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VARIAÇÃO GEOGRÁFICA EM COLORAÇÃO E MORFOMETRIA EM TRÊS ESPÉCIES DE AVES AO LONGO DO INTERFLÚVIO PURUS MADEIRA, AMAZÔNIA OCIDENTAL

FERNANDO HENRIQUE TEÓFILO DE ABREU

Manaus, Amazonas
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Dissertação apresentada ao Instituto Nacional de Pesquisas da Amazônia como parte dos requisitos para obtenção do título de Mestre em Biologia (Ecologia).

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Sinopse: Descrevemos a diversidade fenotípica em coloração e morfometria em três espécies de aves do interflúvio Purus-Madeira, Amazônia Ocidental. Ainda, testamos a associação entre variações no ambiente e variações no fenótipo de aves, sugestivas de processos adaptativos por seleção natural/sexual e também o efeito da distância geográfica na diferenciação dos fenótipos, facilitando o isolamento entre linhagens e, assim, a deriva fenotípica entre as mesmas, ao acaso.

Palavras-chave: Fenótipo, Evolução, BR-319, Pipridae, Thamnophilidae.

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"Nada em biologia faz sentido senão sob a luz da evolução”

Theodosius Dobzhansky

RESUMO

Caracteres de cor e morfometria em muitos grupos de organismos variam com a localização geográfica de suas populações, resultando em variabilidade fenotípica intraespecífica. Embora haja muitos casos já conhecidos de variação geográfica intraespecífica, sobretudo em caracteres acústicos em aves amazônicas, há ainda poucos estudos sobre o tema e ainda muito se especula sobre os fatores determinantes da mesma. Partindo disso, testamos se há variação em cor e morfometria em três espécies de aves amazônicas: *Gymnopithyssalvini, Willisornis poecilinotus* (Thamnophilidae) e *Lepidothrix coronata* (Pipridae) ao longo do interflúvio Purus-Madeira. Em seguida, testamos se mecanismos adaptativos ou estocásticos estão associados à organização dos padrões fenotípicos dessas espécies no interflúvio. Para tal, tiramos medidas colorimétricas da plumagem e morfométricas de machos e fêmeas de 11 localidades ao longo da BR-319. Inserimos, então, as medidas das variáveis colorimétricas e das variáveis morfométricas em duas análises distintas de componentes principais (PCA), e utilizamos modelos lineares generalizados (GLMs) para correlacionar os valores dos escores da PCA de caracteres de cor e morfometria com a variação ambiental (composição de espécies de aves e área basal de árvores) e geográfica (localização geográfica em latitude) ao longo do interflúvio. Em todas as três espécies estudadas, encontramos variação em cor, em brilho e em morfometria entre as localidades amostradas, sendo que em machos da espécie *L. coronata* a variação em cor apresentou a forma de clina. Somente fêmeas de *G. salvini* e de *W. poecilinotus* apresentaram correlação entre a variação em cor e a variação em morfometria entre as localidades estudadas, sugerindo que, de um modo geral, a cor é provavelmente mais lábil que a morfometria e que a expressão destes dois tipos de caracteres é independente e, aparentemente, sujeitas a diferentes mecanismos geradores de variação. Ainda, nas três espécies estudadas mecanismos adaptativos e estocásticos estiveram associados com a evolução dos traços fenotípicos estudados reforçando os indícios de que a ambos processos não são excludentes e podem atuar juntos sobre traços do fenótipo.

Palavras-chave: Fenótipo, Evolução, BR-319, Pipridae, Thamnophilidae.

ABSTRACT

Geographic variation in coloration and morphometry in three species of birds along the Purus-Madeira interfluve, Western Amazon

Characters of color and morphometry in many groups of organisms vary with the geographic location of its population, resulting in intraspecific phenotypic variability. Although there are many known cases of intraspecific geographic variation, especially in acoustic characters in amazonian birds, there are few studies on the subject and much is speculated about the determinants of same. Starting addition, we tested whether there is variation in color and morphometry in three species of Amazonian birds: *Gymnopithys salvini*, *Willisornis poecilinotus* (Thamnophilidae) and *Lepidothrix coronata* (Pipridae) along the Purus-Madeira interfluve. We then test whether adaptive or stochastic mechanisms are associated with the organization of phenotypic patterns of these species in the interfluve. To this end, we took colorimetric and morphometric measures of plumage of males and females from 11 localities along the BR-319. Then insert measures the colorimetric variables and morphometric variables in two distinct principal component analysis (PCA), and we used generalized linear models (GLMs) to correlate the scores of the PCA of characters of color and morphology to environmental variation and geographic over interfluvial. In all three species studied, we found variation in color, brightness and morphometry between sampling localities, and in males of the species *L. coronata* variation presented in the form of color cline. Only females of *G. salvini* and *W. poecilinotus* had correlated between the variation in color and variation in morphometry between the locations studied, suggesting that, in general, the color is likely to be more labile than the morphometry and expression of these two types of characters is independent apparently different generating mechanisms are subject to variation. Still, the three species studied adaptive and stochastic mechanisms were associated with the evolution of phenotypic traits studied reinforcing the evidence that both processes are not mutually exclusive and may act together on the phenotype traits.

Keywords: Phenotype, Evolution, BR-319, Pipridae, Thamnophilidae.

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INTRODUÇÃO

O termo fenótipo foi proposto pela primeira vez em 1911 e utilizado em uma tentativa de diferenciar o genótipo do seu resultado produzido (Johannsen, 1911). Mais tarde essa palavra ganhou utilização mais ampla sendo definida como não somente traços facilmente observáveis, mas sim todos os caracteres de um organismo determinados pela interação do genótipo com o ambiente. Dessa forma, além de cor e morfometria, também traços acústicos, comportamentos aprendidos, produtos bioquímicos e fisiológicos, como o grupo sanguíneo, são considerados como parte importante do fenótipo de um indivíduo (Guenther e Trillmich, 2013).

O fenótipo pode transmitir diversas informações sobre os organismos tais como idade (Val*et. al.*, 2010; D'horta *et al.*, 2012), sexo (Edelaar, 2005), capacidade de luta (Midamegbeetal., 2011) e/ou adaptações específicas de um organismo à sobrevivência no meio em que vive, como camuflagem (Chittka, 2001), mimetismo (Sazima, 2010) e aposematismo (Solano, 2012). No entanto, mesmo entre indivíduos de uma mesma espécie é possível encontrar variação fenotípica (Hill e McGraw, 2006). Essa variação pode se dar em uma mesma localidade (i.e. variação intrapopulacional) (Dale, 2000), entre localidades (i.e. variação interpopulacional) ou variação geográfica ao longo da distribuição de uma espécie - às vezes vezes, esta última surgindo gradualmente em forma de clina (Endler, 1977; Isler *et al.*, 2005).

Os padrões espaciais de variação fenotípica em nível populacional podem surgir por meio de dois principais fenômenos evolutivos: mecanismos adaptativos e/ou estocásticos (Hill &McGraw, 2006). Ambos processos não são excludentes e podem atuar juntos sobre traços do fenótipo (Wang *et al.*, 2013). No primeiro caso, a variação observada nos caracteres é reflexo do grau de variação ambiental experimentada por diferentes populações de uma mesma espécie entre localidades e tem valor adaptativo (e.g. Amezquita *et al.*, 2009). Tal como postula a Teoria dos Gradientes Ambientais, diferenças ambientais bióticas ou abióticas podem ser suficientes para levar a divergência entre populações, podendo levar a especiação por parapatria (Endler, 1977). Nesse caso, a heterogeneidade ambiental é um importante fator que atua como gerador de diversidade fenotípica (Calsbeek *et al.*, 2007; Price, 2008).

De fato, variações ambientais são importantes geradores de pressões adaptativas nos organismos, sejam estas seletivas sobre seus genótipos (Diéguez *et al.*, 1998) ou não, como no caso de polimorfismos com plasticidade fenotípica (West-Eberhard, 1989; Nijhout, 2003). Alguns fenótipos podem refletir a ecologia das espécies determinando, além do uso diferenciado do habitat, diferentes estratégias de exploração de recursos disponíveis no meio (MacArthur e

Pianka, 1966; Levin, 1992). Partindo de uma escala macro-evolutiva para uma micro-evolutiva, é possível que a própria composição de espécies de uma comunidade afete as pressões adaptativas sobre as populações e com isso seus fenótipos (Hoagstrom e Berry, 2008). Isso porque à medida que a composição de espécies competidoras varia, nichos ocupados por determinadas espécies também podem mudar e, com isso, variações fenotípicas podem ocorrer em resposta à tentativa de evitar possíveis competidores (Goldberg e Lande, 2006). Da mesma forma, espécies podem ampliar sua área de forrageamento na ausência de competidores e predadores, sofrendo assim, mudanças nos traços fenotípicos para se adaptar ao novo ambiente biótico disponível (Endler, 1977; Grant e Grant, 1993, 1995, 2003). Deste modo, populações que experimentam condições ambientais distintas podem sofrer pressões adaptativas de diferentes intensidades e levar à variação geográfica por processos evolutivos (por seleção) ou não (plasticidade).

Caracteres fenotípicos importantes para seleção sexual, por sua vez, podem ter efeito contrário ao esperado por adaptação ao ambiente, diminuindo as chances de sobrevivência dos organismos, já que a grande maioria deles gera pressão para uma maior conspicuidade, aumentando o alvo para predação (Andersson 1994; Montgomerie *et al.*, 2001; Ridley, 2006). A evolução de caracteres sexuais (Taylor e McGraw, 2007) pode, no entanto, ocorrer por mecanismos adaptativos ou aleatórios, levando a diferenciações entre populações (Anderson 1994; Ridley, 2006). Desse modo, populações que experimentam condições ambientais similares podem, ainda assim, apresentar variação intra-específica em determinados caracteres resultantes de diferentes recombinações gênicas que derivam para uma frequência maior de determinados alelos em diferentes localidades, ao acaso (Ridley, 2006).

Na Amazônia, muito ainda se especula sobre os fatores determinantes da variação geográfica intraespecífica (Wang e Summers, 2010). O que mais se conhece, de fato, são casos de espécies crípticas, em que fenótipos se mantêm apesar das diferenças genéticas entre linhagens, possivelmente em resposta a pressões seletivas similares sob influência da seleção estabilizadora (O'neill *et al.,* 2011). Apesar de ser bastante comum estudar os processos que agem na Amazônia como um todo, poucos estudos avaliaram os processos determinantes da diversificação biológica com amostragens intensas dentro de um interflúvio. Estudos com essas características adicionarão informações de cunho ecológico e evolutivo para uma região ainda pouco estudada.

Considerando tal problemática, escolhemos três espécies de passeriformes amazônicos como objetos de estudo deste trabalho: *Gymnopthys salvini*, *Willisornis poecilinotus* e *Lepidothrix coronata*. Os táxons estudados se mostram ótimos candidatos para responderem as perguntas em questão por ocorrerem ao longo de todo o transecto estudado e serem objeto de estudos filogeográficos em andamento permitindo comparação subsequente entre dados genéticos e fenotípicos. Partindo disso, testamos o efeito da variação ambiental e da distância geográfica entre localidades na variação fenotípica intraespecífica em cor e morfometria destes táxons ao longo do interflúvio Purus-Madeira, a fim de compreender o papel de mecanismos adaptativos e estocásticos na diferenciação fenotípica. Especificamente testamos as seguintes hipóteses para cada espécie: (1) A variação fenotípica intraespecífica é explicada por variação ambiental, mas não pela distância geográfica entre localidades e, portanto, a variação encontrada seria resultado de adaptação local; (2) Tal variação fenotípica é explicada pela distância geográfica entre localidades, mas não pela variação ambiental e, nesse caso, a variação fenotípica observada entre populações seria resultado de mecanismos estocásticos (deriva) e (3) Ambas, distância geográfica e variação ambiental influenciam a diversificação fenotípica intraespecífica e, assim, esperamos que processos seletivos e estocásticos estejam envolvidos na organização dos padrões fenotípicos destas espécies. Caso não haja variação fenotípica então os fenótipos estudados seriam conservados, e.g. por seleção estabilizadora ou por falta de tempo para divergirem pelos processos mencionados acima.

OBJETIVOS

Objetivo Geral

Investigar a variação fenotípica (cor e morfometria) em três espécies de aves em um transecto latitudinal pouco conhecido da Amazônia ocidental: o interflúvio Purus-Madeira.

Objetivos Específicos

Testar as hipóteses de que:

1) Há variação fenotípica de morfometria e coloração em três espécies de aves ao longo do interflúvio Purus-Madeira, Amazônia Ocidental;

2) A variação fenotípica é explicada por variação ambiental e/ou distância geográfica entre pontos amostrais ao longo do interflúvio Purus-Madeira

CAPÍTULO 1

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Geographic variation in coloration and morphometry in three species of birds along the Purus-Madeira Interfluve, Western Amazon

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ABSTRACT

In many groups of organisms color and morphometry vary with the geographic location of the constituent populations, resulting in intra-specific phenotypic variability. Although there are many known cases of this, especially in acoustic characters in Amazonian birds, there are few studies on the subject, though there is much speculation about the determining factors involved. We first examined whether there is variation in color and morphology between populations of three species of Amazonian birds: *Gymnopithys salvini, Willisornis poecilinotus* (Thamnophilidae) and *Lepidothrix coronata* (Pipridae) along the Purus-Madeira interfluves, then tested whether adaptive or stochastic mechanisms were associated with the phenotypic patterns observed. To this end, we took colorimetric and morphometric measures of plumage of males and females from 11 localities along the BR-319 highway. We then conducted two distinct principal component analysis (PCA) on the colorimetric and morphometric variables, and used generalized linear models (GLMs) to correlate PCA character scores for color and morphology with environmental variation and geographic location within the interfluvium. In all three species studied, we found variation between sampling localities in color, brightness and in morphometry, although in *L. coronata* males color variation occurred in the form of a cline. Only *G. salvini* and *W. poecilinotus* females showed correlation between variation in color and variation in morphology between the study locations, suggesting that the color is likely to be more labile than morphology, that expression of these two types of characters is independent and, apparently, subject to different variation generating mechanisms. In the three species studied, adaptive and stochastic mechanisms were associated with the evolution of the phenotypic traits studied reinforcing the evidence that these processes are not mutually exclusive and may act together on the phenotype traits.

Keywords: Phenotype, Evolution, BR-319, Pipridae and Thamnophilidae.

INTRODUCTION

The term phenotype was first proposed in 1911, and used in an attempt to differentiate the genotype from the physical result it produced (Johannsen 1911). Later, the word gained wider use, being used to define as not only as easily observable traits, but also all the characters of an organism that were determined by the interactions between its genotype and the environment. Hence, in addition to color and morphometry, acoustic traits, innate or learned behaviors, and such physiological and biochemical products as blood group, are also considered important parts of the phenotype of an individual (Guenther and Trillmich 2013).

The phenotype can transmit varied information about an organism, including age (Val et al 2010; D'horta et al 2012), sex (Edelaar 2005), fighting ability (Midamegbe et al 2011) and/or specific adaptations of an organism to survive in the environment they inhabit, such as camouflage (Thery et al 2004), mimicry (Sazima 2010) and aposematic coloration (Solano 2012). However, phenotypic variation is commonly found between individuals of the same species (Hill and McGraw 2006). This variation can occur in the same locality (i.e. intra-population variation), or between localities (inter-population variation, geographic variation) within the distribution of a species (Dale 2000). Sometimes, the latter takes the form of cline (Endler 1977; Isler et al 2005).

At the population level, spatial phenotypic variation can arise through two main evolutionary phenomena: adaptive and stochastic mechanisms (McGraw and Hill 2006). Such processes are not mutually exclusive and may act together on phenotypic traits (Wang et al 2012). In the first instance, the variation observed in characters generally reflects the degree of environmental variation experienced by different populations of the same species at different sites (Amezquita et al 2009). As postulated by the Theory of Environmental Gradients (Endler 1977), biotic or abiotic environmental differences may be sufficient to lead to divergence among populations, and may lead subsequently to speciation by parapatry. Under such circumstances, environmental heterogeneity may be an important factor in generating of phenotypic diversity (Calsbeek et al 2007).

Environmental variation is an important generator of adaptive pressures on organisms. It may act selectively on genotypes (Dieguez et al 1998), though this is often reduced if the polymorphisms in question have strong phenotypic plasticity (West-Eberhard 1989; Nijhout 2003). In general, phenotypes reflect the ecology of the species involved, in terms of the different habitat used and the different strategies deployed to exploit available resources (Levin 1992; MacArthur and Pianka 1966). Along a gradient of macro- to micro-evolutionary scales, it is possible that the *assemblage* species composition to some extent shapes the adaptive pressures experienced by a given population of a particular species, and thus their phenotypes (Hoagstrom and Berry 2008). This is because as the composition of competing species varies, niches occupied by certain species may change, and thus phenotypic variation may occur as a a result of local selection to reduce resource competition (Goldberg and Lande 2006). Similarly, a species may extend its foraging area in the absence of competitors, and changes in phenotypic traits may occur as the species overall adapts to newly available biotic environments (Endler 1977; Grant and Grant 1993, 1995, 2003). Thus, populations that experience different environmental conditions can undergo adaptive pressures of dissimilar intensities, and those may lead to geographic variation by adaptive processes of an evolutionary nature (by selection) or not (plasticity).

Phenotypic characters operating under sexual selection may have appearances that do not seem well-adapted to the environment, and appear to be reducing the survival chances of the organisms possessing them, since the vast majority of such characters increase conspicuousness and hence the chance of successful predation (Andersson 1994; Montgomerie et al 2001; Ridley 2006). The development of sexual characteristics (McGraw and Taylor 2007) can, however, occur by random or adaptive mechanisms, leading to differences between populations (Ridley 2006). For example, populations that experience similar environmental conditions may still have intra-specific variation in certain characters as a result of different genetic recombinations that produce a higher frequency of certain alleles in different locations, at random, by sexual selection (Ridley 2006).

Intra-specific geographic variation is still a relatively unexplored subject and much speculation has occurred about the determinants of such variation (Wang and Summers 2010). Indeed, are best known are cryptic species, in which phenotypes remain very similar despite often pronounced genetic differences; such situations may possibly arise in response to similar selective pressures under the influence of stabilizing selection (David et al 2008; O'Neill et al 2011).

In the Amazon, patterns of biological diversity appear linked to distribution of the region's extensive interfluves. Among the principle hypotheses to explain this are those based on adaptive and/ or stochastic mechanisms (Haffer 1969; Endler 1977; Ribas et al 2012). Though it has been fairly common to study the processes that act on the Amazon as a whole i.e. *between* or *across* interfluves, few studies have evaluated the processes active in biological diversification *within* an interfluve. Studies with these characteristics add information of an ecological and evolutionary nature to a region which, considering the extent of its biological diversity and the variety of evolutionary forces at play, is still comparatively little studied. In this context, we chose three Amazonian passerines as study species: *Gymnopithys salvini* (Thamnophilidae), *Willisornis poecilinotus* (Thamnophilidae), and *Lepidothrix coronata* (Pipridae). The taxa studied show are appropriate candidates to answer the questions at of the study since they (i) occur throughout the studied transect, (ii) are the subject of ongoing phylogeographic studies (so permitting retrospective comparison of genetic and phenotypic data). We (i) estimated patterns of intra-specific phenotypic variation in color and morphology for these taxa within the Purus-Madeira interfluves, and (ii) tested the effect of environmental variation and geographical distance between locations in order to, (iii) understand the role of adaptive and stochastic mechanisms in phenotypic differentiation.

Specifically, we tested the following hypotheses for each species: (1) intraspecific phenotypic variation is explained by environmental variation, but not the geographical distance between locations and therefore any observed phenotypic variation results from local adaptation through habitat filters or niche partitioning; (2) Such phenotypic variation is explained by the geographical distance between localities, but not by environmental variation and, in such cases, observed phenotypic variation between populations results from stochastic mechanisms (drift); (3) Both geographical distance and environmental variation influence intra-specific phenotypic diversification, and so selective and stochastic processes are both involved in structuring phenotypic patterns observed in the study species. If there is no phenotypic variation then the phenotypes studied would be preserved by stabilizing selection, or be the result of a lack of time for divergence to occur by the processes mentioned above.

MATERIALS AND METHODS

Field of study

The study was conducted along a 670 km stretch of the middle and lower regions of the interfluve formed by the Purus and Madeira rivers, central Brazilian Amazonia. The interfluve had previously been analyzed for 21 environmental variables relating to hydrology, landform, vegetation and soil, generating a map with identified 13 different ecoregions (Ximenes 2008). Additionally, other environmental data have been analyzed and described locally to the region, including floristic composition for trees and palms (Emilio 2007) and bird assemblage composition (Menger 2011). Biologically, the interfluve is highly heterogeneous, and this, combined with an absence of spatial autocorrelation (Menger 2011), makes the region very suitable for testing hypotheses regarding the importance of environmental and/or geographical factors in the generation of phenotypic diversity.

The avifauna of the interfluve is part of the Inambari area of endemism (Cracraft 1985), and shows similarity in composition of endemic bird species to the avifauna of the Napo region (Borges 2007). Very little is known about the biology of this region and distributional and taxonomic uncertainties remain for a variety of taxa (Haffer 1992, van Roosmalen et al 2002*)*. However, there have been a number of important recent studies, including that of Menger (2011) which found that birds vary in association with the environment along the interfluves. The study region has high rainfall (2500 mm per year in the central part, 1500 mm in the southern part and 2000 mm in the north), with average annual temperatures of 27°C. The vegetation is a mosaic of terra firme, campina and cerrado, and areas susceptible to flooding. In the center of the interfluve is an 877 km section of the BR-319 (Manaus – Porto Velho) highway running between the municipalities of Careiro de Várzea and Humaitá, near the border with the state of Rondônia (Figure 1). The region represents one well-preserved area of forests, although the area suffers from some major anthropogenic pressure and some stretches of the BR 319 are already occupied, mainly to the north and south. The recent announcement of a project for road construction and paving by the Federal Government also gives cause or grave concern as it can facilitate migration from the "Arc of Deforestation" in central Amazonia, so further threatening these forests (Graça and Fearnside 2009).

Sample design

We conducted sampling at sites using existing infrastructure that PELD (Long Term Ecological Research Project) had installed along the BR 319, as part of the Research Program on Biodiversity - PPBio (http://www.ppbio.inpa.gov.br), created by the Ministry of Science and Technology (MCT). Each PELD site has a set of standardized plots and trails that allow the data collected to be comparable between sites and to be compared to a standardized set of environmental variables measured at each site. Data were collected in 11 modules (study sites), distributed perpendicularly to the BR-319 highway.

Each PELD site constituted a sampling unit. PELD sites have an average distance of 60 km from each other, providing a total extension of 670 km. Each PELD module consists of two parallel transects 5 km in length, separated by 1 km and connected by trails marked from 0 to 5000 m. Each 5 km transect contains six parcels of land spaced 1km from each other. Each sampling plot consists of a center line 250 m in length, which follows the curve of the terrain to minimize environmental variation within the plot.

Data collection

Data on color and morphometry was taken from specimens captured in the field during October and November 2012 and July and October 2013. At each PELD site 10 mist nets (12 x 2.5 m) were set up in four randomly selected plots (total 40 nets per module, open between 6am and noon, for two sequential days. Each individual bird captured was identified to species, sexed, weighed, morphometric measures of interest taken and marked with standard CEMAVE metal rings (in cases where individuals were released). For the three focal species, we collected the first five individuals of each sex, which were deposited as vouchers in the bird collection at the Instituto Nacional de Pesquisas da Amazonia (INPA). When captures at a study site exceeded five individuals a screening procedure was performed and we removed feathers from each region of the body for which coloration metrics were to be recorded (see details below). Birds were then ringed and released.

To provide outgroups for this study, we included phenotypic data of specimens from other interfluves in the phenotypic analyzes (Ipixuna for *G. salvini,* Xingu for *W. poecilinotus* and Japurá for *L. coronata*) with the aim of comparing the data of the phenotype for these focal species studied in the Purus-Madeira clades with other, geographically more distant, areas of endemism.

Color analysis

We obtained color spectra from feathers from current surveys (collected specimens or from feathers of released individuals) or from skins in the bird collections at INPA and MPEG Museu Paraensis Emilio Goeldi, Pará, Belém from previous collections in the Purus-Madeira interfluve and the three additional areas. To quantify color metrics we used a USB-2000 UV-VIS spectrophotometer connected to a fiber-optic (R400-2 UV-VIS), and a PX2 pulsed Xenon light source. We measured plumage color on nine areas of the body: head, neck, chest, abdomen, back, rump, remiges, greater wing coverts and rectrices. Individuals of the species *G. salvini* and *W. poecilinotus* have white spots on the rectrices (lines and circles, respectively), and so respective measurements were also included for each species. As the primary objectives of this study do not include comparison between species, such inclusions do not invalidate the results. Each measurement recorded was the average of ten individual readings of the same area, using an integration time of 20 milliseconds and a zero *boxcar* correction. For *G. salvini* and *W. poecilinotus,* the analysed spectra was between 300-700nm, corresponding to the visual range commonly associated with birds (Cuthill 2006; Pohland and Mullen 2008). For *L. coronata* we used the region between 400-700 nm, corresponding to the spectrum of maximum visual sensitivity associated with the bird family Pipridae (Odeen and Håstad 2013).

We used SpectraSuite software for the collection of spectral data, and changed the extension from .txt files to .transmission with the Avicol program (Gómez 2006), then grouped the spectra and estimated color metrics using PAVO (Maia *et al* 2013) in the R software package (R Core Team 2006). We estimated the coloration pattern of each bird species for each of the 11 study sites using 9 metrics to describe color estimates corresponding to hue (peak location of the color spectrum) and saturation (or color purity) and metric that describes the estimated color brightness (or color intensity) (Table 1) for all analyzed zones. To minimize the influence of correlations between the multiple metrics, we inserted the colorimetric variables of each plumage area into two groups of distinct PCAs, for each species and sex. The first analysis was intended to reduce multidimensionality of the group of variables related to hue and saturation, while the second analysis included only the variable for absolute brilliance.

As in the first PCA we analyzed metrics for each of the color patches (9 or 10 patches), generating matrices with 81 (for *L. coronata*) or 90 colorimetric variables (for *G. salvini* and *W. poecilinotus*) in total. As such large PCAs can generate many axes, each explaining only a small portion of the observed variation, we applied a scaling procedure to select variables, including an initial first PCA of 81 or 90 variables which selected the 10 variables most associated with the first axes (at least 25% of the explained variation). Then we inserted these ten variables into a second PCA and used the first two axes of greatest variation in color (that explained between 75 and 95% of the variation found in the data) as the dependent variable in the statistical analyzes described below. Spots in the crown of *G. salvini* males were very dark with a percentage of average brightness below 2%.These were therefore were excluded from the analyzes of hue and saturation, and were used only in the analysis of brightness.

Morphometric analyzes

From focal species, we took the following morphometric measures: length of beak, beak height and width, length of wing, tarsus and tail. To compare morphological variation between study sites we estimated the mean value for each variable at each location (see details below). We reduced the multidimensionality of the morphometric variables using PCA, and used the first two axes of greatest variation in variable response in the statistical analyzes described below.

To assess the degree of correlation between color and morphology, we performed cross-correlations between the scores of the first two PCA axes of colorimetric variables with the scores of the first two axes of the PCA of morphometric variables. We did this for each species and both sexes. As the data were normally distributed, we applied a parametric correlation test. We used a Pearson Correlation and the Cruzada correlation method (cross-correlation) (Ranta et al 1998).

Phenotypic variation

Standard deviation was calculated to evaluate the extent of phenotypic variation in color brightness and morphometry among study species. We then constructed graphs of variation for each PC axes representing color (hue, saturation and brightness) and morphometry for each study site in order to compare the variation of the traits between locations. As evaluation criteria, we used non-overlapping 95% confidence intervals to determine whether or not there was significant geographic variation (i.e. whether inter-population variation was greater than intra-population variation).

Explanatory variables

To characterize each of the 11 sample sites along the Purus-Madeira interfluve, we use four environmental variables previously collected by Emilio Thaise (palm tree density, percentage of nitrogen and of carbon in the soil), Juliana Schietti (tree basal area) and Juliana Menger (bird community) in the same transects where the current study collected. The environmental variables tested replace a set of local environmental factors and represent differences present between the sampling sites. The environmental variables used were: 1) density of the ten most abundant palm species in the Madeira-Purus interfluve (used as a proxy for floristic variation among sites); 2) basal area of trees (used as a proxy for the variation in vegetation density across the interfluve); 3) species composition of those parts of the avian species assemblage that would indicate the differences in presence/absence of potential competing species and/or predatory species between study sites and also as an environmental indicator; 4) percentage of Carbon and Nitrogen in the soil. As spatial variable, we used the latitudinal geographic coordinates for each locality. The database used is available in the PPBio data repository (see metadata: http://ppbio.inpa.gov.br). We next reduced the dimensionality of the variables 1 (palm) 3 (avian composition) and 4 (N and C percentages in soil) and tested the correlation between the four environmental variables described above. The variable *basal area of trees* was correlated with the variable *percentage carbon and nitrogen in the soil* ($r = 0.75$, P

<0.01) and also with the variable *density of palm trees* (r = 0.83, P <0, 01). Consequently, we chose to keep only the variables *bird community* and *tree basal area* in subsequent analyzes so as to ensure that variables used were more directly associated with the biology of the study species.

Finally, we tested for the presence of spatial autocorrelation in the study region using a correlation test between the variable *basal area of trees* and latitude since Menger (2011) had already noted the absence of spatial autocorrelation between variables *composition of bird species* and *palm trees* against geographic distance along the interfluve. The basal area of trees variable showed poor correlation with latitude (r = -0.18, P = 0.84). Such a result further strengthens the evidence for the absence of spatial autocorrelation in the study area.

Relationship between phenotypes and the Environment and Geography

We built generalized linear models (GLM) to assess whether the phenotype of the bird species studied varies along the Purus-Madeira interfluve and how this is related to environmental heterogeneity or geographical location of the study sites. Using this we related the first two PCA axes for phenotypic variables (color or morphology) with the scores generated from non-metric multidimensional scaling (NMDS) of the environmental variables and the geographic location of study sites. Altogether, we generated three groups of models: one for color *per se* (hue and saturation), one for brightness and one for morphometry, with models generated for each explanatory variable (environmental and geographical) and all combinations totalling seven models in addition to the null model. Subsequently, for choosing the most parsimonious variables (i.e. those that "best" explain the phenotypic variation [color and morphology]), we adopted the criterion of selection by the AIC stepwise algorism (R function "step"). We also use the BiodiversityR package (R function "deviancepercentage") that indicates how much of the data variation is explained by the tested model.

RESULTS

Here we first present the results of analyzes designed to reduce the dimensionality of the phenotypic variables, and then the results of the tested hypotheses themselves, starting with the assessment of phenotypic variation and then the hypotheses concerning the influence of environment and geography on the phenotype of the studied species. Table 2 summarizes the data obtained in the field.

Plumage coloration

For the three species studied, saturation was the color property with the highest variation both between sites (i.e. modules) and between individuals. Of the nine metrics of hue and saturation for 9 or 10 body areas analyzed (a matrix with some 90 variables), only around one-tenth exceeded the extent of variation found in the Principal Components Analysis. All that did related to saturation. The colorimetric metrics most important for the formation of the first two axes of the principal component analysis differed between species and sexes. In males of *G. salvini*, saturation metrics in the red, ultraviolet (UV) and violet spectral regions (S1 variable, Table 1) were the most important factors in the formation of the first two ordination axes, with the percentage axis change being 62% and 13% respectively. Also, most of the within-data variation occurred in the tail, chest, back, neck, rump, remiges and belly areas (70% of the variation) of male *G. salvini*. Also in males of this species, the throat both showed greatest plumage brightness (Bmed = 25.2%) and greatest variation (± SD = 10.8), while the crown was least bright region (Bmed = 2.3 %), and contributed least to the total variance in brightness observed along the interfluve for this species (SD = ± 0.8). In *G. salvini* females, the variables that best accounted for the variation in color saturation were metrics for the blue, violet, UV, yellow and red spectral regions (variable S1, Table 1), but only from the tail and tail bars (20% of the measured body areas). Other metrics associated with the first color PC in the species *per se* were variance in reflectance in the part of the spectral region where colors derived from carotenoid pigments show highest reflectivity (S4 variable table 1), and amplitude-based contrast in differences between highest and lowest reflectance peaks (S5 variable table 1). In addition, the first two PCA axes explained, respectively, 55% and 22% of the variation in brightness data. The belly was the region of brightest plumage in *G. salvini* females (Bmed = 7.4%), and also contributed most to brightness variation (SD = ± 3.1), while the crown was the region with lowest brightness (Bmed = 1.8%), and the lowest contribution to the brightness variation between *G. salvini* females (SD = ± 0.86).

In *W. poecilinotus* males, saturation metrics in the ultraviolet, violet and green (S1 variable, Table 1) were the most important in the formation of the first two PCA axes for color *per se*. The first two PCA axes explained, respectively, 81% and 9% of the data variance in males, and 78% and 13% in females. Additionally, saturation in the green spectral region (S1, Table 1) was present in the first ordination axis only in males and the red was present only in females. Also, 40% of the measured body areas in males (crown, back, neck and rump) and female (neck, chest, remiges and belly) were responsible for most of the color variance. It was also found that white spheres present on the tail of male *W. poecilinotus* were the part of the plumage with the greatest brightness (Bmed = 17.46%), and made the greatest contribution to the variation in brightness (SD = ± 12.12). This contrasted with the dark portion of the tail plumage, which had the lowest brightness values (3.64). The crown was the area with the lowest contribution to the brightness variation in males of this species (SD = ± 1.3). A similar result was observed in *W. poecilinotus* females, where the white spheres on the tail were found to be the brightest part of their plumage (Bmed = 23.54%), while the back had the lowest brightness (Bmed = 2.34). In females, the areas with the highest and lowest variation in brightness were, respectively, the tail spheres (SD = ± 9.7) and rump (SD = ± 1.6).

In *L. coronata* males, the greatest importance in the formation of the first two ordination axes were the saturation metric calculated by summing of the reflectance values 50nm either side of the peak reflectance wavelength (Table S2 1), and saturation metrics for green, blue and yellow (S1 Table 1), plus a variable tint (H1 Table 1). These were responsible for 87% and 7% of data variation, respectively. In *L. coronata* males, 60% of the measured body areas showed greatest variation in color (wing coverts, back, chest, rump and belly). In *L. coronata* males, the crown was the region with the highest plumage brightness, followed by the belly (Bmed = 49% and Bmed = 17.05%, respectively). The neck was the least bright region (Bmed = 2.6 %). The crown was the region that contributed most to the variation in males plumage brightness (± SD = 20.1), while the neck varied least in brightness (SD = ± 1.1). In *L. coronata* females, the first two PCA axes for color *per se* explained 87% and 8% of the data variation. For violet and yellow saturation (S1 Table 1) 60% of the measured body areas (tail, greater coverts, breast, remiges and rump) contained most of the variation in color, and were the most important in the formation of the first two PCA axes. For females, the belly was the plumage region with the highest average brightness (Bmed = 33.9%), while the back had the lowest average brightness (Bmed = 6.4%), compared with other measured regions of the body. The plumage regions with the greatest and lowest variations were, respectively, the belly (SD = ± 10.4) and back (SD = ± 1.8).

Morphometry

Among the species studied, there was a difference between species and between sexes in which morphometric variables were most important in the formation of the first PCA axes. In *G. salvini* males, for example, the first two PCA axes explained 42% and 21% of the morphometric data, respectively. The variables most associated with the first axis were beak height, width and length. In *G. salvini* females, the first two PCA axes explained 37% and 27% of variation in the data. The morphometric variables most associated with the first two axes were beak width, wing length and tail length.

In *W. poecilinotus* males, the first two PCA axes explained 38% and 28% of morphological changes, respectively. The variables most associated with the first axis were beak length and width, and tail length. In *W. poecilinotus* females, the two major PCA axes corresponded to 54% and 25% of variation, respectively. The variables most associated with the first axis were beak height and width, and tail length.

Finally, in *L. coronata* males the variables beak width, height and length, and tarsus length were the morphometric characteristics which varied between individuals in general. In *L. coronata* females, the first axes of the PCA explained 48% and 28% of variation in the data. The variables most associated with the first two PCA axes were wing and tarsus length and beak width.

Only females of *G. salvini* and *W. poecilinotus* showed correlation between color data and morphometric data ($r = 0.69$, $P = 0.026$) and ($r = -0.66$, $P = 0.05$). Males of *G. salvini, W. poecilinotus* showed no correlation between color and morphometry in either gender. *Lepidothrix coronata* showed no correlation for either sex.

Phenotypic Variation between Locations

We found between-location variation in color, brightness and morphology in all the three studied species in about 50% of the first associated PCs of the PCA, except in males of *L. coronata* which showed no variation in brightness and morphometric characteristics between study sites (Figures 3 and 4). For all three species the colorimetric characters which varied most between modules were metric saturation (S1 variable table 1) at different wavelengths (UV, violet, green, blue etc.), while the morphometric characteristics which varied most in *G. salvini* were beak width and wing and tail length, beak width and tail length in *W. poecilinotus* and wing length and width of the beak and of tarsus in *L. coronata*. Other parts varied but showed no significant variation between study sites. This is illustrated in the figures below.

Color and morphology independently varied between study sites. For example, morphometric characters in females of *G. salvini* differed between sites 7 and 9, while brightness and saturation did not vary between them, though they varied between other study sites.

From visual examination, it was apparent that *L. coronata* males showed clinal variation in color, so that males in the northern part of the interfluve were more black (modules 1 and 2, Figure 2), while those in the extreme south were more green (modules 10 and 11, figure 2), and central populations having an intermediate color (modules 7 and 8, figure 2). However, this cline does not appear in colorimetric analysis. This is because what is perceived with the human eye is a gradual change in the amount of black in the plumage of these individuals, while electronic analyzes senses the values of hue and saturation in the plumage of individuals. However, with the color measurements, a discontinuity was noticed in phenotypes break between study sites 2 and 7 which corresponded to the geographic location of the visually perceived change from black to green.

The external groups (Xingu, Japurá and Ipixuna) added to the analyzis did not show a uniform pattern: in some cases there were large phenotypic divergences between them and the samples from the Madeira-Purus interfluve while in other cases variation was slight. The extent of phenotypic variation between exterior groups and samples from study sites in the interfluve showed different results among species (Figures 2, 3 and 4).

Effects of Environment and Geographic Location

Patterns of variation were different in each study species, and differences between sexes were also recorded. In *G. salvini* males, variation in color (in this case, saturation) was better explained by a composite model using both avian community and geographical location variables. This model was significant ($P < 0.05$), and relations were positive (Table 2 Figure 5). Variation in brightness between study sites was best explained by geographical location, although the model was not significant (Table 2). Variation in morphology observed in *G. salvini* males was explained by neither geographic distance nor by environmental variation, so that in this case the null model best explained the observed morphometric variation (Table 2).

The variation in color in female *G. salvini* (in this case, saturation) was significantly related to geographic location ($P < 0.05$) (Table 2). The relationship between the change in latitude and color along the interfluves was positive (Figure 5). The variation in brightness is best explained by the variable ‘’basal area of trees’’ which has a marginally significant positive relationship ($P \approx 0.05$) (Table 2, Figure 6). Morphometric variation (beak width, wing and tail length) was best explained by location along the interfluvial (Figure 7). The relationship between morphometric change and geographical location was positive and marginally significant ($P \approx 0.05$) (Table 2).

In *W. poecilinotus* males, the null model best explained color variation between sampling modules (Table 4). Variation in brightness in *W. poecilinotus* males was best explained by latitude, with which there was a significant negative correlation (Table 2 Figure 6). Morphometric variation was not explained by any of the variables tested with the null model.

In *W. poecilinotus* females, the variation in color (saturation) and brightness responded best to the model that combined the effects of environmental variable ‘’basal area of trees’’ and the latitude of the geographic location (Table 2). The relations were both positive and significant only for coloration (P <0.05) (Figure 5). Morphometric variation was better explained by geographic location, though without significant correlation (Table 2).

Finally, since we found no variation in brightness and morphology in *L. coronata* males, we tested only the colorimetric variation of males in relation to environment and geographical location. Change in color (hue and saturation) were best explained by a full model combining the effect of all explanatory variables (birds community composition, basal area of trees and latitude). Relations were significant (P <0.05), and the bird community variables and latitude showed a positive relationship with the colorimetric variation while the basal area of trees variable was negatively related to coloration variation (Table 2) (Figure 5). For female *L. coronata*, variation in color and brightness was best explained by the null model. Variation in morphometry in *L. coronata* females was best explained by the environmental variable basal area of trees along the Purus-Madeira interfluve (Table 02). This relationship, however, was not significant.

DISCUSSION

Our study is the first to analyze intraspecific phenotypic diversity of birds of the Purus-Madeira interfluve, and one of the few to evaluate the effects of neutral and adaptive mechanisms of phenotypic variation in birds within an Amazonian interfluve. The majority of studies that have tried to explain the sources of intraspecific phenotypic variation in Amazonian birds in interfluves have studied acoustic variability, and those using morphological characters are still in the minority. Some studies have tested more specific aspects, such as Tobias et al (2010) who worked on the theory of sensory direction, with a study of the divergence in acoustic signals of birds in two Amazonian environments: bamboo forest and upland forest. Other studies are based on plumage characters, but use semi-quantitative data that is circumscribed by the limits of human vision. For example, Cabanne et al (2011) studied plumage coloration variation in *Dendocolaptes platyrostris* subspecies in Brazilian, and found that variation in plumage color was associated with ecological factors linked to habitat type rather than to patterns of genetic structure. Our study has is significant as it investigates a topic little studied in the Amazon, and studies on a small spatial scale the microevolution of a non-vocal phenotypic variation. There is still much speculation about the factors controlling intra-specific phenotypic diversity: some studies have shown that historical factors have the main role (e.g. Lee and Mitchell-Olds 2011), while others suggest that selection processes are the most important generators of phenotypic and genetic diversity (Endler 1977; Rudh et al 2007). In our study, we found that geographical location in latitude within the interfluve was the factor that best explained the phenotypic diversity in the studied species. However, for some bird species the synergistic effect of combined stochastic and environmental factors seems to be most important in generating the observed patterns of phenotypic diversity. For example, a recent study by Naka and colleagues (2012) of phenotypic and phylogenetic data in the avian community on either side of the Rio Branco showed that at least 13 pairs of bird taxa from the Guayana Shield (including cases of substitution among species and subspecies). Phylogenetic discontinuities were associated with phenotypic ones, which in turn were related to changes in the landscape. While physical barriers seem to have a fundamental role in the location of phenotypic discontinuities and contact zones in birds studies by Naka colleagues, ecological factors appear to have influenced the patterns also found in their study.

In our study we found that the property of color that showed greatest variation, both between study sites (geographic variation between populations) and between individuals (within population variation) was hue saturation. Low levels of variation for this feature were only found in *L. coronata* males. According to Gomez and Théry (2004), variation of pelage tone is higher in canopy bird species than in those from the understory. This is because the light in the canopy contains a greater range of wavelengths and so can produce visual conspicuousness in chromatic signals over a greater range of tones than the light of the understory. Consequently, the limited range of wavelengths in the understory may favor more achromatic signals such as brightness, and the variation such aspects as saturation between populations of the same understory species can provide a strong foundation for sexual selection (Gomez and Théry 2004). Our results further indicated that males have higher levels of brightness to their feathers when compared to female conspecifics. Such differences in brightness between males and females are due to the different reproductive functions they perform: males in general are exposed to stronger selection for increased conspicuousness, especially those in the Pipridae (Anciães et al 2009), while females tend to be less conspicuous because of their role in offspring care.

Among the models that best explained variation in traits of measured body areas, we found that the variation in brightness of female *G. salvini* was positively related to basal area of trees, indicating that females tend to have brighter feathers in environments with higher tree density and lower light input. In environments with a lower tree density, and thus more light, females of this species tend to have less bright plumage, possibly because they are already conspicuous enough to be detected by conspecifics and syntopic species. In contrast, *W. poecilinotus* males had their brightness variation best explained by a model of negative relation to latitude, indicating that mechanisms such as neutral drift may be involved in the phenotypic difference of this character.

Studies indicate that forests exhibit variation in ambient light and that this can affect communication between animals (e.g. Heindl and Winkler 2003; Théry and Gomez 2004). Light intensity increases along a vertical gradient within forests, with the color of ambient light gradually changing from green in the understory to blue in the canopy. In more open areas the ambient light is white and in habitats more obstructed by vegetation light is green because the light that is transmitted and reflected by vegetation is richer in wavelengths in the 520-620 nm part of the spectrum than is incident light (Endler 1993). In this context, the fewer trees in an environment, the less light is filtered through the canopy and more unsaturated is the light, so that the ambient chromatic environment tends to the white. Conversely, the greater the number of trees, the more intense the chromatic filter, this makes color more saturated, so that the ambient chromatic environment tends to the green. Our results revealed that variation in saturation in female *W. poecilinotus* was positively correlated with the basal area of trees. This indicates that females of this species have more saturated colors in environments of green light (more saturated ambient color) and have less saturated colors in lighter environments (less saturated ambient color). It has been known for some time that in certain kinds of Thamnophiliidae females present conspicuous characters, whereas the males are not or barely distinguishable from one another. This variation was called by Hellmayr (1929) of heterogynism and played an important role among birds of this family. Despite the pressures of sexual selection on females to greater conspicuity still has been a little investigated topic and often neglected, we believed that it must have importance in females which compete for mates through advertising their attractiveness or in direct female-female contests (Espmark et al 2000). Most birds see well in ultraviolet. Knowing that the metrics of saturation of ultraviolet (Table S1 1) for the chest and remiges regions were most associated with the first PCA axes in female *W. poecilinotus*, we hypothesized that this should facilitate communication between conspecifics or potential competitors (other birds) without that females of this species are conspicuous, and therefore vulnerable, to vision-based predators. The effect of signalling to conspecifics and predators was investigated in *Dendrobates pumilio* arrowpoison frogs by Siddiqi et al (2004), who showed the relevance of different selection pressures on the evolution of visual signals.

We found the opposite in *L. coronata* where variation in hue and saturation, most notably in males, was negatively related to tree basal area. From analysis PCs we conclude that the metrics most associated with the first PCA axes were saturation in the green region of the spectrum in four different body areas (wing coverts, breast, rump and belly), in the blue region for one body area (belly), and in a yellow region for one body area (belly) (S1, Table 1). Male *L. coronata* have the most at the site with the highest number of trees. Here the chromatic environment is obstructed and consequently ambient light becomes more saturated (green). Males have more unsaturated coloration (mixed with other colors) when the surroundings are more saturated (green) and more saturated color (tending towards green) when the environment light is unsaturated (tending to white). Consequently, they are more conspicuous against the ambient light environment, which facilitates communication between conspecifics, and thus the fitness by the action of natural selection via sexual selection.

Among the phenotypic differences, we find that, in all species, beak characters had the greatest morphometric variation, suggesting that this may be the most labile of the morphometric traits studied. Studies of the differences in sizes and shapes of beaks of finch species on the Galapagos Islands have found variation in these traits to be closely linked to the availability of specific food resources between islands and between seasons (Grant and Grant 2003).

Flight-related characters (wing and tail) varied in females of all species and in *W. poecilinotus* males, but only in *G. salvini* was the variation significantly explained by one of the proposed models (variation in geographic location). In the other cases variation is considered to have more influenced such stochastic mechanisms as genetic drift. Other characters that varied in the other species studied were not associated with the tested environmental variables and, in the case of *G. salvini* and *W. poecilinotus* males, also not associated with latitude. Models evaluating the role of geographic distance were not good predictors of variation in phenotype or morphology of the three study species. A relationship between latitude and morphometric variation was found only in *G. salvini* females, but was only marginally significant. In *G. salvini* males, and the remaining species, none of the tested variables significantly explained the observed morphometric variation. Our results therefore suggest that variations in the sampled factors had little direct affect on variation in the studied morphometric traits. This indicates that these variables directly affected neither the ecology of the studied species (e.g. foraging mode or physiology), nor the autoecological aspects associated with phenotypic traits studied here. Morphometric characters in birds can be classified into different categories determined by the role they play. For example, wings and tail are characters associated with flying and evolve to increase the efficiency of flight and maximally reduce energy demand. A study that sought to understand the relationship ecomorphology and morphometric characteristics in *Thamnomanes* (Thamnophilidae) species showed that larger wings are related to greater efficiency during long flights, while wing size is limited by vegetation density in which the antshrikes forage (Schulenberg 1983). Moreover, differences in tarsus length and beak were related directly to diet and foraging strategies (Schulenberg 1983).

A study that investigated the effect of selective and stochastic processes in the divergence of *Gymnopithys spirurus* morphological characters found that tree density can influence morphometric character divergence in this species (Mila et al 2009). However, *G. spirurus* does occur in the understory, since it is more arboreal than *G. salvini* or *W. poecilinotus* (which forage on or near the ground taking advantage of prey flushed by army ants). Possibly the fact that they are species that exploit the lower strata of the forest, rather than trunks like *G. spirurus*, means that the density of trees does not exercise such a direct effect on the ecology of these species.

For *L. coronata*, the best model, although not significant, that explained morphometric variation in females was the tree basal area, a variable that represents the density of vegetation between sampling sites. We did not record geographical variation in morphology between samples modules for male *L. coronata*. According Durães et al (2007), adult male *L. coronata* use of the forest is restricted to small areas around leks, so that they are much more sedentary than females. Perhaps for this reason, the density of vegetation exerts a lesser effect on the morphology of flight in the males than females, which have to travel longer distances and move through more cluttered vegetation when in search of resources.

Finally, a special case of variation in phenotypic plasticity of phenotypic traits is an adaptive mechanism in which the same genotype can express different phenotypes depending on the environmental circumstances (West-Eberhard 1989; Nijhout 2003). The polymorphism, a type of plasticity restricted to morphology (Fowlie and Kruger 2003; Galeotti et al 2003; Berman et al 2005; Gray and McKinnon 2007), is one of the processes that contribute most to the generation of phenotypic variation and can evolve under the influence of interactions between organisms (Agrawal 2001), and do so relatively quickly (Pimm 1988). However, its evolution, as is the case with all variable organisms, tends to be restricted since they are adapted to specific environmental conditions and therefore their fitness is restricted to certain sub-sets of the environment (West-Eberhard 1989). Our study did not investigate whether the observed variation in the phenotype of the studied species would result from phenotypic plasticity, since we currently have no knowledge of the genetic structuring of the study species in the region investigated. However, ongoing studies of the relationship between genetic variability found in the species studied with phenotypic variation described here will help us to better understand the effect of other evolutionary processes such as phenotypic plasticity in these birds.

This work suggests that even within an interfluve, where, at least for birds, the presence of barriers to gene flow is limited when compared between interfluves, geographical distance combined with the effects of environmental heterogeneity is sufficient to generate intraspecific divergence in phenotypic traits. In this study we found that there is geographic variation in coloration and morphology in three species of birds along the Purus-Madeira interfluve, especially for *L. coronata* where males show a variation in color shaped cline, following a gradient of black individuals in the extreme north of the interfluve to green in its extreme south. However, different phenotypic traits within species and also within the same sex seem to evolve independently as a result of the performance of different evolutionary mechanisms such as drift and natural / sexual selection.

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FIGURES

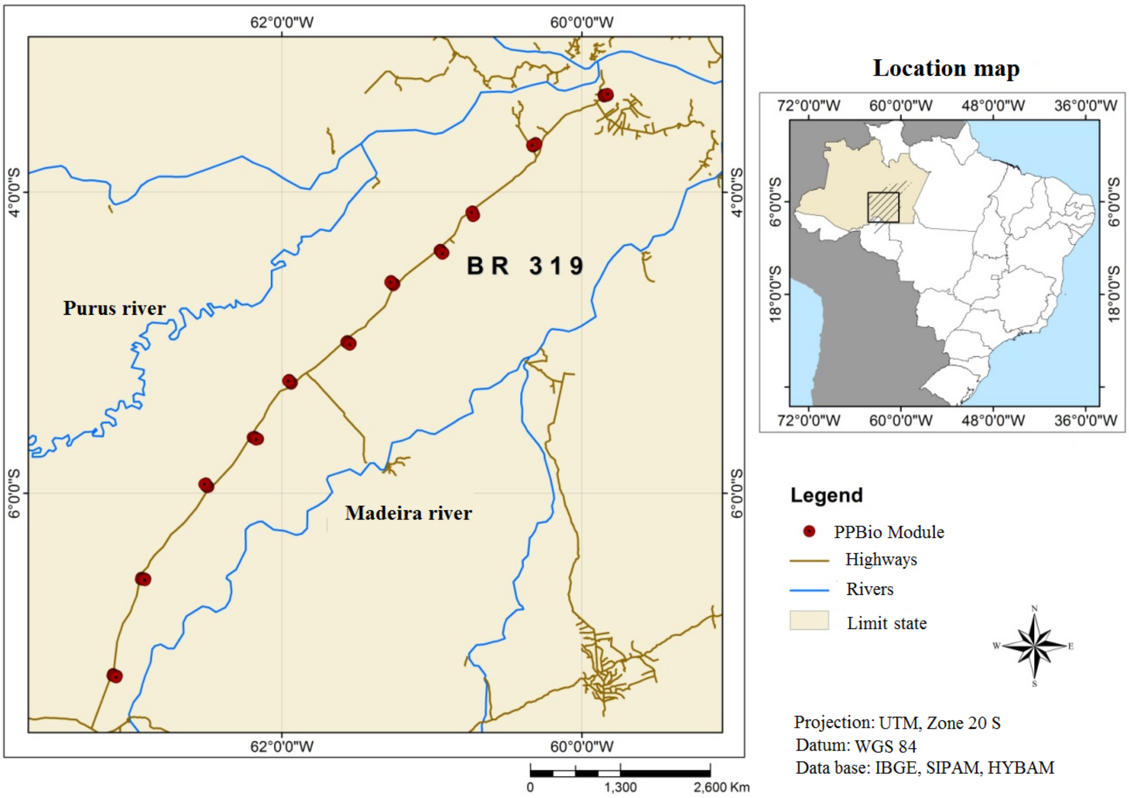


Figure 1: Map of the study area in the Madeira-Purus interfluve showing the locations of the 11 sampling units (red dots)

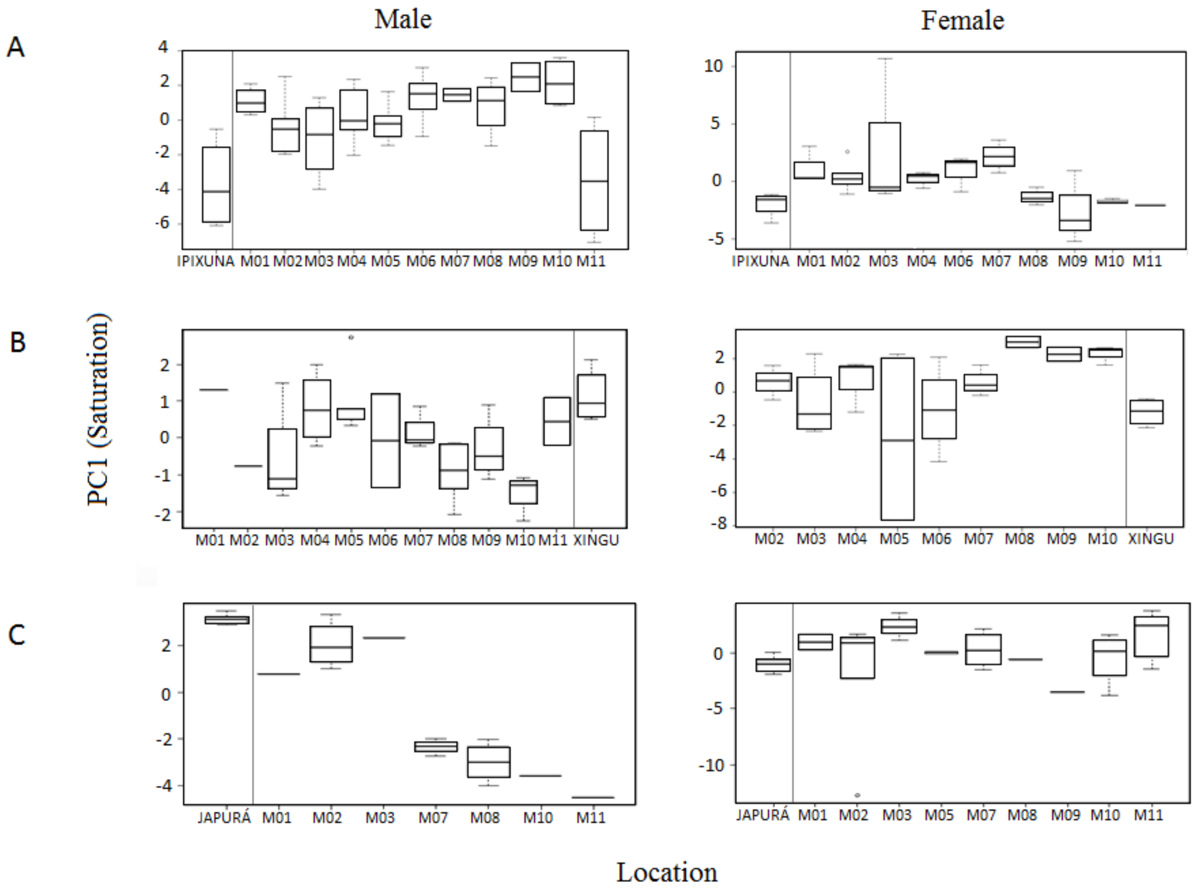


Figure 2: Geographic variation in saturation in A) *Gymnopithys salvini* B) *Willisornis poecilinotus* and C) *Lepidothrix coronata* among the sampling sites. The boxes around the median represent the standard deviation and the bars above the box represent the confidence intervals of 95%.

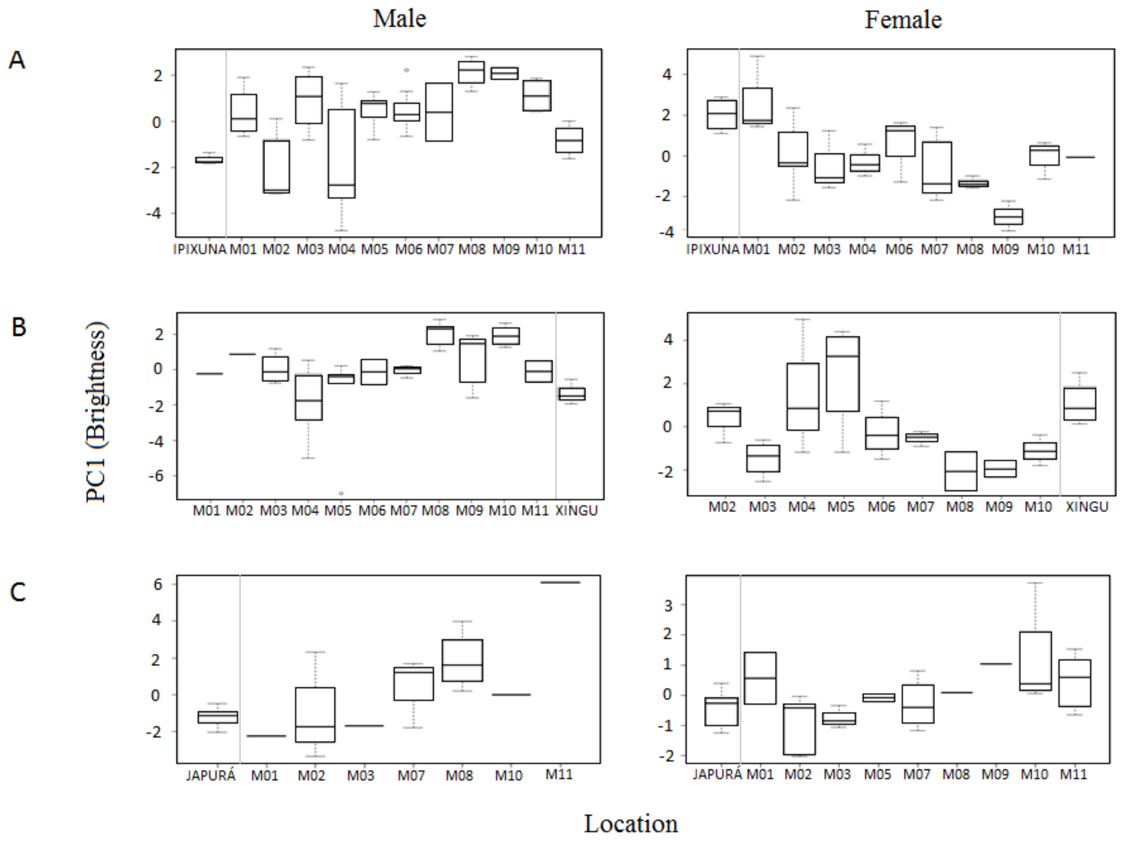


Figure 3: Geographic variation in brightness in A) *Gymnopithys salvini* B) *Willisornis poecilinotus* and C) *Lepidothrix coronata* among the sampling sites. The boxes around the median represent the standard deviation and the bars above the box represent the confidence intervals of 95%.

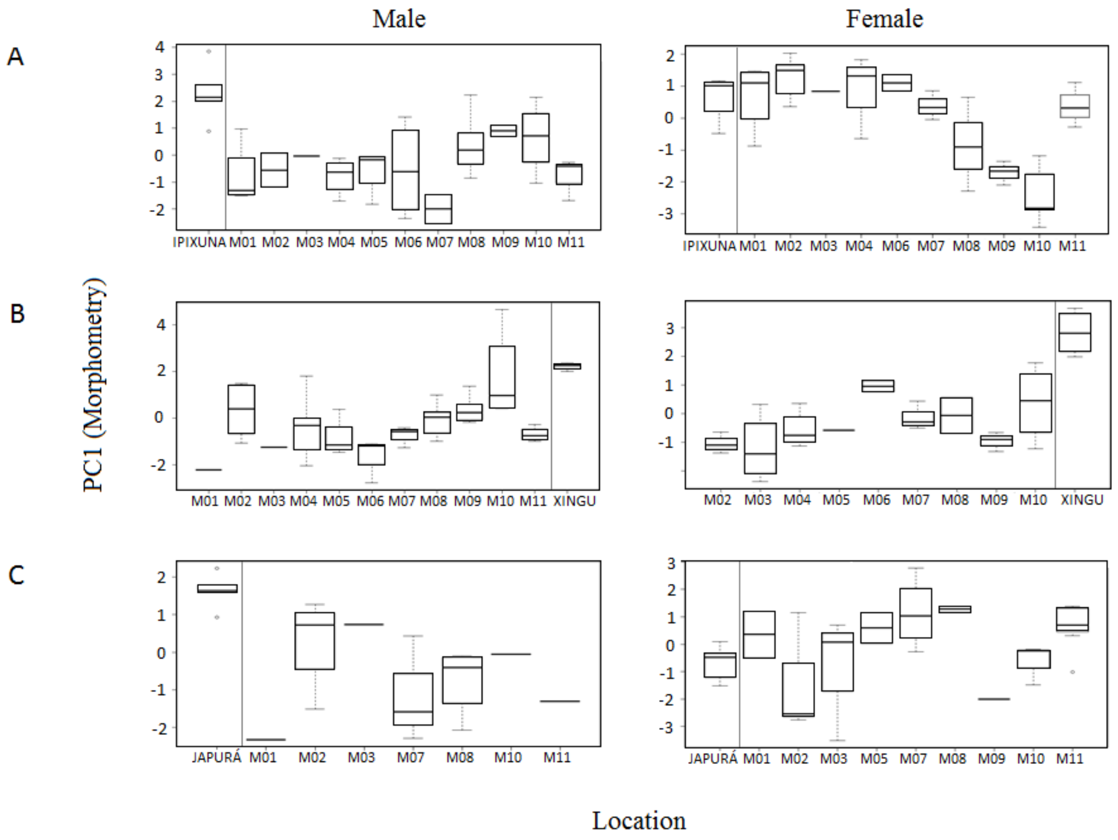


Figure 4: Geographic variation in morphometry in A) *Gymnopithys salvini* B) *Willisornis poecilinotus* and C) *Lepidothrix coronata* among the sampling sites. The boxes around the median represent the standard deviation and the bars above the box represent the confidence intervals of 95%.

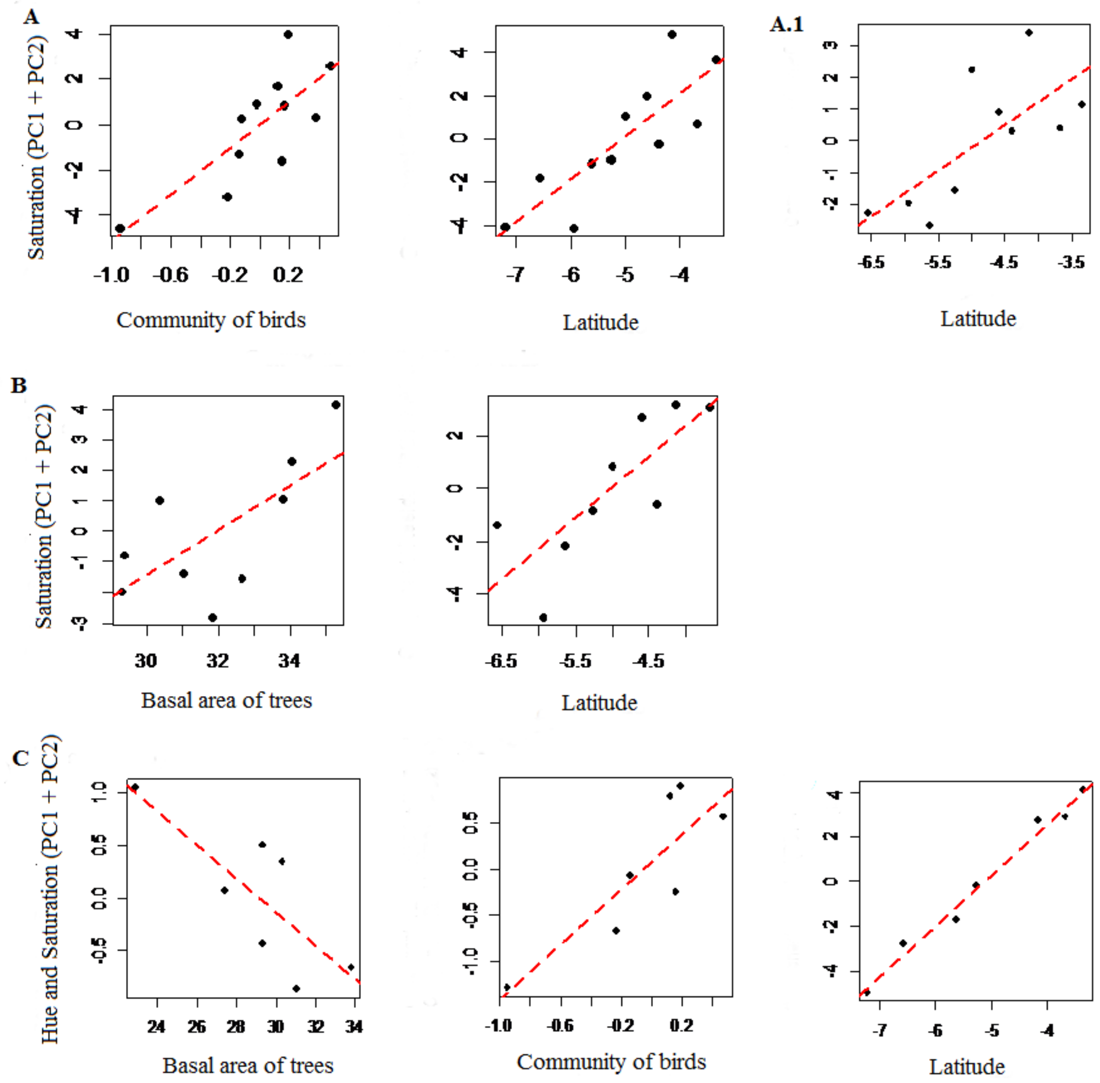


Figure 5: Scores PC1+PC2 of color in relation to explanatory variables tested for significant models in A) males *Gymnopithys salvini* A.1) Females of *G. salvini* B) females of *Willisornis poecilinotus* and C) Males of *Lepidothrix coronata*.

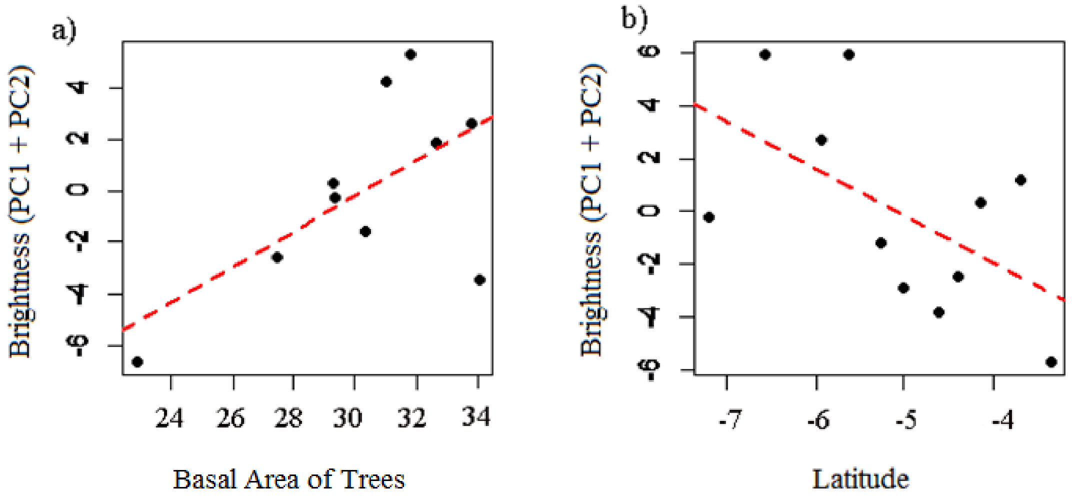


Figure 6: Scores PC1+PC2 of brightness in relation to explanatory variables tested for significant models in A) female of *Gymnopithys salvini* and B) males of *Willisornis poecilinotus.*

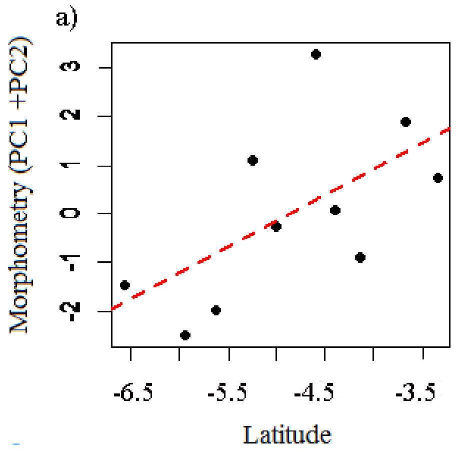
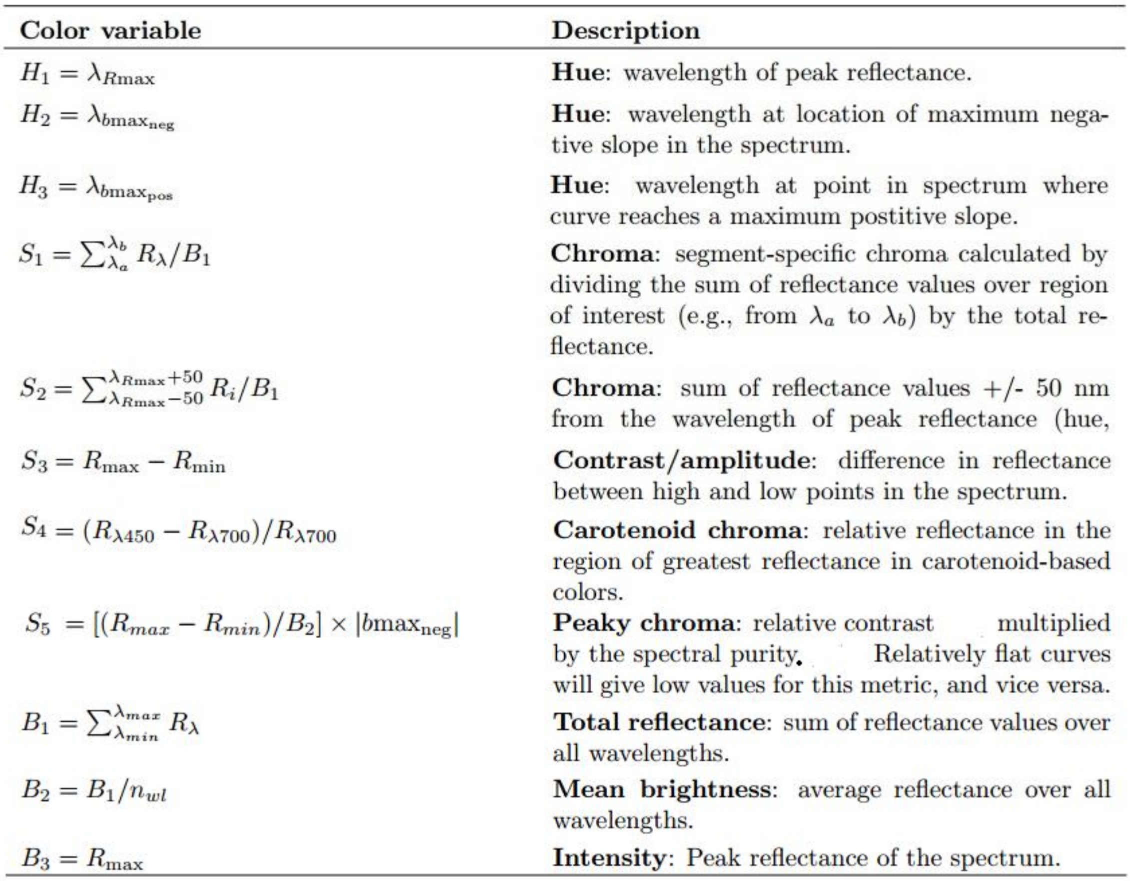


Figure 7: Scores PC1+PC2 of morphometry in females of *Gymnopithys salvini* relation to Latitude

TABLES

Table 1: Color variables calculated by summary function in PAVO and used for brigthness, hue and chroma estimates from the species in this study (adapted from Maia et al 2013).



Color variable	Description
$H_1 = \lambda_{R\max}$	**Hue**: wavelength of peak reflectance.
$H_2 = \lambda_{b\max_{\text{neg}}}$	**Hue**: wavelength at location of maximum negative slope in the spectrum.
$H_3 = \lambda_{b\max_{\text{pos}}}$	**Hue**: wavelength at point in spectrum where curve reaches a maximum postitive slope.
$S_1 = \sum_{\lambda_a}^{\lambda_b} R_\lambda / B_1$	**Chroma**: segment-specific chroma calculated by dividing the sum of reflectance values over region of interest (e.g., from λ_a to λ_b) by the total reflectance.
$S_2 = \sum_{\lambda_{R\max}-50}^{\lambda_{R\max}+50} R_i / B_1$	**Chroma**: sum of reflectance values +/- 50 nm from the wavelength of peak reflectance (hue,
$S_3 = R_{\max} - R_{\min}$	**Contrast/amplitude**: difference in reflectance between high and low points in the spectrum.
$S_4 = (R_{\lambda 450} - R_{\lambda 700}) / R_{\lambda 700}$	**Carotenoid chroma**: relative reflectance in the region of greatest reflectance in carotenoid-based colors.
$S_5 = [(R_{max} - R_{min})/B_2] \times \lvert b\max_{\text{neg}} \rvert$	**Peaky chroma**: relative contrast multiplied by the spectral purity. Relatively flat curves will give low values for this metric, and vice versa.
$B_1 = \sum_{\lambda_{min}}^{\lambda_{max}} R_\lambda$	**Total reflectance**: sum of reflectance values over all wavelengths.
$B_2 = B_1 / n_{wl}$	**Mean brightness**: average reflectance over all wavelengths.
$B_3 = R_{\max}$	**Intensity**: Peak reflectance of the spectrum.

Table 2: Predictions of the influence of environmental variables and geographic location on the phenotypic variation of the taxa studied. Results of GLM (coefficients associated with the t value in parentheses) and the significance level for each variable of the best models models. Colorimetric and morphometric data given are the most related to the first PC (colorimetric metric descriptions in Table 1 are available). For models the following explanatory variables were used: CB) Community of Birds; BA) Basal area of trees and GL) Geographic Location in latitude. Coefficients provided for in each case multiple variables (color, brightness, morphometry) refer to mixed models.

Species	**Sex**	**N**	**colorimetric variables (PC1)**	**morphometric variables (PC1)**	**Explanatory variables**	**Color**	**Brightness**	**Morphometry**
G. salvini	Male	49	S1 in tail, chest, back, neck, rump, rêmige e abdomen	Height, width and length of beak	CB	3.964 (1.518)	.	.
					BA	.	.	.
					GL	2.233** (2.694)	1.3145 (1.485)	.
					AIC	55.13	61.65	45.86
					P-value	0.029**	0.181	.
					Model variation explained (%)	47.81	19.68	.
G. salvini	Female	34	S1, S4, S5 in tail and tail lines	width of bill and length of the wing and tail	CB	.	.	.
					BA	.	0.6861* (2.229)	.
					GL	1.1439** (-(2.591)	.	1.061*(2.127)
					AIC	38.14	54.61	40.59
					P-value	0.0357**	0.0574*	0.0704
					Model variation explained (%)	45.63	38.32	36.13
W. poecilinotus	Male	41	S1 in head, back, neck and rump	Width and length of bill and tail	CB	.	.	.
					BA	.	.	.
					GL	.	-1.77* (-2.048)	
					AIC from model	57.74	61.13	47.24
					P-value	.	0.0769	.
					Model variation explained (%)	.	31.78	.

Species	Sex	N	colorimetric variables (PC1)	morphometric variables (PC1)	Explanatory variables	Color	Brightness	Morphometry
W. poecilinotus	Female	29	S1 in neck, chest, remige e abdomen	Height and width of the beak and tail length	CB	.	.	.
					BA	0.7295* (2.301)	-0.895 (-1.721)	.
					GL	2.3206** (3.2)	-2.484 (-2.07)	-0.9094 (-1.615)
					AIC from model	41.48	50.38	36.21
					P-value	0.31	0.3865	0.18
					Model variation explained (%)	70.49	52.94	27.15
L. coronata	Male	17	S1, S2, H1 greater wing coverts	.	CB	1.49523 (1.619)	.	.
					BA	-0.15953 (-1.837)	.	.
					GL	2.27254***(8.734)	.	.
					AIC from model	16.99	.	.
					P-value	0,012	.	.
					Model variation explained (%)	98.04	.	.
L. coronata	Female	30	S1 in tail, greater wing coverts, chest, remige e rump	Width of the beak and wing length and tarsus	CB	.	.	.
					BA	.	.	0.2627 (1.701)
					GL	.	.	.
					AIC from model	48.76	51.18	37.51
					P-value	.	.	0.135
					Model variation explained (%)	.	.	29.24

CONCLUSÃO

Este trabalho sugere que mesmo no interior de um interflúvio onde, pelo menos para as aves, a presença de barreiras ao fluxo gênico é limitada quando comparada entre interflúvios, a distância geográfica combinada com efeitos da heterogeneidade ambiental são suficientes para gerar divergência sutil em traços fenotípicos intraespecíficos. Nesse estudo constatamos que há variação geográfica em coloração e morfometria nas três espécies de aves estudadas ao longo do interflúvio Purus-Madeira, com destaque para a espécie *L. coronata*onde os machos apresentam uma variação em cor em forma de clina, seguindo um gradiente de preto em indivíduos no extremo norte do interflúvio até verde no extremo sul. Ainda, diferentes traços fenotípicos numa mesma espécie e também no mesmo sexo parecem evoluir de forma independente em consequência da atuação de diferentes mecanismos evolutivos como deriva e seleção natural/sexual.

APÊNDICE

Escores das Análises de Componentes Principais (PCA) para as caracteristicas fenotípicas cor, brilho e morfometria das espécies *Gymnopithys salvini*, *Willisornis poecilinotus* e *Lepidothrix coronata*

Cor

Gymnopithys salvini

macho

Variável	**PC1**	**PC2**
S1.red_cauda	-0.30984	-0.15362
S1.reddorso	-0.24551	-0.52007
S1.redgarganta	-0.35893	0.231862
S1.Uvgarganta	0.360181	-0.35097
S1.violetgarganta	0.362313	-0.34524
r.achievedgarganta	-0.36089	0.34551
S1.redpeito	-0.28918	-0.09421
S1.redremige	-0.29964	-0.25736
S1.reduropigio	-0.28242	-0.3479
S1.Uvventre	0.265486	0.30705

fêmea

Variável	**PC1**	**PC2**
S1.bluecauda	0.31889	0.309224
S1.redcauda	-0.3321	-0.30243
S3cauda	-0.31684	-0.33296
S6cauda	-0.31221	-0.31472
S9cauda	0.335782	0.218627
S1.Uvcaudabarra	-0.30342	0.429
S1.violetcaudabarra	-0.30373	0.431144
S1.bluecaudabarra	-0.31561	0.109047
S1.yellowcaudabarra	0.302601	-0.33219
S1.redcaudabarra	0.319241	-0.25108

Cor

Willisornis poecilinotus

macho

Variável	**PC1**	**PC2**
r.maxgarganta	-0,2837	0,14232
S1.Uvgarganta	0,27196	0,16438
S1.violetpeito	0,30598	0,20124
S1.Uvdorso	-0,3235	-0,2017
S1.verdegarg	0,29732	0,15342
S1.violedorso	0,31534	0,03287
S1.reduropigio	-0,2964	-0,1032
r.maxuropigio	-0,3429	-0,1843
S1.violetventre	0,30877	-0,1327
r.maxventre	-0,3223	0,42376

fêmea

Variável	**PC1**	**PC2**
r.maxgarganta	-0,2444	0,16415
S1.Uvpeito	0,33196	0,20762
S1.violetpeito	0,33598	0,21195
r.maxpeito	-0,334	-0,2297
S1.Uvremige	0,33782	0,17302
S1.violetremige	0,33776	0,16511
S1.redremige	-0,342	-0,0706
r.maxremige	-0,314	-0,1115
S1.violetventre	0,28194	-0,6306
r.maxventre	-0,2871	0,60002

Cor

Lepidothrix coronata

macho

Variável	PC1	PC2
H1cobasa	0.28739	-0.0777
S1.greencobasa	-0.3158	0.33328
S3cobasa	-0.3342	0.1049
S3dorso	-0.3185	0.15756
S1.greenpeito	-0.2943	0.57092
S1.greenuro	-0.3149	0.19553
S1.blueventre	0.32596	0.28441
S1.greenventre	-0.3175	-0.4217
S1.yellowventre	-0.3269	-0.2874
r.achievedventre	-0.3238	-0.3762

fêmea

Variável	PC1	PC2
S1.Uvcauda	-0.308	-0.4898
S1.violetcauda	-0.3097	-0.4829
S1.Uvcobasa	-0.3125	0.33976
S1.violetcobasa	-0.3116	0.34333
S1.violetpeito	-0.2995	0.30077
S1.Uvremige	-0.3327	-0.1294
S1.violetremige	-0.3331	-0.1317
S1.yellowremige	0.32085	0.22093
S1.Uvuro	-0.3162	0.25365
S1.violeturo	-0.3165	0.23628

Brilho

Gymnopithys salvini

macho

Variável	PC1	PC2
Bmedcauda	0.075915	0.107771
Bmedcaudaesfera	0.094955	-0.47087
Bmedcobasa	0.257896	0.178926
Bmedcoroa	0.223789	-0.18909
Bmeddorso	0.268507	-0.12043
Bmedgarganta	0.260988	0.196178
Bmedpeito	0.28365	-0.02225
Bmedremige	0.183038	-0.1929
Bmeduro	0.296446	0.006219
Bmedventre	0.163023	0.328882

fêmea

Variável	PC1	PC2
Bmedcauda	-0.26139	0.11393
Bmedcaudaesfera	-0.02511	0.356382
Bmedcobasa	-0.21908	0.024853
Bmedcoroa	-0.28629	0.095271
Bmeddorso	-0.26456	-0.08086
Bmedgarganta	-0.16896	-0.26438
Bmedpeito	-0.22453	-0.07044
Bmedremige	0.000324	-0.51059
Bmeduro	-0.26135	-0.04678
Bmedventre	-0.28953	0.077674

Brilho

Willisornis poecilinotus

macho

Variável	PC1	PC2
B1cauda	-0.27805	-0.02979
B1caudabola	-0.11002	0.400029
B1cobasa	-0.10373	-0.46276
B1coroa	-0.22336	-0.26495
B1dorso	-0.27454	0.13788
B1garganta	-0.27448	0.023039
B1peito	-0.22275	0.103674
B1remige	-0.14171	-0.02496
B1uro	-0.26259	-0.08891
B1ventre	-0.24611	0.126217

fêmea

Variável	PC1	PC2
B1cauda	-0.26082	-0.00492
B1caudabola	0.040929	-0.49794
B1cobasa	-0.24181	-0.08032
B1coroa	-0.25472	0.091232
B1dorso	-0.24865	-0.18666
B1garganta	-0.25678	0.138824
B1peito	-0.14765	-0.26977
B1remige	-0.19815	-0.24119
B1uro	-0.25559	0.121197
B1ventre	-0.22976	0.193681

Brilho

Lepidothrix coronata

macho

Variável	PC1	PC2
B1cauda	0.236232	-0.16918
B1cobasa	0.247743	-0.01442
B1coroa	0.230611	-0.25288
B1dorso	0.259079	-0.0822
B1garganta	0.231309	0.337847
B1peito	0.236583	0.294997
B1remige	0.188782	-0.40811
B1uro	0.238676	0.176129
B1ventre	0.245834	0.041571

fêmea

Variável	PC1	PC2
B1cauda	0.273159	0.18106
B1cobasa	0.334543	-0.01524
B1coroa	0.120294	-0.2394
B1dorso	0.314905	0.156574
B1garganta	0.229652	-0.24845
B1peito	0.205106	0.138058
B1remige	0.124522	0.401532
B1uro	0.20738	-0.37649
B1ventre	0.215659	-0.03733

Morfometria

Gymnopithys salvini

macho

Variável	PC1	PC2
bico_comp	-0.3385	0.48224
bico_alt	-0.5369	-0.0514

fêmea

Variável	PC1	PC2
bico_comp	0.20471	-0.6575
bico_alt	0.516	0.05541

bico_larg	-0.5956	0.04123
asa	-0.3438	0.29389
cauda	0.32085	0.52653
tarso	0.14597	0.63205

bico_larg	0.49051	-0.0737
asa	-0.437	-0.2162
cauda	-0.5086	-0.0381
tarso	-0.0396	-0.7148

Morfometria

Willisornis poecilinotus

macho

Variável	PC1	PC2
bico_comp	-0.4679	-0.2756
bico_alt	-0.5811	-0.0327
bico_larg	-0.5253	-0.3143
asa	0.15993	-0.7056
cauda	0.36655	-0.5654
tarso	0.08643	0.08161

fêmea

Variável	PC1	PC2
bico_comp	-0.4124	-0.3041
bico_alt	-0.3677	-0.2327
bico_larg	-0.5356	-0.2286
asa	0.41278	-0.5096
cauda	0.48524	-0.3153
tarso	-0.0451	-0.6648

Morfometria

Lepidothrix coronata

macho

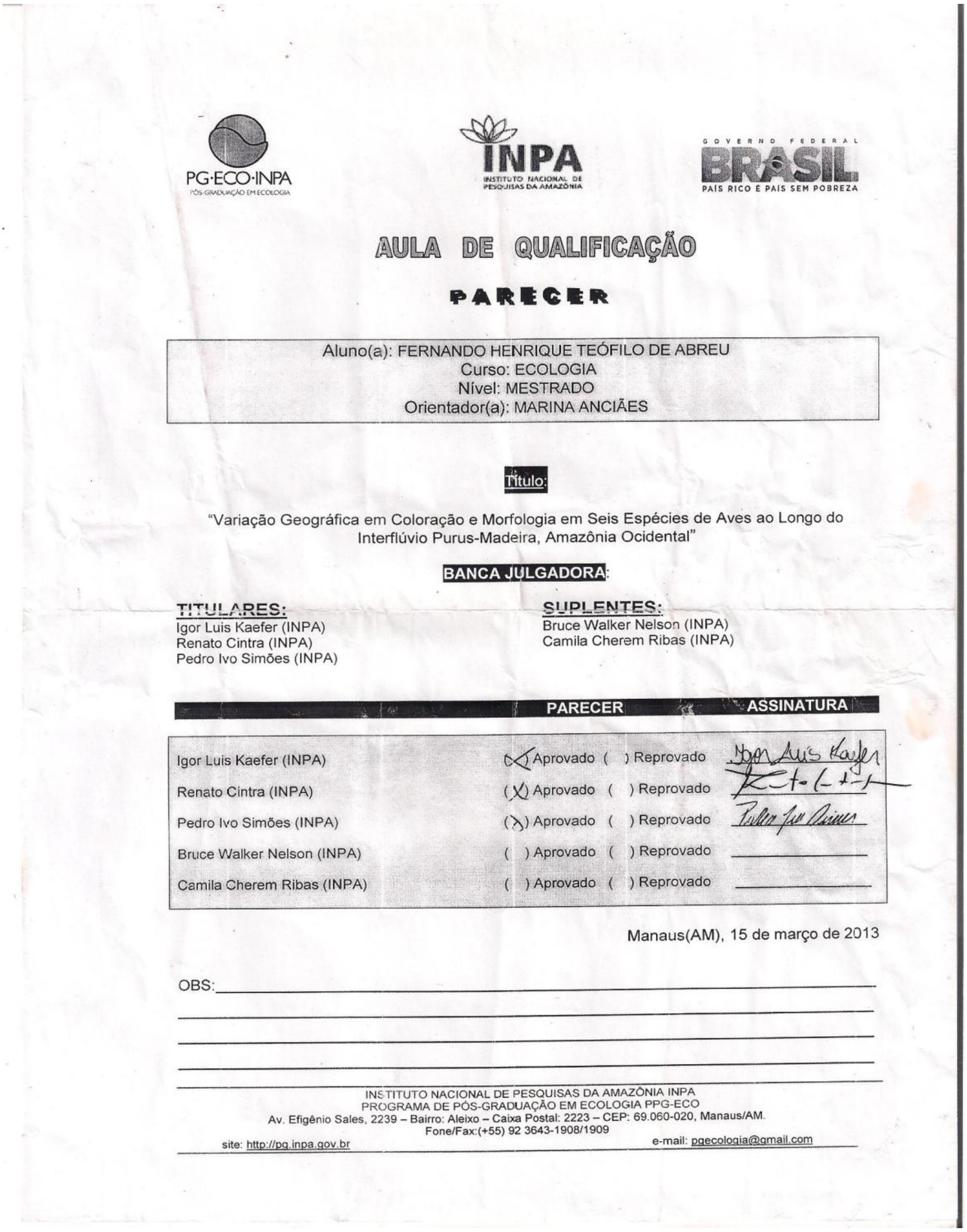
Variável	PC1	PC2
Cauda	-0.523	0.13395
Asa	-0.3319	-0.5468
Tarso	-0.2494	0.31247
Bico_alt	0.45654	-0.3779
Bico_comp	-0.4996	0.12686
Bico_larg	-0.31	-0.6531

fêmea

Variável	PC1	PC2
Cauda	0.48926	-0.2215
Asa	0.53258	0.13831
Tarso	-0.3728	0.35424
bico_altura	-0.4023	-0.1839
bico_comprimento	-0.1888	0.66022
bico_largura	0.37487	0.58016

ANEXOS

Ata da aula de qualificação



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PAÍS RICO É PAÍS SEM POBREZA

AULA DE QUALIFICAÇÃO

PARECER

Aluno(a): FERNANDO HENRIQUE TEÓFILO DE ABREU
Curso: ECOLOGIA
Nível: MESTRADO
Orientador(a): MARINA ANCIÃES

Título:

"Variação Geográfica em Coloração e Morfologia em Seis Espécies de Aves ao Longo do Interflúvio Purus-Madeira, Amazônia Ocidental"

BANCA JULGADORA:

TITULARES:	SUPLENTES:
Igor Luis Kaefer (INPA)	Bruce Walker Nelson (INPA)
Renato Cintra (INPA)	Camila Cherem Ribas (INPA)
Pedro Ivo Simões (INPA)	

	PARECER	ASSINATURA
Igor Luis Kaefer (INPA)	(X) Aprovado ( ) Reprovado	Igor Luis Kaefer
Renato Cintra (INPA)	(X) Aprovado ( ) Reprovado	
Pedro Ivo Simões (INPA)	(X) Aprovado ( ) Reprovado	
Bruce Walker Nelson (INPA)	( ) Aprovado ( ) Reprovado	
Camila Cherem Ribas (INPA)	( ) Aprovado ( ) Reprovado	

Manaus(AM), 15 de março de 2013

OBS:

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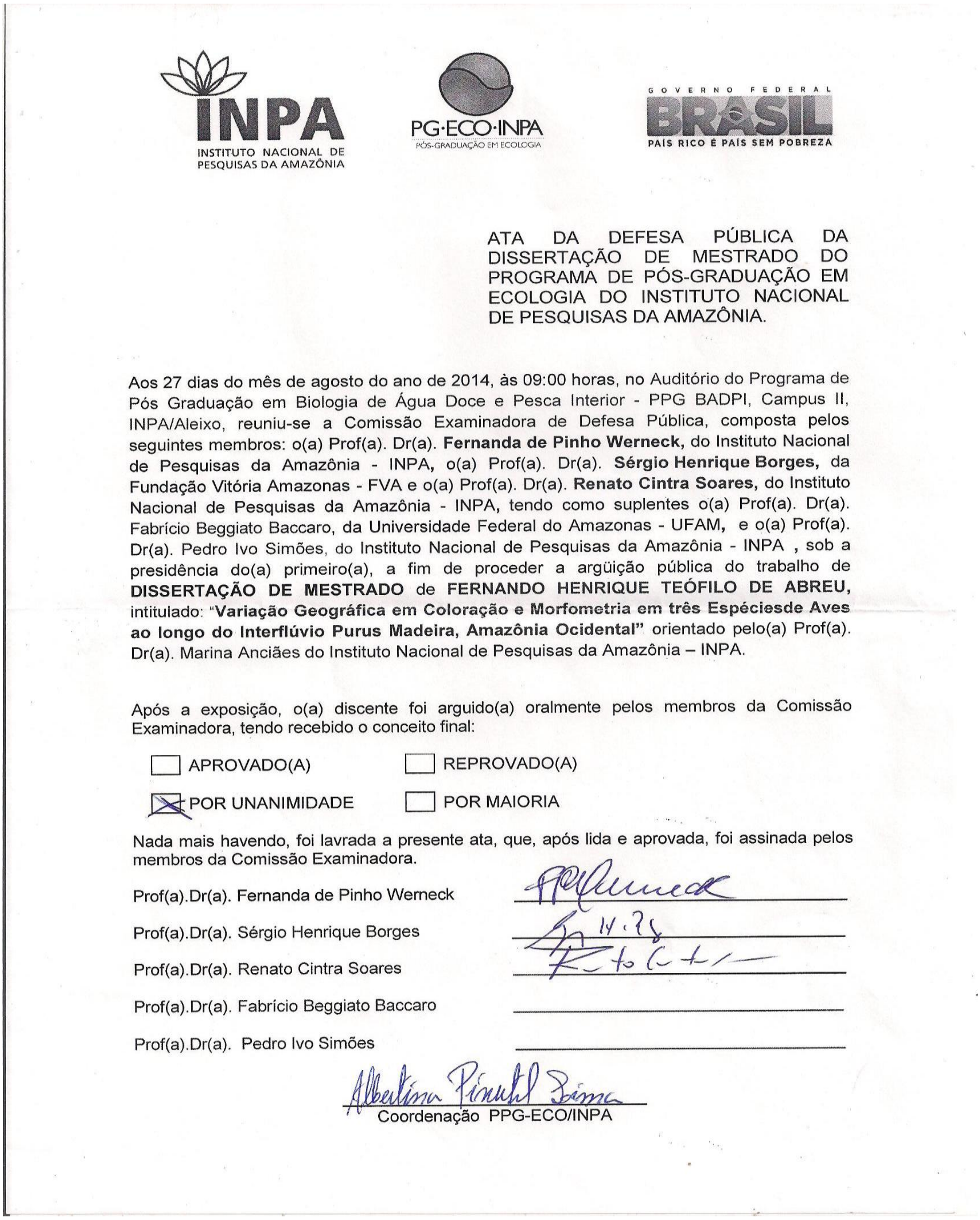
Ata da Defesa Pública



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ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 27 dias do mês de agosto do ano de 2014, às 09:00 horas, no Auditório do Programa de Pós Graduação em Biologia de Água Doce e Pesca Interior - PPG BADPI, Campus II, INPA/Aleixo, reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o(a) Prof(a). Dr(a). **Fernanda de Pinho Werneck,** do Instituto Nacional de Pesquisas da Amazônia - INPA, o(a) Prof(a). Dr(a). **Sérgio Henrique Borges,** da Fundação Vitória Amazonas - FVA e o(a) Prof(a). Dr(a). **Renato Cintra Soares,** do Instituto Nacional de Pesquisas da Amazônia - INPA, tendo como suplentes o(a) Prof(a). Dr(a). Fabrício Beggiato Baccaro, da Universidade Federal do Amazonas - UFAM, e o(a) Prof(a). Dr(a). Pedro Ivo Simões, do Instituto Nacional de Pesquisas da Amazônia - INPA , sob a presidência do(a) primeiro(a), a fim de proceder a argüição pública do trabalho de **DISSERTAÇÃO DE MESTRADO** de **FERNANDO HENRIQUE TEÓFILO DE ABREU,** intitulado: "**Variação Geográfica em Coloração e Morfometria em três Espéciesde Aves ao longo do Interflúvio Purus Madeira, Amazônia Ocidental"** orientado pelo(a) Prof(a). Dr(a). Marina Anciães do Instituto Nacional de Pesquisas da Amazônia – INPA.

Após a exposição, o(a) discente foi arguido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

☐ APROVADO(A) ☐ REPROVADO(A)

☒ POR UNANIMIDADE ☐ POR MAIORIA

Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof(a).Dr(a). Fernanda de Pinho Werneck ____________

Prof(a).Dr(a). Sérgio Henrique Borges ____________

Prof(a).Dr(a). Renato Cintra Soares ____________

Prof(a).Dr(a). Fabrício Beggiato Baccaro ____________

Prof(a).Dr(a). Pedro Ivo Simões ____________

Albertina Pimentel Lima
Coordenação PPG-ECO/INPA