming their mitochondrial origin. The nuclear markers generated a database of 1566 bps, with 390 bps for the GAPDH-I3, 537 bps for the FGB-I5, and 639 bps for the MB-I2. Overall, we found two polymorphisms sites in the GAPDH-I3 fragment and seven in the FGB-I5 sequence, although we found no polymorphic sites in the MB-I2. The concatenated genes were arranged in the following order: FGB-I5, COI, GAPDH-I3, MB-I2, ND2 and ND3, and the program indicated that the codons provided the best arrangement for the understanding of partitioning the data. We identified the GTR+I+G model as the optimum evolutionary model for the FGB-I5 sequences, the HKY+I+G model for the COI gene (codon 1), the K80+I for COI (codon 2), and the F81 model for COI (codon 3). We also identified the F81+I model as the optimum for the GAPDH-I3 sequences, the F81 model for MB-I2, the HKY+I model for ND2 (codon 1), the F81 model for ND2 (codon 2), and the HKY for the ND2 (codon 3) sequence, as well as the HKY for the ND3 sequence (codons 1 and 2) and the K80 model for ND2 (codon 3).

The topologies produced by the mitochondrial (Online Resource 3) and multilocus (Fig 1) datasets are congruent, with two main clades, one that groups the *P. aureola* specimens and the hybrid (Pfa40), and the other that includes the *P. fasciicauda* specimens. The topology based on the nuclear data alone (not shown) did not provide a clear phylogenetic interpretation of the two species, given that the intrageneric variation in the nuclear genes did not differentiate *P. fasciicauda* from *P. aureola*. Given this, the potential hybrid did not present heterozygous sites that allowed the contribution of the parental ancestors to be interpreted. This precludes the confirmation of *P. fasciicauda* as the paternal lineage, based on the genetic data. All the sequences generated in the present study were deposited in GenBank (www. ncbi.nlm.nih.gov) under access numbers MN128472 through MN128514 for mitochondrial genes and MN166326 through MN166360 for nuclear genes (Online Resource 1).

Discussion

Based on the integrated analysis of morphological, molecular, and zoogeographic data, we conclude that the hybrid specimen was the product of a process of hybridization between *Pipra fasciicauda* and *Pipra aureola*, with a subsequent introgression event involving the *P. aureola* lineage, given that the specimen in question presents more morphological similarities with *P. aureola*. This indicates the possible existence of a sympatric zone between the two taxa, given that a specimen of *P. fasciicauda* (Pfa41) was collected at the same site as the potential hybrid (Fig 1), which was previously considered to be part of the geographic distribution of only *P. fasciicauda*. Sympatry between closely-related piprids has been reported previously in the Amazonian basin. In particular, this species is distributed in the region of the mouth of the Tapajós River (Haffer 1970, 1997), and the lower Xingu and Tocantins rivers (Ridgely and Tudor 1994), which is the region where the potential hybrid analyzed in the present study was collected. However, no integrated analysis of the morphological and molecular data had been applied previously to specimens from this region to confirm the possible existence of interspecific hybrids. Haffer (1970) concluded that an ample hybrid zone between *P. aureola* and *P. fasciicauda* certainly did not exist, but that the occurrence of isolated hybrids in contact zones may eventually be detected. The results of the present study corroborate this hypothesis.

Interspecific hybridization in the genus *Pipra* was confirmed by Haffer (1970), who concluded that the morphological description of a hybrid, denominated *P. heterocerca*, was in fact the result of a cross between *P. aureola* and *P. filicauda*. The hybrid described in the present study, which appears to be the result of the crossing of *P. fasciicauda* and *P. aureola*, provides additional evidence that this type of hybridization is not unusual in this genus. Some authors have discussed the circumstances that may promote heterospecific mating. Aspects of the behavior and evolution of *P. fasciicauda* and *P. aureola* may have facilitated hybridization, in particular, the similarities of their prenuptial rituals, which include the formation of leks and the elaborate courtship behavior, together with the morphological similarities of the different forms. Analyzing vocal repertoires, Curé et al. (2010) found that closely-related species may interact and respond to each other, despite their other differences. As sister taxa, it seems likely that *P. fasciicauda* and *P. aureola* will have similar vocalizations (Sick 1997), and PVC conducted tests (unpublished data) of song recognition in these two manakin species (*P. fasciicauda* and *P. aureola*) and in hybrids from the Acará area, and obtained high rates of response from hybrids to the songs and calls of both parent species, as well as positive responses using records of *P. fasciicauda* and *P. aureola* to attract each other. Alatalo et al. (1990) also highlighted a combination of plumage and vocal traits that contribute to hybridization, by causing errors in the recognition of conspecific males.

Our analysis on the morphology of the hybrid indicates a plumage pattern shared by *P. aureola* and *P. fasciicauda*, with more characters being similar to *P. aureola*. The genetic data also indicated that this species represented the maternal lineage. This suggests that, following hybridization, a process of introgression occurred through secondary crosses with the *P. aureola* lineage. It thus seems likely that the hybrid is not the product of a first generation cross, given that the only trait it shares with *P. fasciicauda* is the white band on the tail, which is never observed in *P. aureola*. Introgression events have been reported in manakins (Dias et al. 2018; Weir et al. 2015), which indicates that phylogenetic proximity and recent divergence times facilitate the maintenance of the fertility of the hybrids, and the subsequent occurrence of introgression events.

We emphasize the importance of understanding how the efficacy of interspecific sexual interactions influences the evolution of a hybrid zone, especially given that the potential backcrossing of hybrids with either parent species may eventually lead to introgression (Rezende et al. 2013). In this case, hybridization and introgression are mechanisms that may have a direct influence on the evolution of this bird group and, depending on the effectiveness of pre- and post-zygotic barriers, may also affect genetic variation and speciation, creating fundamental difficulties for the delimitation of taxa and the interpretation of their phylogenetic relationships (Toews et al. 2018, Dias et al. 2018).

Reference List

Alatalo R, Eriksson D, Gustafsson L, Lundberg A (1990) Hybridization between pied and collared flycatchers: sexual selection and speciation theory. Journal of Evolutionary Biology 3:375–389

Brumfield RT, Jernigan RW, McDonald DB, Braun JM (2001) Evolutionary implications of divergent clines in an avian (*Manacus*: Aves) hybrid zone. Evolution 55:2070-2087

Curé C, Aubin T, Mathevon N (2010) Intra-sex vocal interactions in two hybridizing seabird species (*Puffinus* sp.). Behavioral Ecology Sociobiology 64:1823-1837

De Kort SR, Den Hartog PM, Ten Cate C (2002) Vocal signals, isolation and hybridization in the vinaceous dove (*Streptopelia vinacea*) and the ring-necked dove (*S. capicola*). Behavioral Ecology Sociobiology 51:378-385

Dias C, Lima KA, Araripe J, Aleixo A, Vallinoto M, Sampaio I, Schneider H, Rêgo PS (2018) Mitochondrial introgression obscures phylogenetic relationships among manakins of the genus *Lepidothrix* (Aves: Pipridae). Molecular Phylogenetics and Evolution 126:314-320

Gill F and D Donsker (Eds) (2019) IOC World Bird List (v 9.2). Doi 10.14344/IOC.ML.9.2. http://www.worldbirdnames.org/. Accessed 01 July 2019

Grant PR, Grant BR (1992) Hybridization of Bird Species. Science 256:193-197

Graves GR (1993) A new hydrid manakin (*Dixiphia pipra* × *Pipra filicauda*) (Aves: Pipridae) from the Andean foothills of eastern Ecuador. Proceedings of The Biological Society of Washington 106:436-441

Haffer J (1970) Art-Entstehung bei einigen Waldvögeln Amazoniens. Journal Für Ornithologie 111:285-331

Haffer J (1997) Alternative models of vertebrate speciation in Amazonia: an overview. Biodiversity and Conservation 6:451-476

Hall TA (1999) BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754-755. https://doi.org/10.1093/bioinformatics/17.8.754

Lanfear RB, Calcott SY, Ho S (2012) Guindon. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29:1695-1701

Novaes FC, Lima MFC (2009) Aves da Grande Belém: municípios de Belém e Ananindeua, Pará. Museu Paraense Emílio Goeldi, Belém, Brasil

Parkes KC (1961) Intergeneric hybrids in the family Pipridae. The Condor, 63:345-350

Rezende MA, Vasconcelos MF, Nogueira W, Silva JC, Becho DP, Silva LF, Souza TO (2013) Novas Ocorrências de Híbridos entre *Chiroxiphia caudata* e *Antilophia galeata* em Minas Gerais, Brasil, com a Primeira Descrição de uma Fêmea Híbrida e Comentários sobre os Riscos da Hibridação. Atualidades Ornitológicas 174:33-39

Ridgely RS, Tudor G (1994) The Birds of South America. The Suboscine Passerines. Austin, United States of America: University of Texas Press 2

Sick H (1997) Ornitologia Brasileira. Nova Fronteira, Rio De Janeiro

Smithe FB (1975) Naturalist's color guide. American Museum of Natural History, New York

Toews DPL, Walsh J, Campagna L (2018) Population Genomics of Birds: Evolutionary History and Conservation. In: Population Genomics. Springer, Cham pp.1-40

Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27:171-180

Weir JT, Faccio MS, Pulido-Santacruz P, Barrera-Guzmán AO, Aleixo A (2015) Hybridization in headwater regions, and the role of rivers as drivers of speciation in Amazonian birds. Evolution 69:1823-1834

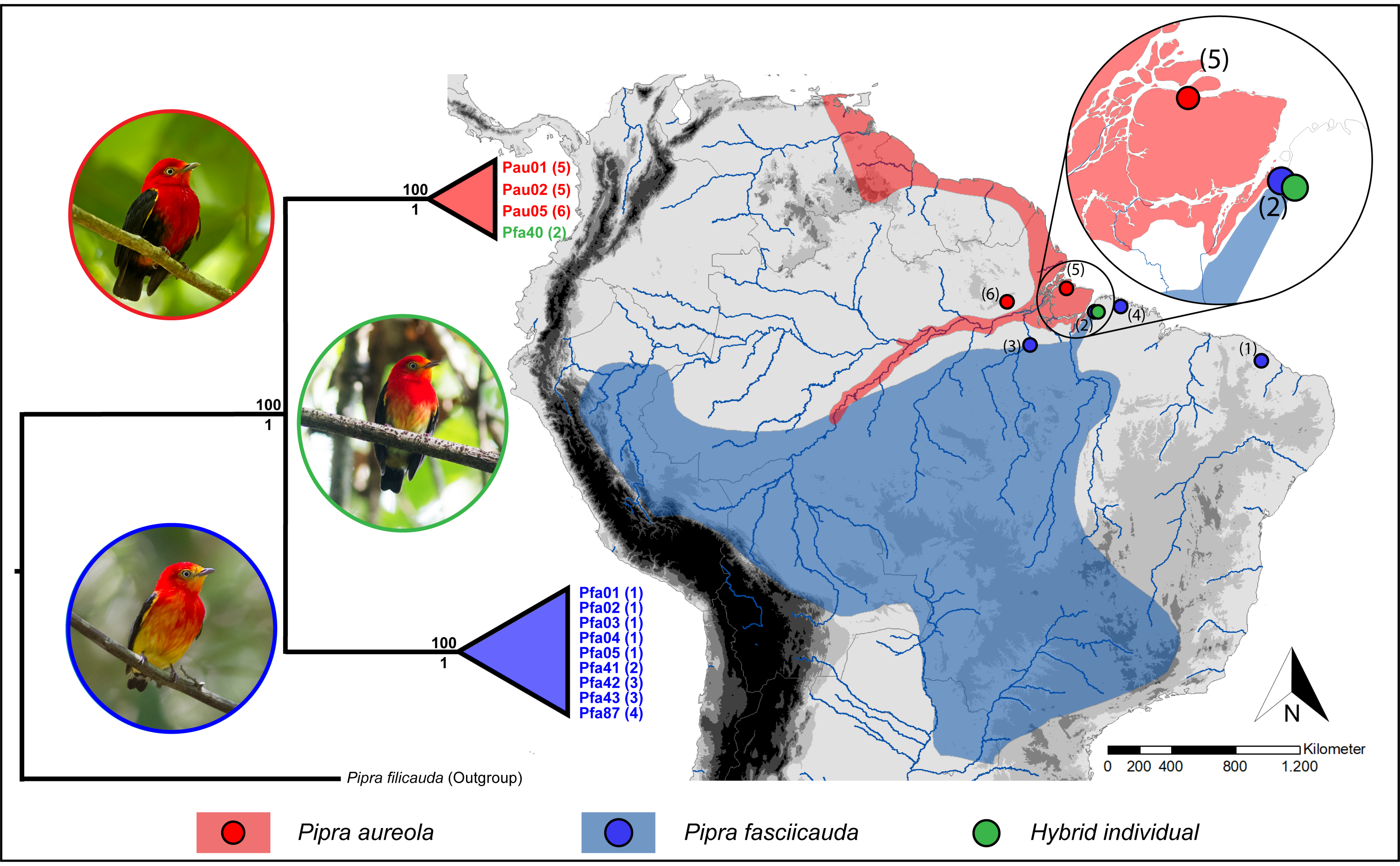
Figure Caption

Fig 1 Geographic distribution of *Pipra fasciicauda* (blue) and *Pipra aureola* (red), showing the collecting sites of the genetic data analyzed in the present study (Online Resource 1) and the topology of the Maximum Likelihood and Bayesian Inference analyses of *Pipra fasciicauda*, *Pipra aureola* and the potential hybrid, based on the multilocus data (ND2, ND3, COI, FGB-I5, MB-I2, GAPDH-I3) with *Pipra filicauda* as the outgroup. The numbers within parentheses correspond to those of the collecting sites identified in the Online Resource section, and the inset in the upper right portion of the map is a closeup of the region of Acará, where the specimens of the potential hybrid and typical *P. fasciicauda* were collected. Photographs are provided in the phylogenetic tree to show the parental species and the potential hybrid, *Pipra aureola*, outlined in red (credit: Ciro Albano), *Pipra fasciicauda*, outlined in blue, and the potential hybrid from the Acará region, outlined in green (credit: Pablo Vieira Cerqueira). The numbers above and below the branches correspond to the nonparametric bootstrap and posterior probabilities, respectively. The species distribution shape files were adapted from BirdLife International-Data Zone

Fig 2 Specimens showing plumage characters of each parental species,with the red and blue colors representing *Pipra aureola* and *Pipra fasciicauda*, respectively, and the potential hybrid in green. A) Dorsal view, B) Ventral view, and C) Closeups of the dorsal portion of the tail, showing the pattern of the white band: *P. fasciicauda* presents a complete white band, *P. aureola* lacks the band, and the potential hybrid presents an incomplete band with white marks on only the three outer rectrices. Voucher numbers: *Pipra fasciicauda* (MPEG 35883), Hybrid individual (MPEG 82869) and *Pipra aureola* (MPEG 47486)

Table 1 Comparison of the plumage coloration of *Pipra fasciicauda*, *Pipra aureola*, and the potential hybrid, including the crown, throat, breast, flanks, and the presence of the white band on the tail.

		Pipra fasciicauda	Hybrid	*Pipra aureola*
Crown		Geranium (#12) and Orange Yellow (#18)	Geranium (#12)	Geranium (#12)
Throat		Orange Yellow (#18) finelly tinged of Flame Scarlet (#15)	Flame Scarlet (#15)	Flame Scarlet (#15)
Breast		Flame Scarlet (#15)	Geranium (#12)	Geranium (#12)
Flanks		Orange Yellow (#18)	Jet Black (#89) Less extensive than in *P. aureola*	Jet Black (#89)
Presence of white tail bands	Dorsal view	Yes	No (Only in the three outer rectrices)	No
	Ventral view	Yes	Yes	No


100
1
Pau01 (5)
Pau02 (5)
Pau05 (6)
Pfa40 (2)
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1
Pfa01 (1)
Pfa02 (1)
Pfa03 (1)
Pfa04 (1)
Pfa05 (1)
Pfa41 (2)
Pfa42 (3)
Pfa43 (3)
Pfa87 (4)
Pipra filicauda (Outgroup)
(5)
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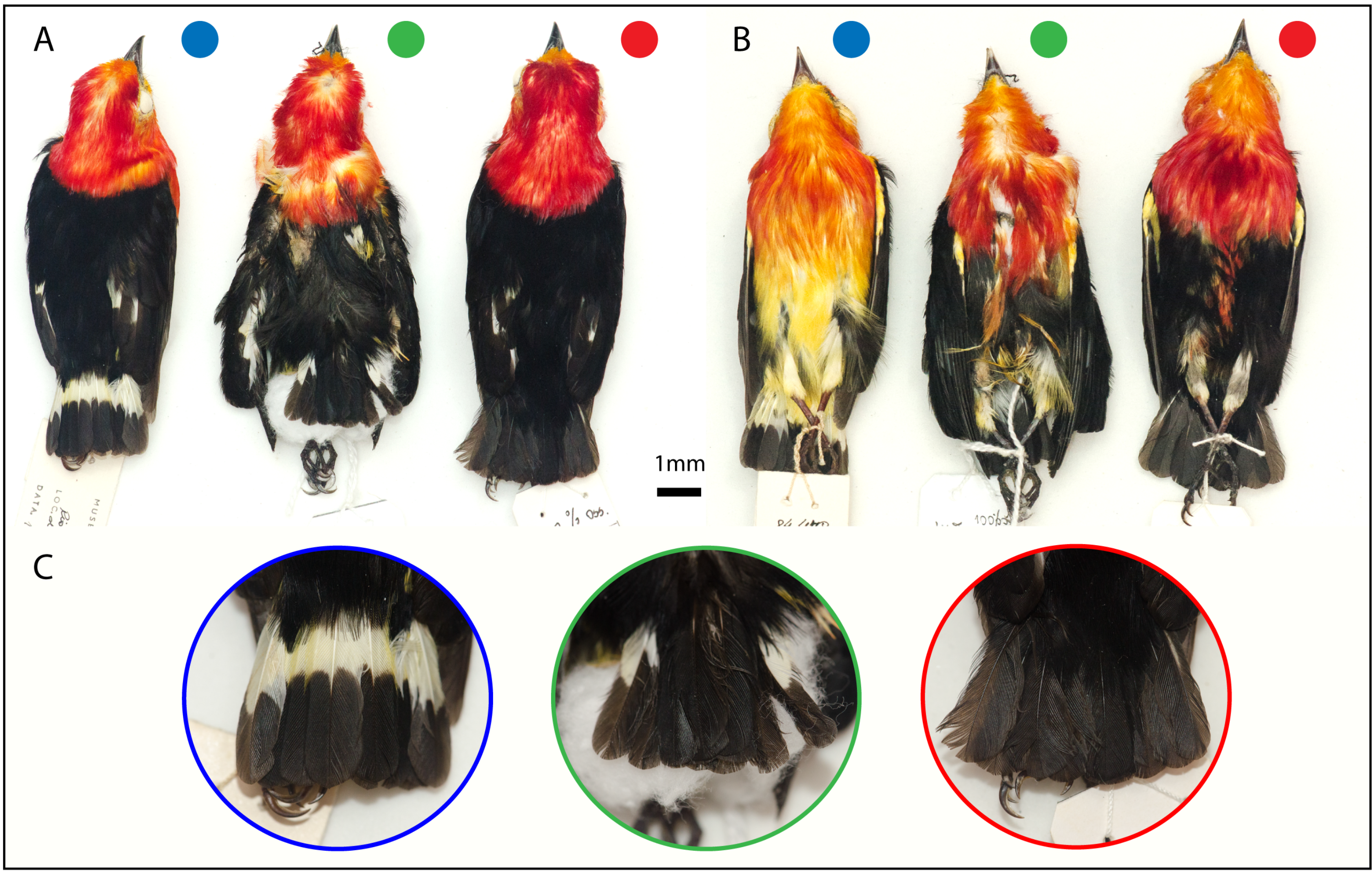
N
Kilometer
0 200 400 800 1.200
Pipra aureola
Pipra fasciicauda
Hybrid individual


A
B
1mm
C

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Online Resource 1. The *Pipra aureola* and *Pipra fasciicauda* specimens analyzed in the present study and *Pipra filicauda* used as out-group, with information on the identification of the specimens and the markers sequenced with their respective GenBank access codes. The markers not sequenced in each individual are indicated by a minus (-) sign. The specimens were obtained from the ornithological collections of the Goeldi Museum (Museu Paraense Emílio Goeldi: MPEG*) in Belém and the National Institute for Amazonian Research (INPA) in Manaus, Brazil.

Collect point (Map)	Taxon	Laboratory code	Tombo	Collection	Locality	Geographical coordinates	ND2	ND3	COI	FGB-I5	MB-I2	GAPDH-I3
01	*P. fasciicauda*	Pfa01		MPEG*	Brasil, Ceará, Guaramiranga, Serra de Baturité, Parque das Trilhas	04° 16' 06" S; 38° 56' 14" W	MN128475	MN128506	MN128491	MN166352	MN166326	MN166340
01	*P. fasciicauda*	Pfa02		MPEG*	Brasil, Ceará, Guaramiranga, Serra de Baturité, Parque das Trilhas	04° 16' 06" S; 38° 56' 14" W	MN128476	MN128507	MN128492	MN166353	MN166327	MN166341
01	*P. fasciicauda*	Pfa03		MPEG*	Brasil, Ceará, Guaramiranga, Serra de Baturité, Parque das Trilhas	04° 16' 06" S; 38° 56' 14" W	MN128477	MN128508	MN128493	MN166354	MN166328	MN166342
01	*P. fasciicauda*	Pfa04		MPEG*	Brasil, Ceará, Guaramiranga, Serra de Baturité, Parque das Trilhas	04° 16' 06" S; 38° 56' 14" W	MN128478	MN128509	MN128494	MN166355	MN166329	MN166343
01	*P. fasciicauda*	Pfa05		MPEG*	Brasil, Ceará, Guaramiranga, Serra de Baturité, Parque das Trilhas	04° 16' 06" S; 38° 56' 14" W	MN128479	MN128510	MN12849	MN166356	MN166330	MN166344
02	Ambiguous individual	Pfa40		MPEG*	Brasil, Pará, Acará, Comunidade Menino Jesus	01°29'14" S; 48°18' 57" W	MN128480	MN128511	MN128496	MN166357	MN166331	MN166345
02	*P. fasciicauda*	Pfa41		MPEG*	Brasil, Pará, Acará, Comunidade Menino Jesus	01°29'14" S; 48°18' 57" W	-	MN128512	MN128497	MN166358	MN166332	MN166346

03	*P. fasciicauda*	Pfa42	55524	MPEG*	Rio Xingu, Altamira, Ilha da Taboca, UHE Belo Monte	3°22'63”S, 51°57'57”W	MN128481	MN128513	MN128498	MN166359	MN166333	MN166347
03	*P. fasciicauda*	Pfa43	55525	MPEG*	Rio Xingu, Altamira, Ilha da Taboca UHE Belo Monte	3°22'83”S, 51°57'15”W	MN128482	MN128514	MN128499	MN166360	MN166334	MN166348
04	*P. fasciicauda*	Pfa87		MPEG*	Primavera, Pará		MN128483	-	MN128500	-	-	-
05	*P. aureola*	Pau01	A8491	MPEG*	Brasil, Pará, Ilha do Marajó, Município de Chaves, Fazenda Anjos	00°11'18,6"S; 49°53'11,3"W	MN128472	-	MN128488	MN166349	MN166335	MN166337
05	*P. aureola*	Pau02	58085	MPEG*	Brasil, Pará, Ilha do Marajó, Município de Chaves, Fazenda Anjos	00°11'18,6"S; 49°53'11,3"W	MN128473	-	MN128489	MN166350	-	MN166338
06	*P. aureola*	Pau05	66561	MPEG*	Brasil, Pará, Almeirim, FLOTA do Paru	00°56'S; 53°14'W	MN128474	MN128505	MN128490	MN166351	MN166336	MN166339
	P. filicauda	Pfi12	INPA A 611	INPA	ESEC Juami-Japurá; boca do Rio Juami; margem direita do Rio Japurá, ca 70 km WNW Japurá		MN128484	-	MN128501	-	-	-
	P. filicauda	Pfi13	INPA A 962	INPA	Ilha do Barbado, Rio Solimões, ca 60 km E de Tefé		MN128485	-	MN128502	-	-	-
	P. filicauda	Pfi15	INPA A 1052	INPA	Parque Nacional Viruá, margem direita do Rio Anauá, localidade "Campinho"		MN128486	-	MN128503	-	-	-

P. filicauda	Pfi16	INPA A 2095	Caracaraí; Vista Alegre; margem esquerda do Rio Branco; ca 26 Km W da base do Parque Nacional do Viruá	MN128487	-	MN12850	-	-	-

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Online Resource 2 Genes analyzed in the present study, with their respective primers and nucleotide sequences

Gene	Primer	Sequence	Reference
ND3	L10755	5’ GACTTCCAATCTTTAAAATCTGG 3’	Chesser 1999.
	H11151	5’ GATTTGTTGAGCCGAAATCAAC 3’	Chesser 1999.
ND2	L5216	5’ GGCCCATACCCGRAAAT 3’	Hackett 1996.
	H6313	5’ CTCTTATTTAAGGCTTTGAAGGC 3’	Hackett 1996.
COI	FISHF1	5’ TCAACCAACCACAAAGACATTGGCAC 3’	Ward et al. 2005.
	COIA	5’ AGTATAAGCGTCTGGGTAGTC 3’	Palumbi and Benzie 1991.
FGB-I5	FIB5	5’ CGCCATACAGAGTATACTGTGACA 3’	Driskell and Cristidis 2004.
	FIB6	5’ GCCATCCTGGCGATTCTGAA 3’	Driskell and Cristidis 2004.
MB-I2	MYO2	5’ GCCACCAAGCACAAGATCCC 3’	Heslewood et al. 1998.
	MYO3F	5’ GCAAGGACCTTGATAATGACTT 3’	Slade et al. 1993.
GAPDH-I3	G3PDH13b	5’ TCCACCTTTGATGCGGGTGCTGGCAT 3’	Fjeldsa et al. 2003.
	G3PDH14b	5’ AAGTCCACAACACGGTTGCTGTA 3’	Fjeldsa et al. 2003.

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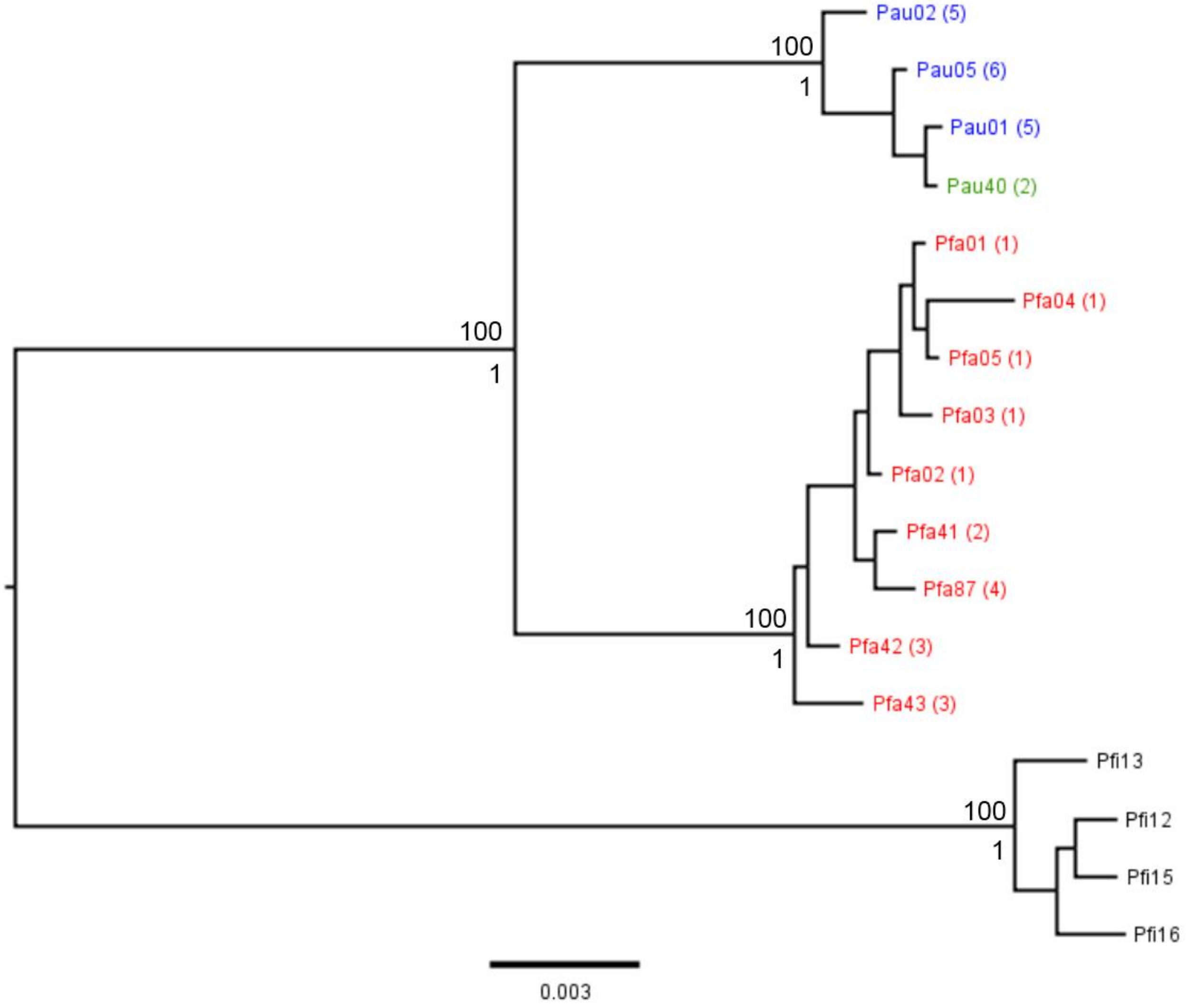

Online Resource 3 Bayesian inference topology of *Pipra fasciicauda* (Pfa) and *Pipra aureola* (Pau), with *a posteriori* values below the branches and bootstrap values above.

The topology was rooted by *Pipra filicauda* (Pfi)