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NAYRON FRANCÊS DO NASCIMENTO

Phylogeography and population genetics of the Needle-billed hermit (*Phaethornis philippii*, Aves, Trochilidae)

Belém
2020

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Dissertação apresentada ao Programa de Pós-Graduação em Zoologia, do convênio da Universidade Federal do Pará e Museu Paraense Emílio Goeldi, como requisito parcial para obtenção do título de Mestre em Zoologia.
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BELÉM, 2020

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“Transformar o simples em complicado é fácil, porém transformar o complicado em simples exige muita criatividade”

(Edward Elric)

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FILOGEOGRAFIA E GENÉTICA POPULACIONAL DO RABO-BRANCO-AMARELO (*PHAETHORNIS PHILIPPII*, AVES, TROCHILIDAE)

RESUMO

Phaethornis philippii é uma espécie monotípica de beija-flor, com distribuição nas florestas úmidas da Amazônia Ocidental e Central. Ocorrendo a leste do Peru, norte da Bolívia e parte do Brasil, ao sul do Solimões e do rio Amazonas e a oeste do rio Tapajós. Apesar de ter sido incluída em alguns trabalhos anteriores, a história evolutiva da espécie ainda não foi bem elucidada. Para isso, no presente trabalho, buscamos revisar a taxonomia da espécie através de caracteres morfológicos e moleculares, além de propor uma hipótese filogenética mais densa para as linhagens agrupadas em *P. philippii* e investigar sua história de diversificação, comparando-a com cenários biogeográficos já propostos para a Amazônia. Nossas análises filogenéticas e de genética de populações revelaram seis linhagens reciprocamente monofiléticas em *P. philippii* e sugerem que a diversificação ocorreu durante o Pleistoceno entre aproximadamente 2.5 e 0.3 milhões de anos. Nossos resultados mostram que *Phaethornis philippii* parece ter dificuldades em atravessar algumas barreiras, como os principais rios da Amazônia, e que mesmo rios relativamente mais estreitos como o Aripuanã, Jiparaná e Tarauacá podem representar barreiras à dispersão, reforçando a hipótese de que os "mini-interfluvios" também têm uma influência notável na estruturação e diversificação de aves na Amazônia. Nosso estudo lança luz sobre a dinâmica populacional histórica dessa espécie, que também pode ter sido influenciada por variações climáticas durante Pleistoceno tardio, onde encontramos evidências de eventos recentes que influenciaram na diversificação de linhagens em *P. philippii*, associados principalmente à cursos atuais e históricos de rios amazônicos.

Palavras-chave: Diversificação, História Evolutiva, Estrutura Populacional, Diversidade Críptica, Beija-flor.

PHYLOGEOGRAPHY AND POPULATION GENETICS OF THE NEEDLE-BILLED HERMIT (*PHAETHORNIS PHILIPPII,* AVES, TROCHILIDAE)

ABSTRACT

Phaethornis philippii is a monotypic species of hummingbird, with distribution in the humid forests of western and Central Amazonia. It occurs in eastern Peru, northern Bolivia and Brazil, south of the Solimões and Amazon rivers and west of the Tapajós river. Despite being included in some previous phylogenetic works, the evolutionary history of the species has not yet been fully elucidated. Here, we review the taxonomy of the species through morphological and molecular characters, in addition to proposing a denser phylogenetic hypothesis for the lineages grouped in *P. philippii* and to investigate its history of diversification, comparing it with biogeographic scenarios already proposed for the Amazon. Our phylogenetic and population genetic analyzes revealed six reciprocally monophyletic lineages within *P. philippii* and suggest that diversification in this group occurred during the Pleistocene between 2.5 and 0.3 mya. Our results show that *Phaethornis philippii* may have difficulties crossing barriers such as major Amazon rivers. Even narrower rivers such as Aripuanã, Jiparaná and Tarauacá can represent barriers to dispersal, reinforcing the hypothesis that "mini-interfluves" also have a notable influence regarding the structuring and diversification of birds in the Amazon. Our study sheds light on the historical population dynamics of this species, which may have also been influenced by climatic variations during the late Pleistocene, where we found evidence of recent events influencing the diversification of lineages within *P. philippii*, mainly associated to current and historical courses of Amazonian rivers.

Keywords: Diversification, Evolutionary History, Population Structure, Cryptic Diversity, Hummingbird.

INTRODUCTION

Phylogeography involves the study of the distribution of species lineages, seeking to reconstruct their evolutionary history, making it possible to correlate phylogeny with biogeographic patterns and processes that somehow affected a particular distribution (Avise 2000). The use of the phylogeographic approach coupled with molecular data has revealed patterns on the evolutionary history of bird species in the Neotropical region (Thom and Aleixo 2015; Ferreira et al. 2017; Araújo-Silva et al. 2017), which have resulted in changes on the taxonomic status of some lineages (Cerqueira et al. 2016). When molecular data is integrated with phylogeographic approaches and phenotypic variation analyzes, we can test hypotheses about the processes that drove species diversification in the tropical forests (Moritz et al. 2000), besides allowing for the evaluation of possible intraspecific genetic structures.

Population genetics studies have used phylogeography as an important tool to produce data for understanding the geographical limits of genetic diversity, as well as contributing to the knowledge about the population structure and evolutionary history of several taxa (Avise and Walker 1998; Vallinoto et al. 2017; Silva et al. 2018). Studies show that the loss of a population may compromise the survival of a species on a local scale (Ehrlich and Daily 1993; Ceballos and Ehrlich 2002; McConkey and Drake 2006), causing a possible loss of genetic diversity (Lacy 1987), emphasizing the relevance that population genetic studies can generate for the knowledge of the rich existing biodiversity.

Studies indicate that the neotropical region is one of the most biodiverse regions of the planet (Antonelli and Sanmartín 2011), and that the main source of this biodiversity is the Amazon (Antonelli et al 2018). Many taxonomic groups occur exclusively in the neotropical region, the hummingbird family (Trochilidae), for example, also occurs mainly in this region, and represents one of the most numerically significant families within the avifauna (Stiles 1995). Trochilidae is known worldwide for their unique sustained hovering skills and phenotypic beauty (Altshuler and Dudley 2002). Hummingbirds are also of great ecological importance, just as other pollinators, while feeding on nectar or insects, act as pollen dispersers (Stiles 1981; Bawa 1990; Cronk and Ojeda 2008), contributing significantly to the diversity and evolution of flora in tropical forests that are sometimes pollinated exclusively by hummingbirds (Stiles 1981; Feinsinger 1987; Lagomarsino et al. 2017; Zambon et al. 2019).

In this way, the genus *Phaethornis* Swainson, 1827, groups species that have a brownish color; an orange, red or yellow jaw; a slightly curved downward or straight beak; and the first pair of rectricces longer than the others (Elliot 1878). The genus is composed of 25 described species

(Hinkelmann and Schuchmann 1997; del Hoyo 1999; McGuire 2014), among them *Phaethornis philippii*, target of this work, which is a monotypic species of hummingbird that occurs in the understory of lowland *terra firme* forest, sometimes in *várzea* and bamboo thickets (Hinkelmann et al. 2020). Occurring in eastern Peru, northern Bolivia and part of Brazil, south of the Solimões and Amazonas river and west of the Tapajós river (Hinkelmann et al. 2020) (Figure 1), this species presents the lower body parts with ocher-yellowish tones and the end of the rectrices rusty (Figure 2A).

The most recent phylogenetic hypotheses proposed for species of the genus *Phaethornis* included a few samples of *P. philippii* (Hinkelmann and Schuchmann 1997; del Hoyo 1999; McGuire 2014; Araújo-Silva et al. 2017). Thus, in the most complete molecular phylogeny for the group obtained so far (Araújo-Silva et al. 2017), samples of the entire *P. philippii* distribution were not included, as this was not the target species of that study. However, even with a low sampling (N = 2) of *P. philippii*, Araújo-Silva et al. (2017) verified the existence of significant genetic divergence between these two individuals sampled, showing that some degree of cryptic diversity must exist in *P. philippii*, highlighting the need of a denser sampling for this species, which could potentially provide a better resolution on the evolutionary history of the entire genus *Phaethornis*.

Here, we present the most complete molecular data set yet on *Phaethornis philippii* to: (1) elucidate the phylogeography of the species using mitochondrial and nuclear molecular markers and compare this information with morphological data to review its taxonomy; (2) understand the genetic diversity and population structure within lineages and the relationships among them; and (3) use this information to investigate whether there is a relationship between landscape evolution and the diversification of *Phaethornis philippii* by comparing it with biogeographic models on biotic diversification already proposed for the Amazon (Ribas et al. 2012; Hayakawa and Rossetti 2015; Ruokolainen et al. 2019; Silva et al. 2019).

MATERIAL AND METHODS

Sampling, DNA extraction and sequencing

We studied a total of 76 specimens of *Phaethornis philippii* (Figure 1, Appendix 1) available from the Fernando Novaes Ornithological Collection (MPEG – Belém, Pará, Brazil), Instituto Nacional de Pesquisas da Amazônia (INPA - Manaus, Amazonas, Brazil), Museu de Zoologia da Universidade de São Paulo (MZUSP - São Paulo, Brazil), and the Louisiana State University Museum of Natural Science (LSUMZ - Louisiana, United States). Our sampling includes individuals distributed beyond the species' known distribution (IUCN 2016; Hinkelmann et al. 2020), the map shown below is already adapted to our samples distribution.

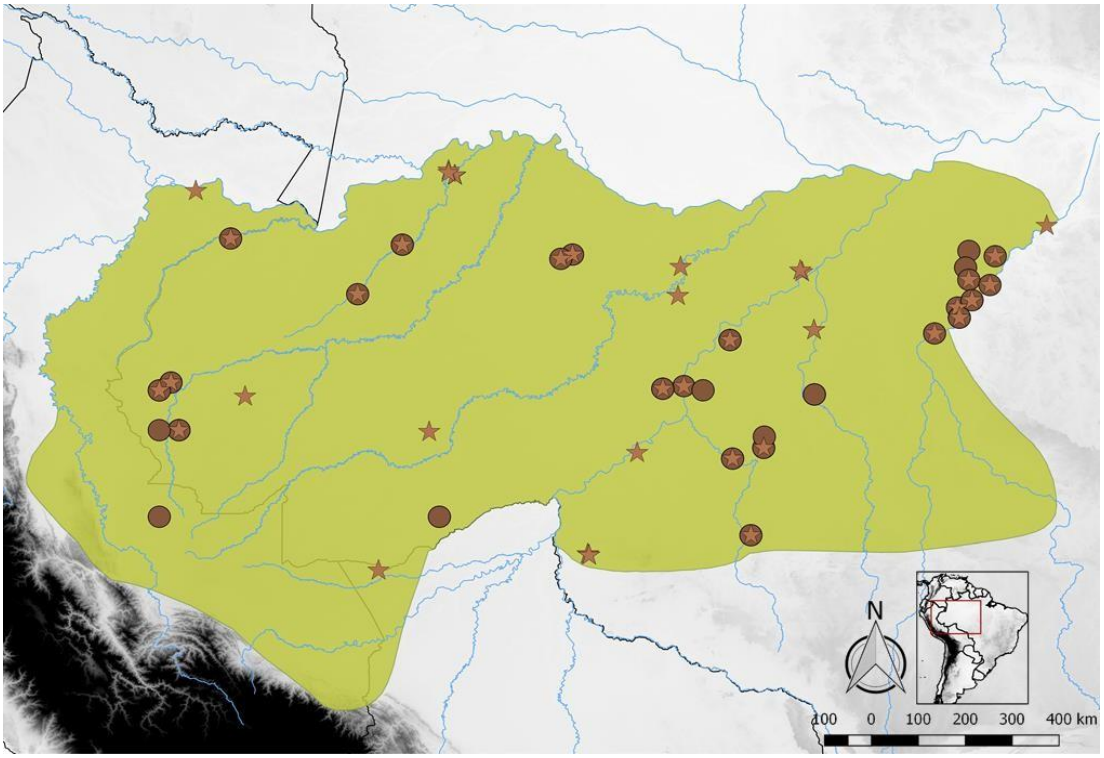

Figure 1 – Map showing the distribution of specimens sampled in this work. Stars represent the location of specimens included in the molecular data set; circles represent specimens used in the morphological database; stars in circles represent specimens included in both molecular and morphological databases. The green area represents the geographical distribution of the species (modified from International Union for Conservation of Nature - IUCN 2016).

We sequenced 54 specimens of *P. philippii* (muscle tissue) and 54 study skins were sampled for morphological analyses for almost the entire species range (Appendix 1). As outgroups, we used sequences of *Phaethornis koepckeae* and *Phaethornis bourcieri major*, which were previously found to be the phylogenetically closest species to *P. philippii* (McGuire et al. 2014; Araújo-Silva et al. 2017). We used the phenol-chloroform protocol for DNA extraction, and PCR amplification was performed using standard procedures (Sambrook et al. 1989). Three nuclear (intron 5 of β-fibrinogen [BF5], glyceraldehyde-3-phospho-dehydrogenase intron 11 [G3PDH] and the Transforming Growth Factor Beta 2 [TGFB2]) and two mitochondrial (NADH dehydrogenase subunit 2 [ND2] and cytochrome *b* [CYTB]) markers were used. The primers used are shown in Table 1.

The amplifications for the different genes were made according to the following protocol: an initial step of 5 minutes at 95ºC for temperature homogenization and DNA double helix denaturation, followed by 35 cycles of 1 minute at 95°C; 1 minute at the optimal annealing temperature for each of the primers (Table 1); 1 minute at 72°C for extension; and one step for final extension at 72°C for 10 minutes. Amplification products were sequenced using the "Big Dye Terminator Cycle Sequencing Standart v3.1" kit on Applied Biosystems ABI3130 automated sequencer according to the

manufacturer's specifications. Both strips of each fragment were sequenced for greater accuracy in analyzes.

Table 1 - Primers and annealing temperatures used for each sequenced marker.

Marker	*Primer* code	5'- 3'	Annealing temperature
ND2	L 5215	TATCGGGCCCATACCCCGAAAAT	58ºC
	H 6313	CTCTTATTTAAGGCTTTGAAGGC	
CYTB	L 14841	GCTTCCATCCAACATCTCAGCATGATG	54ºC
	H 16065	AACTGCAGTCATCTCCGGTTTACAAGAC	
BF5	S 713	CGCCATACAGAGTATACTGTGACAT	55ºC
	AS 767	GCCATCCTGGCGATCTGAA	
G3PDH	G3PL890	ACCTTTAATGCGGGTGCTGGCATTGC	75ºC
	G3PH950	CATCAAGTCCACAACACGGTTGCTGTA	
TGFB2	TGFB2-5 F	TTGTTACCCTCCTACAGACTTGAGTC	55ºC
	TGFB2-6 R	GACGCAGGCAGCAATTATCC	

Phylogenetic analyses

The nucleotide sequences were edited using Geneious v9.0.5 (Kearse et al. 2012) and the alignment was done using MAFFT v7 (Katoh and Standley 2013). For haplotype reconstruction of nuclear genes we used PHASE v2.1, accepting results of probability >70% (Stephens et al. 2001). The evolutionary models and partitions that best explained the evolution of the sequenced genes were obtained from Partition Finder v.1.1.1 (Lanfear et al. 2012). We used all genes (Table 1) sequenced for 54 individuals to perform a Bayesian inference analysis (BI) implemented in MrBayes 3.2.6 (Ronquist et al. 2012) with two independent runs with four Markov chains, twenty million generations and sampling trees every 500 generations. The first 20% of trees were discarded as burnin. We used this tree as a guide to test lineages boundaries on multilocus coalescent methods.

Molecular dating and species tree estimation

We run a multilocus analysis on BEAST v1.6 (Drummond et al. 2012) to estimate the divergence times among *P. philippii* lineages. The resulting species tree (ST) was estimated using all individuals for which at least one mitochondrial and one nuclear gene were sequenced. The evolutionary models were the same used in BI. We used two different rates (substitution rates of 0.0105, SD = 0.0034 for CYTB from Weir and Schluter [2008] and substitution rates of 0.0123, SD = 0.45 for ND2 from Smith and Klicka [2013]), which were used to estimate the rates of the other

genes. We assumed an independent uncorrelated lognormal relaxed clock and changed two priors (species.popMean and species.Yule.birthRate) from 1/x to log-normal distribution with Log (mean) = 4.0 and Log (SD) = 2.0. We performed a run of 5 x 10^7 generations with parameters sampled every 1000 generations. *BEAST analyses were conducted on the CIPRES Science Gateway (Miller et al. 2010). The Tracer v1.5 software was used to determine when the analyzes reached convergence in the estimated parameters and to check if the values of Effective Sample Sizes (ESS) were greater than the recommended threshold of 200 (Drummond and Rambault 2007). We used TreeAnnotator 1.8.0 (Drummond et al. 2012) to summarize the distribution of the maximum clade credibility tree and discard the first 25% trees as burnin.

Molecular Species delimitation

We used the program Bayesian Phylogenetics and Phylogeography (BPP v3.4) (Yang 2015), which models multilocus sequence data to generate probabilities for lineages identified a priori as potential species-level taxa (Yang and Rannala 2010). Considered one of the best performing coalescent-based methods for species delimitation evaluated (Camargo et al. 2012; Rannala 2015; Leaché et al. 2019), BPP uses a Bayesian modeling approach to generate a posterior distribution of speciation models where the number of species in each model varies. We tested two different algorithms and multiple combinations of prior distributions representing different population sizes (θ) and ages for the root of the species tree (τ_0) (Leaché and Fujita 2010) as follows: (run1) large ancestral population sizes and deep divergences (h and s gamma priors G(1, 10) and G(1, 10)); (run2) small ancestral population sizes and shallow divergences among species (h and s gamma priors G(2, 2000) and G(2, 2000)); (run3) large ancestral population sizes and shallow divergences among species (h and s gamma priors G(1, 10) and G(2, 2000)) (Table 2). We ran the analyses for 5 $\times 10^5$ generations, sampling parameters every five generations and discarding the first 25 000 as burn-in. We used topology obtained under the species tree analyses as the guide tree. Each analysis was run at least twice to confirm consistency of results (Yang 2015).

Population genetics analyses

Mean pairwise *p*-distances within and among clades recovered by the phylogenetic analyses were calculated using the mtDNA dataset only in MEGA v10.1 (Kumar et al. 2018). Haplotype networks were constructed in HaploViewer (Salzburger et al. 2011) to visualize genealogical relationships among individuals of each sequenced gene, which were coded following the topologies recovered by the BI.

We used a Bayesian Analysis of Population Structure (BAPS v 6.0.) (Corander et al. 2008) to estimate the level of geographic structure within *P. philippii*. The genetic clusters probabilities (from

K = 1 to K = 10) were surveyed with the concatenated nuclear dataset under a mixture analysis followed by an admixture analysis. The program was run ten times for each value of K. The admixture analysis was performed using 100 iterations, a minimum of three individuals per population, 200 reference individuals for each population, and 20 iterations of reference individuals. We also ran the analysis with the concatenated mitochondrial database to check how the database behaved. We also used Extended Bayesian Skyline Plot (EBSP) (Heled and Drummond 2010), implemented in BEAST, to analyze population size dynamics through time. The rate of 2.1% of nucleotide substitutions per million years (Weir and Schluter 2008) was used for the mtDNA with the relaxed clock and uncorrelated lognormal priors. The evolutionary models for each locus of each population were estimated in Partition Finder v.1.1.1 (Lanfear et al. 2012).

Morphological analysis

For morphological data, 54 study skins were evaluated. The measurements were performed using a digital caliper with an accuracy of 0.1 mm. Five characters were examined for each specimen: length of the right wing, length of the tail, and length, height and width of the beak. Three consecutive measurements were made of each character studied in each specimen and averaged between them to minimize measurement errors. The arrangement of the groups tested for differences in the morphological analyzes followed that obtained in the genetic analyzes. The PAST 3.11 program (Hammer et al. 2001) was used to perform a Principal Component Analysis (PCA). We also analyzed possible variations in plumage color and compared our results with Piacentini (2011), following the definition of colors in Smithe (1975).

RESULTS

Data characteristics and phylogenetics analyses

We obtained a multilocus dataset of 3232 base pairs (bp) for 54 individuals of *P. philippii*, of which 1615 bp were of mitochondrial genes; 398bp of G3PDH; 592bp for BF5 and 627bp for TGFB2. No base saturation was detected for any of the sequenced genes. The nucleotide substitution models most suitable for the sequences were: HKY + I for the ND2 and CYTB genes and JC + I for the BF5, G3PDH and TGFB2 genes.

The IB topology recovered the monophyly among all individuals of *P. philippii* sequenced in this study. The IB (Figure 2b) recovered most relationships with high statistical support and supported the existence of six major geographically distinct clades (named A, B, C, D, E and F) (Figure 2d). Clades A and B (green and orange colors respectively) are shown as a sister group of Clade C (yellow color), which together group all individuals located west of the Madeira river and form the sister group of clade D (blue color), which groups samples to the east, near the left bank of the Tapajós

river. Clades E and F (brown and purple colors respectively) form the sister group to the other clades and are located east of the Madeira river to the left bank of the Aripuanã river. However, the node joining the clades found west of the Madeira river and that found on the left bank of the Tapajós is statistically poorly supporting, indicating that a sister relationship between clades A+B+C and clades E+F cannot be ruled out (Figure 2b).

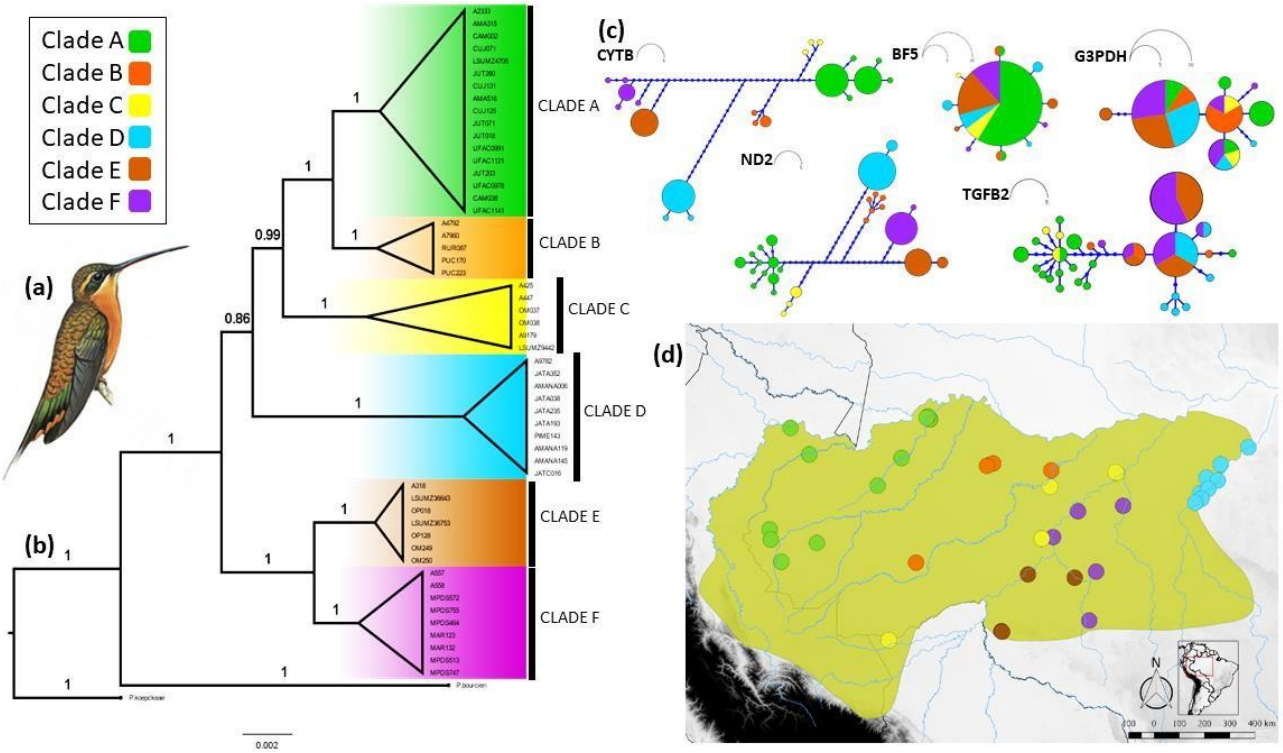

Figure 2 - Results of molecular analyses. (a) *Phaethornis philippii* (modified from Handbook of the Birds of the World available at: http://www.hbw.com/). (b) Multilocus phylogenetic hypothesis obtained through Bayesian Inference. (c) Haplotype networks estimated for each gene sequenced. (d) Map with the distribution of samples used in molecular analyzes. For detailed information on the samples included in the analyzes see Appendix 1.

Molecular dating

We recovered a multilocus coalescent tree for *P. philippii* using three independent loci (2 mitochondrial and 2 nuclear; Figure 3). Compared to the BI tree, the multilocus species tree recovered a slightly different topology, indicating an initial separation of Clade D during the Pleistocene around 2.0 million years ago (mya), suggesting that Clade D is the sister group of all remaining *P. philippii* clades. In both topologies, IB and ST, *P. philippii* and *P. koepckeae* are more closely related to each other than to *P. bourcieri*.

The oldest divergence time estimated was 3.7 mya and comprises the separation of *P. bourcieri* with the group *P. philippii* + *P. koepckeae*. The second divergence occurred approximately between 3.7 and 2.2 mya, separating *P. koepckeae* from all clades of *P. philippii*. Between 2.3 and 1.4 mya the populations located west of the Madeira river (clades A, B and C) were separated from

clades E and F located in the Madeira-Aripuanã interfluvium (east of the Madeira river), and this is the divergence with the lowest posterior probability recovered in the ST (PP = 0.67). Around 1.2 mya, clade C diverged from the clade grouping lineages A and B. Subsequently, clades A and B diverged from each other around 1.0 and 0.3 million years. Finally, the separation of clades E and F occurred around 0.6 mya.

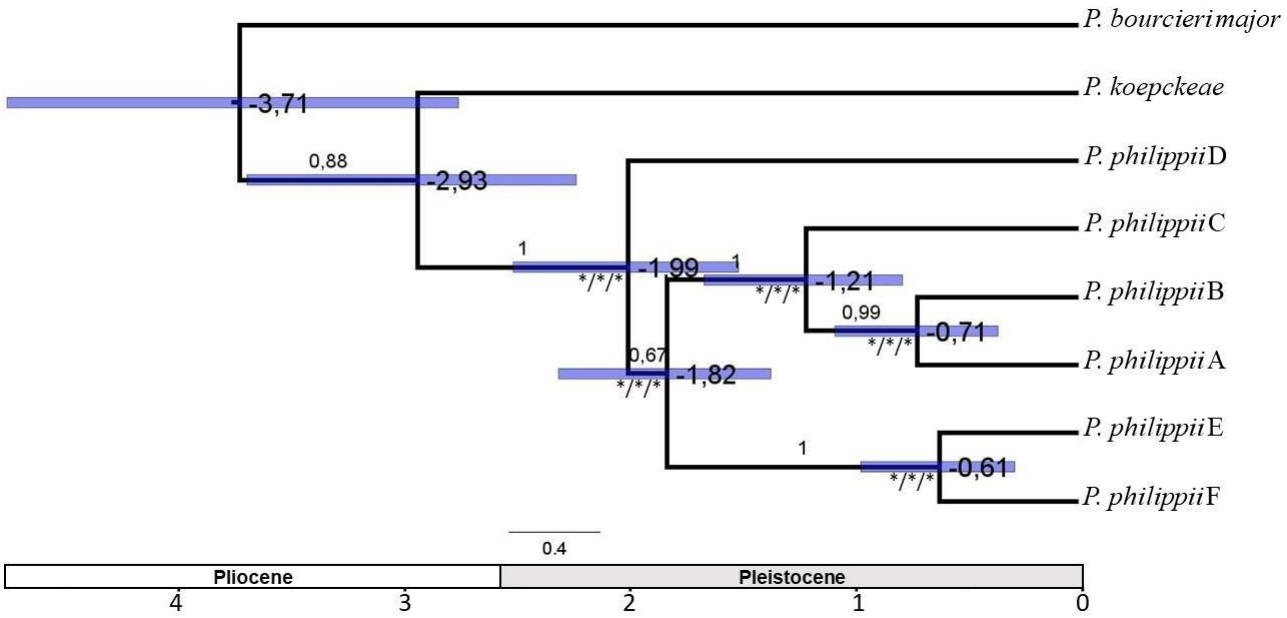

Figure 3 - Multilocus species tree analysis. Numbers on top of branches represent posterior probability values. Numbers on nodes represent the median divergence time in millions of years whereas bars denote the confidence interval of the estimated divergence times. Asterisks below branches represent maximum posterior probability values (≥0.99) obtained for three different coalescence scenarios tested on the species tree topology with BPP. Numbers on the time scale below represent millions of years.

Species delimitation

We obtained similar results in all BPP analyzes (Table 2) using the two algorithms and the three different combinations of τ and θ values. The model with the highest PP indicated that there are six species (nodes recovered with PP> 0.99 for all three prior sets) present within *P. philippii*. All six likely species recovered were strongly supported (PP> 0.99). This six species scheme had the highest posterior probability (equal to 1.0), with the second highest being the one with 5 species (equal to 0.001%) grouping the clades E and F.

Table 2 - Results of BPP analyses showing posterior probabilities of different divergence schemes tested for the lineages recovered in *Phaethornis philippii*. ε, α and m are different values of fine-tune parameters. Θ is a prior for the population size parameter and τ for divergence time at the root of the species tree.

BPP Posterior Probability						
	Algorithm 0 (ε = 2)			Algorithm 1 (α = 2, m = 1)		
Clade	Run 1 Θ= G(1,10) τ= G(1,10)	Run 2 Θ=G(2,2000) τ=G(2,2000)	Run 3 Θ=G(1,10) τ=G(2,2000)	Run 1 Θ=G(1,10) τ=G(1,10)	Run 2 Θ=G(2,2000) τ=G(2,2000)	Run 3 Θ=G(1,10) τ=G(2,2000)
A	1,00000	1,00000	1,00000	1,00000	1,00000	1,00000
B	1,00000	0,99999	0,99999	1,00000	0,99999	0,99999
C	1,00000	0,99999	0,99999	1,00000	1,00000	0,99999
D	1,00000	1,00000	1,00000	1,00000	1,00000	1,00000
E	0,99999	0,99999	1,00000	1,00000	1,00000	1,00000
F	0,99999	0,99999	1,00000	1,00000	0,99999	1,00000

Population genetics and historical demography

The levels of uncorrected genetic divergence (*p*-distance) within and between clades A, B, C, D, E and F range from 0.06% to 0.4% and 1.5% to 4.54% respectively (Table 3). The greatest divergence between pairs of populations within *P. philippii* was between clades D and F (4.54%).

Table 3 - Pairwise uncorrected genetic divergences (*p*-distances) among different clades of *P. philippii* and the two closest species *P. koepckeae* (*P.k*) and *P. bourcieri* (*P.b*). A, B, C, D, E and F - clusters recovered from previous analyzes.

	A	B	C	D	E	F	*P.k*	*P.b*
A	0,32%							
B	1,85%	0,30%						
C	2,94%	2,99%	0,40%					
D	3,89%	3,75%	4,21%	0,06%				
E	3,16%	3,48%	3,43%	4,13%	0,06%			
F	3,29%	3,37%	3,40%	4,54%	1,50%	0,27%		
P.k	4,29%	4,12%	4,72%	5,38%	4,83%	4,83%	-	
P.b	5,49%	5,33%	6,14%	6,12%	5,98%	6,06%	5,88%	-

The median-joining networks (Figure 2c) show clear phylogeographic structure for both mitochondrial genes (ND2 and CYTB), indicating that there are several haplotypes restricted to each lineage that are separated by several mutational steps. On the other hand, haplotype networks for nuclear markers do not have a well-defined structure. In the haplotype network for the TGFB2 nuclear gene, some geographic signal was present, but there were haplotypes shared between geographically non-adjacent populations, as in a haplotype shared by populations E, F (east of the Madeira river) and B (west of the Purus river). In the haplotype networks of BF5, a widespread haplotype is shared by most lineages, with several other haplotypes connected to it by one or two mutational steps. The

G3PDH network has three main haplotypes shared by all lineages, with other haplotypes connected to them by one or two mutational steps.

In our BAPS analysis using the nuDNA database only (Figure 4), the optimal value of K recovered was 2, where one of these clusters corresponds geographically to all individuals located west of the Madeira river, while the other involves all individuals east of the Madeira river. In contrast, when using the mtDNA database, the analysis recovered six clusters (K = 6) (Appendix 2), suggesting a genetic and geographic structure similar to previous phylogenetic analyzes. The admixture levels recovered by the analysis were low in both cases. In our historical demography analyzes using EBSP (Figure 5), we recovered patterns of recent expansion in three clades during the Holocene (clades A, C and D). However, for clades E and F, the analyzes favored scenarios of demographic stability. No results were generated for clade B due to the small sample size available for this lineage.

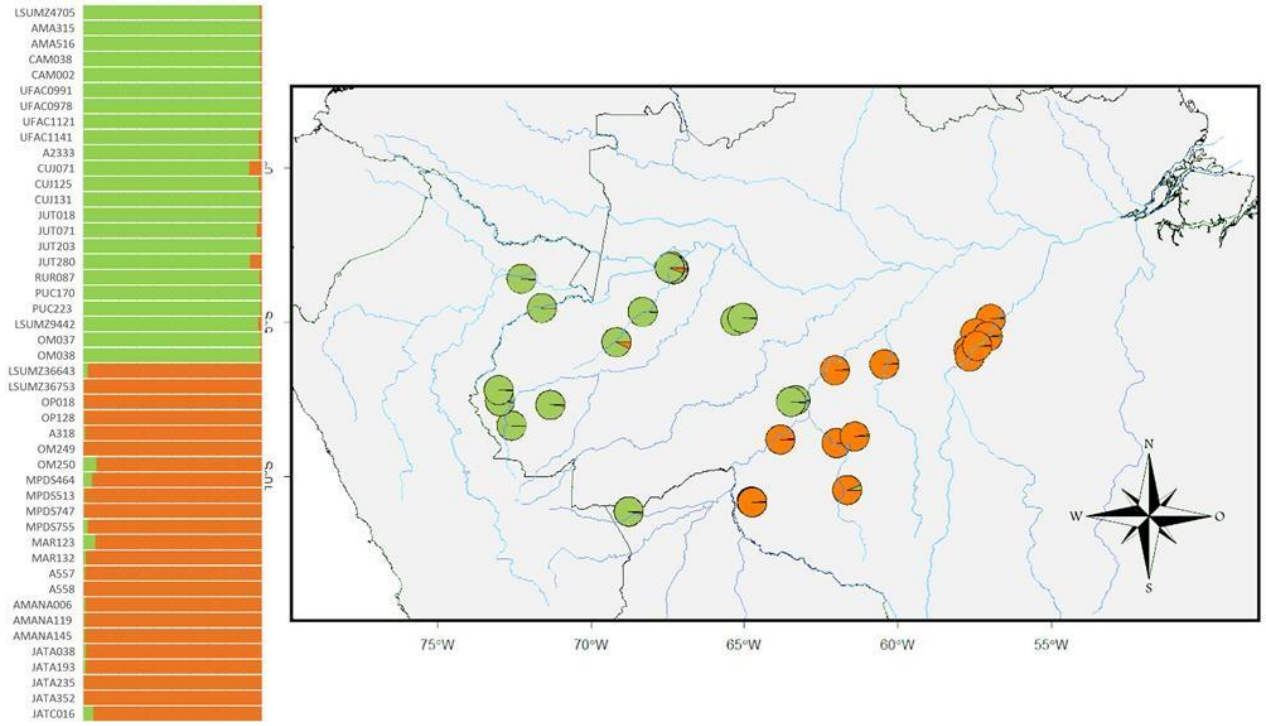

Figure 4 - Geographic distribution of the two clusters (K = 2) and individual's admixture proportion as revealed by a Bayesian Analysis of Population Structure (BAPS). Bar graph representing the population structure analysis obtained for *P. philippii* with the concatenated nuclear database. The colors represent the populations recovered by the phylogenetic analyses. The same bars are plotted on the map in pie chart format, according to the location of each individual.

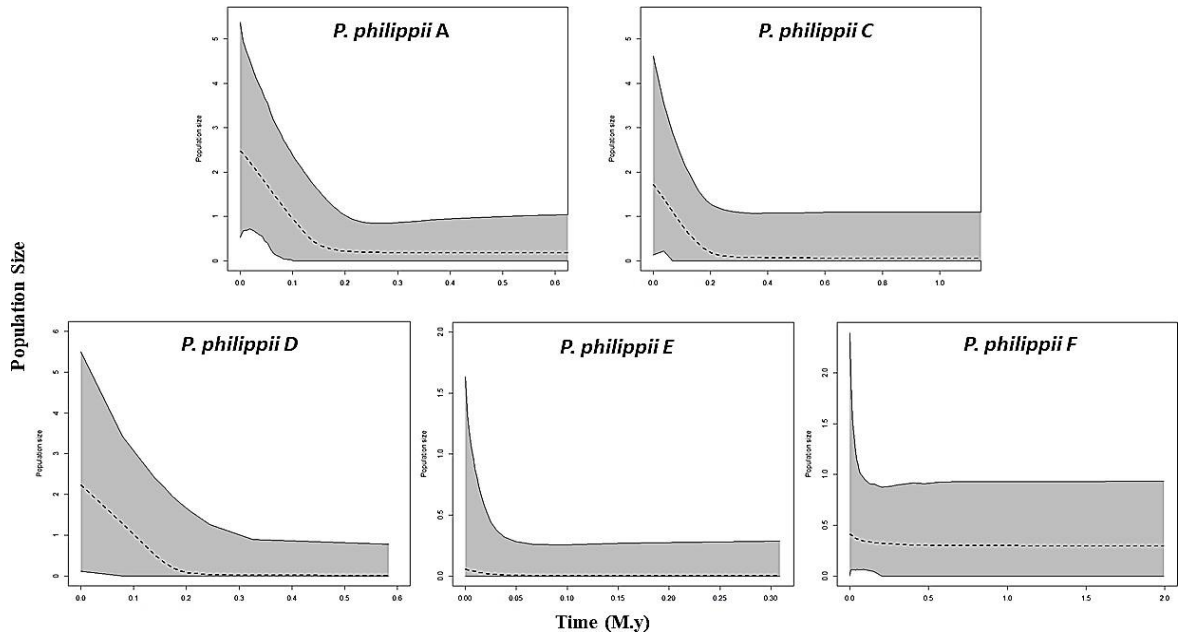

Figure 5 - Demographic histories of clades A, C, D, E and F of *Phaethornis philippii* inferred through extended Bayesian skyline plots (EBSP) based on mtDNA (CYTB and ND2), BF5, TGFB2 and G3PDH sequences. Black solid lines represent median values and dashed lines correspond to 95% confidence intervals. The X axis corresponds to time in millions of years before present, while the Y axis represents estimated effective population sizes (*Ne*) in a logarithmic scale.

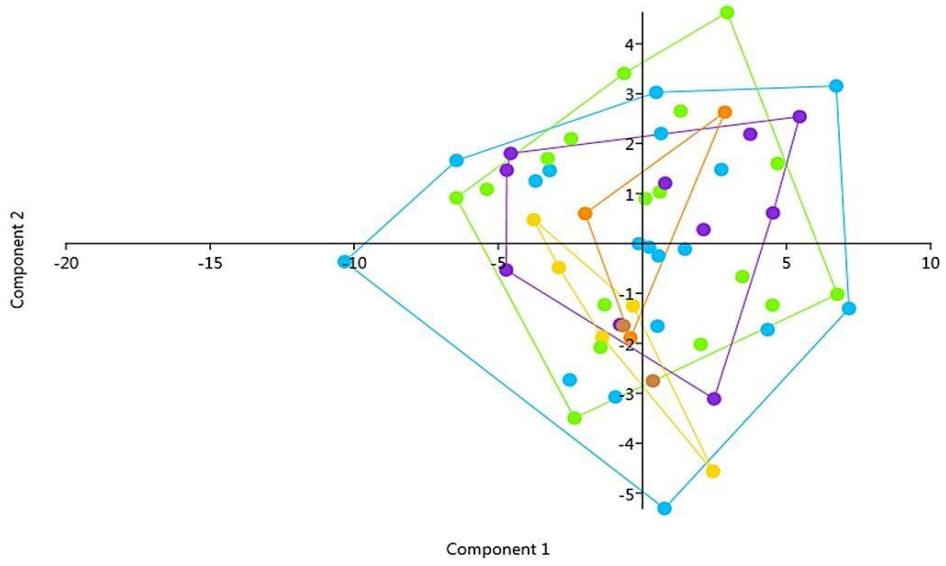

Figure 6 - Principal Component Analysis (PCA) showing the ordination of the specimens studied based on morphometric characters. Points are colored according to the groups recovered by the phylogenetic analysis.

Morphometrics

Morphological differentiation among lineages of *P. philippii* is not evident based on morphometric analyses. When all the traits were analyzed, the first two components of the PCA explained 91.309% of the variance (PC1 67.896%, PC2 23.413%). Tail length was the most

informative trait for PC1, whereas the wing and beak length were the most informative for PC2 (Figure 6). Regarding the plumage characters, it was not possible to identify diagnostic plumage color attributes for each population, although individuals that occur in the Amazonas-Madeira interfluvium seem to have a tendency to present darker tones in the ventral and dorsal colors when compared to individuals that occur to the east of the Madeira river.

DISCUSSION

Phylogeny, population structure and morphology

The results from our molecular analyzes revealed a never-documented discovery for the monotypic species *Phaethornis philippii*, where six reciprocally monophyletic and strongly supported clades were recovered. It was also possible to assess the phylogenetic relationships of *P. philippii* with the most closely related species (*P. koepckeae* and *P. bourcieri major*) (Figure 2b), where our results are consistent with those obtained by other authors where *P. philippii* is phylogenetically closest to *P. koepckeae* than *P. b. major* (Hinkelmann and Schuchmann 1997; McGuire 2014). Araujo-silva et al. (2017) recovered this configuration with the concatenated multilocus BI and ML trees (with *P. philippii* and *P. koepckeae* as sister taxa), but not with their coalescent multilocus species tree, where *P. koepckeae* and *P. bourcieri major* were recovered as sisters (Araújo-silva et al. 2017).

Six *P. philippii* lineages can also be distinguished in haplotype networks based on the mitochondrial dataset. For both CYTB and ND2 genes, unique haplotypes sort out all six parapatric populations throughout Amazonia. In contrast, the networks estimated for nuclear markers retrieved widely shared haplotypes among populations that are not close geographically (Figure 2c), an expected outcome, since this type of marker depicts a relatively older evolutionary history than the mitochondrial genes (Antonelli 2010). The mitochondrial genetic divergence between some of these recovered lineages (*p*-distance: D/C = 4.21%; D/F = 4.54%) is even greater than that between *P. koepckeae* and some clades of *P. philippii* (*p*-distance: *P.k*/clade A = 4.29%; *P.k*/clade B = 4.12%), showing that the interspecific genetic divergence between these two sister species is similar to the differences among lineages of *P. philippii* (Table 3).

The BAPS analysis using only nuDNA (Figure 4) revealed a different pattern of geographic structuring than that provided by the concatenated dataset. Essentially, BAPS recovered two major groups in *P. philippii,* one limited to the Inambari and the other to the Rondônia endemism. In contrast, when considering the mtDNA database only (Appendix 2), the six groups recovered were the same as those revealed by the phylogenetic analyzes. Therefore, while the mitochondrial and the concatenated datasets suggest that several Amazonian rivers delimit genetically distinct lineages of

P. philippii, the nuclear data supports the Madeira river as the major biogeographic divide for this species. Considering the different modes and rates of evolution between mitochondrial and nuclear markers, these apparently conflicting results can be easily reconciled with the notion that the oldest divergence in *P. philippii* occurred across the Madeira, while the other rivers corresponded to more recent divisions. Supporting this view is the fact that neither the BI nor the ST phylogenies recovered the basal-most relationships within *P. philippii*, which involved the sister group relationship to clade D, with strong support (Figures. 2 and 3). Therefore, it is also possible that clade D is actually sister to clades E + F from the right bank of the Madeira, agreeing with the results of BAPS based exclusively on the nuclear data. At any rate, both nuclear and mitochondrial datasets suggest that the Madeira, Aripuanã, Purus, Jiparaná and Juruá rivers may be acting as barriers preventing or limiting gene flow between lineages of *P. philippii*. All individuals sampled, regardless of the database considered, have low levels of admixture, which can be graphically detected only in the nuclear database (Figure 2c). In these particular cases, clades sharing haplotypes were not geographically close or adjacent to each other, which can be better explained by lack of lineage sorting / retention of ancestral polymorphism instead of gene flow. Other instances of deeply divergent and reciprocally allopatric populations separated by the Madeira river have been documented for birds (Aleixo 2004; Fernandes et al. 2013; Thom and Aleixo 2015; Ferreira et al. 2017; Schultz et al. 2019).

Our historical demography estimates (Figure 5) suggest recent population growth during the late Pleistocene for nearly all *P. philippii* clades (i.e. A, C, D, E and F; due to very small sample sizes, no EBSP were inferred for clade B). However, for clades E and F, the confidence intervals do not rule out the possibility of population stability. Demographic fluctuations induced by climate change may have influenced this history of recent diversification in *P. philippii*, as well as documented for several neotropical birds (Ribas et al. 2018; Soares et al. 2019). Population size recoveries after climatic changes in the last glacial maxima, dated to ca. 31 kya and 25 kya, may be directly linked to this trend of population increase in the late Pleistocene (Cheng et al. 2013; Wang et al. 2017).

On the other hand, our plumage analyses among *P. philippii* clades did not result in any detected diagnostic variation. In the case of *P. philippii*, both darker and lighter specimens were found within the same genetically delimited clades, supporting the interpretation that plumage color intensity varies at the individual level, with no consistent taxonomic value, as found by Piacentini (2011) based on a larger series of more than 200 *P. philippii* specimens. Similar results were also recovered by our PCA analysis (Figure 6) with morphometric data, where none of the populations previously defined within *P. philippii* based on the genetic data could be separated by the characters measured in this study.

Historical diversification of *Phaethornis philippii*

Many hypotheses have been proposed to explain diversification patterns in Amazonia (Gascon et al. 2000; Haffer 2001). Currently, there is a growing consensus that major Amazonian rivers are potential barriers to dispersal and, therefore, can promote speciation by vicariance (Wallace 1852; Gascon et al. 2000; Cortés-Ortiz et al. 2003; Ribas et al. 2012; Boubli et al. 2015; Schultz et al. 2017). Amazonian rivers also seem to have an important role in the diversification of some clades of *P. philippii*, but not in all. Studies have shown that the history of a river's course is very dynamic through time and that the current conformation of many Amazonian rivers may more recently than expected (Almeida-Filho and Miranda 2007; Hayakawa and Rossetti 2015; Ruokolainen 2019; Silva et al. 2019).

The genetic structure within clades E and F is not strictly delimited by the Jiparaná river, since two samples of clade E (OM 249 and OM 250) occur on the right bank of that river, which serves as the western limit of clade F. Therefore, clades E and F overlap slightly in distribution and co-occur on the right bank of the Jiparaná river (Figure 2b), which on this stretch (ca. 100 km upriver from its confluence with the Madeira river) seems to have had a very complex and dynamic history, with multiple course changes inferred during the Quaternary (Hayakawa and Rossetti 2015). Thus, based on the distribution of *P. philippii* clades, our data corroborate the hypothesis that the Jiparaná river was located eastward of its modern position (Hayakawa and Rossetti 2015). A similar pattern was found in *Rhegmatorhina hoffmannsi*, where two recently split lineages within the species overlapped their distributions extensively in the mini-interfluve bound by the Jiparaná and Madeira rivers, indicating that river course changes in this area affected upland *terra-firme* birds consistently and probably during very recent times (Ribas et al. 2018). However, in contrast to *P. philippii*, in *R. hoffmannsi* lineages overlapped on the left bank of the Jiparaná rather than on the right bank, which could reflect interspecific differences in historical population sizes and rates of introgression between populations isolated from each other before rivers course shifts or even incomplete sampling.

For all other *P. philippii* lineages recovered in this study, the current conformation of Amazonian rivers seem to have had a major role on their diversification, where clade D seems to be geographically limited to the Madeira-Tapajós interfluvium (area of endemism Rondônia), and clades A, B and C largely limited to the Solimões-Juruá, Juruá-Purus, Purús-Madeira interfluves, respectively (all these within the Inambari area of endemism). However, clade A seems to have expanded into the adjacent interfluvium (Juruá-Purus), possibly by the headwaters of the Juruá river (Juruá-Envira region).

Our molecular dating results and the BPP analysis confirmed a significant degree of coalescence for all clades within *P. philippii*. We recovered splits ranging from 4.7 to 0.3 mya, a timeframe when several other speciation events seem to have occurred among Amazonian birds (Silva et al. 2019). The oldest split recovered is dated in the Pliocene (4.7 to 2.7 mya) and involves a separation between *P. bourcieri major*, which occurs east of the Tapajós river, and its sister group *P. koepckeae* and *P. philippii*, distributed west of the Tapajós. The confidence intervals of this diversification do not overlap with the estimated for the formation of the Tapajós river as a barrier based on previous bird phylogenies (Ribas et al. 2012; Fernandes et al. 2014), showing that different taxa do not respond in the same way to the influence of major Amazonian rivers (Kopuchian et al. 2019; Silva et al. 2019). The next split occurred around 3.6 to 2.2 mya (mean 2.93 mya), separating *P. koepckeae* (which occurs on the eastern Andes foothills in Peru) from the clades of *P. philippii*. The first divergence within *P. philippii* occurred around the Plio-Pleistocene border between 2.5 and 1.5 mya (mean 1.99 mya), separating clade D (within the Madeira-Tapajós interfluvium) from clades A, B, C (which occur to the west of the Madeira river), plus E and F (occurring west of Aripuanã). As in *P. philippii*, studies show that several groups have endemic Madeira-Tapajós interfluvium lineages that diversified during the latest Pliocene / Early Pleistocene (Thom and Aleixo 2015; Araújo-silva et al. 2017; Ribas et al. 2018; Schultz et al. 2019; Silva et al. 2019).

A split around 1.3 to 2.3 mya (mean 1.82 mya) occurred between clade E+F (occurring in the Madeira-Aripuanã interfluve) and clades A, B and C (which occur in the Inambari endemism). During this period, at least some part of the modern course of the Madeira river could have been established, which may have promoted this diversification (Ribas et al. 2012; Fernandes et al. 2014; Ferreira et al. 2017). More recently, around 1.2 mya, clade C diverged from clades A and B, becoming limited to the Purús-Madeira interfluvium. A similar pattern of diversification in the same region was also found for *Malacoptila rufa*-lineage R8 by Ferreira et al. (2017). Finally, between 1.0 and 0.3 mya (mean 0.71 mya) clades A and B diverged from each other. Clade A appears to have invaded the adjacent interfluvium by crossing the headwaters of the Juruá river, but it may be limited to the left bank of the Tarauacá river, its major eastern tributary. A study showed that a primate species has distribution limits consistent with the Juruá and Tarauacá rivers (Nunes and Serrano-Villavicencio 2017), but a better sampling on both banks of the Tarauacá river must be verified to check the effectiveness of this possible barrier for gene flow between *P. philippii* lineages.

CONCLUSION

Here, we obtained statistically well supported molecular evidence for the existence of undocumented cryptic evolutionary lineages within *P. philippii* with independent histories, some as divergent from each other as they are from their sister species *P. koepckeae*. Additionally, the diversification of these lineages seems to have been greatly influenced by events of vicariance related to the formation and dynamics of Amazonian rivers during the Pleistocene. Our study supports the Plio-Pleistocene model of diversification in Amazon, where we found evidence of recent events influencing the diversification of lineages within *P. philippii*, and sheds light on the historical population dynamics of this species, which may have also been influenced by climatic variations during the late Pleistocene, as well as documented for several neotropical taxa. Our results are also of practical relevance for the conservation of *P. philippii*. Even with the lack of clear morphological diagnoses among its highly divergent cryptic lineages, the genetic variability documented here must be considered when reviewing this species conservation status.

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APPENDICES

Appendix 1 - *Phaethornis philippii* samples used in the present work. DNA- Samples used for the molecular database, Morph.- Samples used for the morphological database, Inst.- institution (MPEG: Museu Paraense Emílio Goeldi; INPA: National Amazon Research Institute; LSUMZ: Louisiana State University Museum of Natural Science), Lineage- Named according to the clusters found in the molecular analyzes, Locality- Locality Information (plotted on the map in Figure 1).

Inst.	DNA	Morph.	Sample	Clade	Locality
INPA	x		A 2333	A	Brasil, Amazonas, Resex do Rio Gregório; margem direita do Rio Gregório; Comunidade 1o de Junho; ca 196 km SW Eirunepé
MPEG	x	x	AMA 315	A	Brasil, Amazonas, Atalaia do Norte, Estirão do Equador (04o31'49"S;71o36'58,5"W)
MPEG	x		AMA 516	A	Estirão do Equador, Atalaia do Norte (04o31'49"S;71o36'58,5"W)
MPEG	x	x	CAM 002	A	Guajará (07°23'42,7"S;72°45'33,3"W)
MPEG	x	x	CAM 038	A	Guajará (07°23'42,7"S;72°45'33,3"W)
MPEG	x	x	CUJ 071	A	RDS Cujubim, margem E Rio Jutaí (05o38'19"S,69o10'59"W)
MPEG	x	x	CUJ 125	A	Brasil, Amazonas, Jutaí, RDS Cujubim, margem E Rio Jutaí (04o39'14"S,68o19'38"W)
MPEG	x	x	CUJ 131	A	RDS Cujubim, margem E Rio Jutaí (04o39'14"S,68o19'38"W)
MPEG	x		JUT 018	A	Jutaí, RESEX do Rio Jutaí, Comunidade São João do Acurau (03°16'45,1"S;67°19'01,3"W)
MPEG	x		JUT 071	A	Jutaí, RESEX do Rio Jutaí, Comunidade São João do Acurau (03°16'16,1"S;67°19'30,6"W)
MPEG	x		JUT 203	A	Jutaí, ESEC Jutaí/Solimões, Capivara (03°10'32,8"S;67°23'28,5"W)
MPEG	x		JUT 280	A	Jutaí, ESEC Jutaí/Solimões, Pati (03°13'24"S;67°26'30"W)
LSUMZ	x		LSUMZ 4705	A	Peru,Loreto Department,S Rio Amazonas, ca 10 km SSW mouth Rio Napo on E. bank Quebrada Vainilla
MPEG		x	UFAC 0758	A	Senador Guiomard, Br 364 km 25, Fazenda Experimental Catuaba (10°04'S;67°37'W)
MPEG	x	x	UFAC 0978	A	Porto Walter, Igarapé Cruzeiro do Vale, Colônia Dois Portos (08o20'35,7"S72o36'19,7"W)
MPEG	x	x	UFAC 0991	A	Porto Walter, Igarapé Cruzeiro do Vale, Colônia Dois Portos (08o20'35,7"S72o36'19,7"W)
MPEG		x	UFAC 1001	A	Porto Walter, Igarapé Cruzeiro do Vale, Colônia Dois Portos (08o20'35,7"S72o36'19,7"W)
MPEG		x	UFAC 1003	A	Porto Walter, Igarapé Cruzeiro do Vale, Colônia Dois Portos (08o20'35,7"S72o36'19,7"W)
MPEG		x	UFAC 1043	A	Porto Walter, Igarapé Cruzeiro do Vale, Colônia Dois Portos (08o20'35,7"S72o36'19,7"W)
MPEG		x	UFAC 1047	A	Porto Walter, Igarapé Cruzeiro do Vale, Colônia Dois Portos (08o20'35,7"S72o36'19,7"W)
MPEG	x	x	UFAC 1121	A	Mâncio Lima, Estrada do Barão, Comunidade S. Domingos (07o33'10,0S;72o59'00,6"W)
MPEG	x	x	UFAC 1141	A	Mâncio Lima, Estrada do Barão, Comunidade S. Domingos (07o33'10,0S;72o59'00,6"W)
MPEG		x	UFAC 1171	A	Mâncio Lima, Estrada do Barão, Comunidade São Domingos (07°33'10"S;72°59'00,6"W)
INPA	x		A 4792	B	Margem esquerda do Rio Purus; Flona Purus; Rio Inauini; "Colônia Vista Alegre"; ca 45 km NW Boca do Acre

INPA	x		A 7960	B	Margem esquerda do Rio Purus; Reserva Biológica do Abufari; "Turiaçu"; ca 73 km NW de Tapauá
MPEG	x	x	PUC 170	B	Tefé, Base Petrobras/Urucu, Papagaio (04o 51'S, 65o 04'W)
MPEG	x	x	PUC 223	B	Tefé, Base Petrobras/Urucu, Papagaio (04o 51'S, 65o 04'W)
MPEG	x	x	RUR 087	B	Brasil, Amazonas, Coari, Rio Urucu, Estrada do NIT (04o56'05"S;65o17'29,5"W)
INPA	x		A 425	C	Comunidade Bela Vista, Lago Xadá, margem esquerda do Rio Madeira, 40 km a 250° (SWS) de Novo Aripuanã
INPA	x		A 9179	C	Margem direita do Rio Purus; "Igarapé do Jacinto"; ca 10 km S de Tapauá
INPA	x		A447	C	Brasil, Amazonas, 40 km a 250° (SWS) de Novo Aripuanã, Comunidade Bela Vista, Lago Xadá, margem esquerda do Rio Madeira
LSUMZ	x		LSUMZ 9442	C	Bolivia,Pando Department,Nicolás Suarez; 12 km by road S of Cobija, 8 km W on road to Mucden
MPEG	x	x	OM 037	C	Brasil, Amazonas, Humaitá, margem esquerda Rio Madeira, Ipixuna (07o31'06"S;63o20'16"W)
MPEG	x	x	OM 038	C	Humaitá, margem esquerda Rio Madeira, Ipixuna (07o31'06"S;63o20'16"W)
MPEG		x	OM 039	C	Humaitá, margem esquerda Rio Madeira, Ipixuna (07o31'06"S;63o20'16"W)
MPEG		x	UFAC 0769	C	Senador Guiomard, Br 364 km 25, Fazenda Experimental Catuaba (10°04'S;67°37'W)
MPEG		x	UFAC 0963	C	Porto Walter, Igarapé Cruzeiro do Vale, Colônia Dois Portos (08o20'35,7"S72o36'19,7"W)
MPEG		x	30997	D	Apuí, margem direita Rio Aripuanã, foz Rio Guariba, Vista Alegre (07°38'S;60°26'W)
MPEG		x	30998	D	Apuí, margem direita Rio Aripuanã, foz Rio Guariba, Vista Alegre (07°38'S;60°26'W)
MPEG		x	30999	D	Apuí, margem direita Rio Aripuanã, foz Rio Guariba, Vista Alegre (07°38'S;60°26'W)
MPEG		x	31000	D	Apuí, margem direita Rio Aripuanã, foz Rio Guariba, Vista Alegre (07°38'S;60°26'W)
MPEG		x	31001	D	Apuí, margem direita Rio Aripuanã, foz Rio Guariba, Vista Alegre (07°38'S;60°26'W)
INPA	x		A 9782	D	Itaituba; margem esquerda do Rio Tapajós; A-P2
MPEG	x	x	AMANÃ 006	D	Brasil, Pará, Itaituba, FLONA Amanã, margem direita Igarapé Montanha (4o52'58,7"S;56o58'10,1"W)
MPEG		x	AMANÃ 074	D	Itaituba, FLONA Amanã, margem direita Rio Amanã, Pista de Pouso São Pedro (4o46'41,1"S;57o28'24,7"W)
MPEG		x	AMANA 112	D	Itaituba, FLONA Amaná, margem direita Igarapé Porquinho (5o06'35,7'S; 57O32'14,9"w)
MPEG	x	x	AMANA 119	D	Itaituba, FLONA Amaná, Maranhense (5o21'08,7"S;57o28'30"W)
MPEG	x	x	AMANA 145	D	Jacareacanga, Transamazônica, ponte sobre o Rio Igarapé Preto (5o54'12,6"S;57o41'30,1"W)
MPEG	x		JAT(A) 038	D	Jacareacanga, margem esquerda Rio Tapajós, Vila Mamãe-anã (05o45'31,5"S;57o24'45,8"W)
MPEG		x	JAT(A) 047	D	Jacareacanga, margem esquerda Rio Tapajós, Vila Mamãe-anã (05o45'31,5"S;57o24'45,8"W)
MPEG	x	x	JAT(A) 193	D	Penedo, margem esquerda Rio Tapajós (05o27'21,6"S;57o04'12"W)
MPEG		x	JAT(A) 206	D	Jacareacanga, Vila São Martins, margem esquerda Tapajós (06o06'14,88"S;57o39'23,75"W)
MPEG		x	JAT(A) 210	D	Jacareacanga, Vila São Martins, margem esquerda Tapajós (06o06'14,88"S;57o39'23,75"W)

MPEG	x	x	JAT(A) 235	D	Jacareacanga, Vila São Martins, margem esquerda Tapajós (06o06'14,88"S;57o39'23,75"W)
MPEG	x	x	JAT(A) 352	D	Jacareacanga, Vila Mamãe anã, margem esquerda Tapajós (05o45'31,5"S;57o24'45,85"W)
MPEG	x	x	JAT(C) 016	D	Jacareacanga, margem esquerda Rio Tapajós, Comunidade São Martim (06o06'22,4"S;57o39'19,92"W)
MPEG	x	x	PIME 143	D	Jacareacanga, Terra do Burandir (06.24685oS;057.87498oW)
INPA	x		A 318	E	Brasil, Rondônia, ca 10 km sudoeste de Porto Velho, margem direita do Rio Madeira
LSUMZ	x		LSUMZ 36643	E	Brazil,Rondônia,Reserva Biologica Rebio Ouro Preto, ca 70 km E Guajara'-Mirim
LSUMZ	x		LSUMZ 36753	E	Brazil,Rondônia,Reserva Biologica Rebid Duro Preto, ca 70 km E Guajara'-Miram
MPEG	x	x	OM 249	E	Machadinho D'Oeste, margem direita Rio Jiparaná (08o54'32,9"S;62º10'0,03"W)
MPEG	x	x	OM 250	E	Machadinho D'Oeste, margem direita Rio Jiparaná (08o54'32,9"S;62º10'0,03"W)
MPEG	x		OP 018	E	Rio Ouro Preto, margem esquerda, Guajará-Mirim, Reserva Biológica Ouro Preto (10o50'S, 64o45'W)
MPEG	x		OP 128	E	Rio Ouro Preto, margem esquerda, Guajará-Mirim, Reserva Biológica Ouro Preto (10o50'S, 64o45'W)
INPA	x		A 557	F	Brasil, Amazonas, Margem esquerda do Rio Aripuanã, Rio Arauazinho, Igarapé Três Jacus, 127 km S Novo Aripuanã
INPA	x		A558	F	Brasil, Amazonas, 127 km S Novo Aripuanã Margem esquerda do Rio Aripuanã, Rio Arauazinho, Igarapé Três Jacus
MPEG	x	x	MAR 123	F	Manicoré, Rodovia do Estanho, km 137 (08o41'44,4"S, 61o24'16,6"W)
MPEG	x	x	MAR 132	F	Brasil, Amazonas, Manicoré, Rodovia do Estanho, km 137 (08o41'44,4"S, 61o24'16,6"W)
MPEG		x	MAR 285	F	Manicoré, Rodovia do Estanho, Fazenda Copeares (08o28'20,8"S, 61o23'35,7"W)
MPEG	x	x	MPDS 464	F	Brasil, Rondônia, Jiparaná, Igarapé Lurdes, Aldeia Gaviões (10o26'S, 61o39"W)
MPEG	x	x	MPDS 513	F	Município de Jiparaná, Igarapé Lurdes, Aldeia Gaviões (10o26'S, 61o39"W)
MPEG	x	x	MPDS 572	F	Município de Humaitá, T. Indígena Parintintin, Aldeia Pupunha (07o28'S, 62o56'W)
MPEG		x	MPDS 711	F	Município de Humaitá, T. Indígena Parintintin, Aldeia Traíra-Bacupaí (07o33'S, 62o34'W)
MPEG	x	x	MPDS 755	F	Brasil, Amazonas, Humaitá, Território Indígena Ipixuna, Aldeia Canavial, Miriti (06o33'S, 62o03'W)
MPEG		x	MPDS 756	F	Humaitá, Território Indígena Ipixuna, Aldeia Canavial, Miriti (06°33'S;62°03'W)
MPEG	x	x	MPDS747	F	Brasil, Amazonas, Humaitá, Território Indígena Ipixuna, Aldeia Canavial, Miriti (06o33'S, 62o03'W)

Appendix 2 - Geographic distribution of the six clusters (*K* = 6) and individual's admixture proportion as revealed by Bayesian Analysis of Population Structure (BAPS). Bar graph representing the population structure analysis obtained for *P. philippii* with the concatenated mitochondrial database. The colors represent the populations recovered by the phylogenetic analyses. The same bars are plotted on the map in pie chart format, according to the location of each individual.