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SISTEMÁTICA E BIOGEOGRAFIA DO COMPLEXO *AUTOMOLUS INFUSCATUS*
(AVES:FURNARIIDAE): TESTANDO HIPÓTESES DE DIVERSIFICAÇÃO PARA O
NEOTRÓPICO

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Manaus - Amazonas

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NEOTRÓPICO

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Sinopse:

Realizou-se uma análise filogenética do complexo *Automolus infuscatus* (Aves:Furnariidae) para identificar a diversidade críptica dentro do grupo bem como as relações entre as linhagens e o tempo de divergência entre elas. Realizou-se análises demográficas e biogeográficas para acessar a história evolutiva das linhagens e associar os padrões encontrados às hipóteses de diversificação propostas para aves das terras baixas Neotropicais.

Palavras-chave: sistemática molecular, diversidade críptica, diversificação, relógio molecular, Neotrópico

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Resumo

Recentes revisões do gênero de aves neotropicais *Automolus* e da família Furnariidae indicaram a parafilia de *A. infuscatus* e revelaram um complexo de espécies englobando *A. infuscatus*, *A. ochrolaemus*, *A. paraensis*, *A. leucophthalmus*, *A. lammi* e *A. subulatus*, o último historicamente classificado no gênero *Hyloctistes*. O conhecimento detalhado da taxonomia, distribuição geográfica, relação filogenética e idade das divergências de um táxon possibilita explorar sua história evolutiva e testar diferentes cenários de diversificação. Diferentes hipóteses biogeográficas foram propostas para explicar os padrões de distribuição encontrados na biota das florestas de terras baixas neotropicais, onde as espécies do complexo habitam. Essas hipóteses, em geral, relacionam a diversificação das linhagens à evolução geológica da paisagem e à ciclos de expansão e retração florestal associados à variações climáticas. Nesse contexto, inferimos as relações filogenéticas, tempos de divergência e biogeografia do complexo *A. infuscatus* buscando desvendar a diversidade críptica dentro do complexo e revelar sua história evolutiva. Para isso sequenciamos dois marcadores mitocondriais (ND2 e cytb) e três nucleares (ACO, G3PDH e Fib7) compreendendo 302 indivíduos pertencentes a todas as espécies do complexo e a maioria das subespécies descritas. Nossas análises suportam a parafilia de *A. infuscatus*, indicando a existência de pelo menos dois clados distintos não relacionados proximamente. As demais espécies foram recuperadas como monofiléticas. No entanto, uma diversidade intraespecífica bem estruturada foi encontrada com 19 linhagens sugerindo uma grande diversidade críptica dentro das espécies descritas. *A. subulatus* foi recuperado dentro do complexo, corroborando sua manutenção no gênero. Os padrões de distribuição das linhagens encontradas combinam com padrões de distribuições conhecidos para aves de florestas de terras baixas neotropicais. Apesar de alta congruência entre distribuições de diferentes linhagens, com diversas linhagens irmãs separadas atualmente pelas mesmas barreiras, a incongruência temporal entre as divergências de linhagens co-separadas pelas mesmas barreiras revela uma história evolutiva complexa. Enquanto eventos mais antigos podem estar relacionados com o surgimento de barreiras como os Andes e os grandes rios amazônicos, diversos eventos mais recentes sugerem dispersão após a consolidação dessas barreiras. Nossas análises sugerem que o complexo teve sua origem a cerca de 6 milhões de anos (Ma) e que habitava o oeste amazônico entre o Mioceno superior e o início do Plioceno. Considerando o hábito ripário das espécies do clado irmão, o surgimento e as primeiras diversificações do complexo podem estar relacionadas ao estabelecimento de florestas de terra firme na região conforme ela mudava de uma planície de inundação para um sistema fluvial.

Abstract. Systematics and Biogeography of the *Automolus infuscatus* complex (Aves:Furnariidae): Testing diversification hypotheses to the Neotropic.

Recent revisions of the avian neotropical genus *Automolus* and the Furnariidae family pointed to the paraphyly of *A. infuscatus* and revealed a species complex comprising *A. infuscatus*, *A. ochrolaemus*, *A. paraensis*, *A. leucophthalmus*, *A. lammi* and *A. subulatus*, the latter historically classified in the genus *Hyloctistes*. The detailed knowledge of the taxonomy, geographic distribution, phylogenetic relationship and divergence times of a taxon allows to explore its evolutionary history and test different scenarios of diversification. Different biogeographical hypotheses were proposed to explain the patterns of distribution found in the neotropical lowland forests biota, where the species of the complex inhabit. These hypotheses, generally, relate lineages diversification to the geological evolution of the landscape and cycles of forestry expansion and retraction associated with climatic variations. In this context, we inferred the phylogenetic relationships, divergence times and biogeography of the *A. infuscatus* complex seeking to unveil the cryptic diversity within the complex and reveal its evolutionary history. To do that we sequenced two mitochondrial (ND2 and cytb) and three nuclear markers (G3PDH, ACO, Fib7) comprising 302 individuals belonging to all species in the complex and most described subspecies. Our analysis support the paraphyly of *A. infuscatus*, indicating the existing of at least two distinct clades not closely related. The remaining species were all recovered as monophyletic. Notwithstanding, a well structured intraspecific diversity was found with 19 lineages suggesting a great cryptic diversity within the described species. *A. subulatus* was recovered within the complex, corroborating its positioning inside the genus. The patterns of distribution encountered match with known distribution patterns of neotropical lowland birds. In spite of the high congruence between distributions of different lineages, with several sister lineages currently separated by the same barriers, the temporal incongruence between divergences of lineages co-separated by the same barriers reveals a complex evolutionary history. While older events might be related with the emergence of barriers such as the Andes and major amazonian rivers, younger events suggest dispersal after the consolidation of those barriers. Our analysis suggest that the complex had its origin around 6 million years (Ma) and inhabited Western Amazonia in Late Miocene-Early Pliocene. Considering the riparian habit of species in its sister clade, the rise and early diversifications of the complex may be related to the establishment of *terra firme* forests as it changed from a floodplain to a fluvial system.

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INTRODUÇÃO GERAL

A biogeografia é o ramo da ciência que se dedica ao estudo da distribuição e evolução de organismos no tempo e no espaço (Ball, 1976). De uma forma geral, quando organismos apresentam padrões congruentes de distribuição isso pode indicar uma história evolutiva comum. Há séculos biólogos e naturalistas tentam entender esses padrões e desvendar os eventos responsáveis por eles. Para isso fez-se necessário a integração de um vasta gama de disciplinas como sistemática filogenética, geologia, biologia molecular e evolutiva, paleontologia e ecologia. Nos últimos anos a biogeografia passou por grandes avanços devido a popularização de técnicas laboratoriais que facilitam a extração e sequenciamento do DNA, evolução dos métodos analíticos e acúmulo crescente de informações geológicas e paleoclimáticas (Donoghue & Moore, 2003; Riddle et al., 2008; Baker et al. 2014).

Atualmente, filogenias datadas permitem investigar o tempo de divergência de linhagens e, assim, testar hipóteses de vicariância em relação a eventos conhecidos (Crisp et al. 2010). No entanto, sem um bom conhecimento taxonômico não se pode ter precisão na dimensão da diversidade, na distribuição das linhagens e na relação evolutiva entre elas, gerando interpretações errôneas de eventos de diversificação (Bates & Demos, 2001; Rull, 2011; Smith et al., 2013). Esta questão é especialmente relevante para regiões tropicais onde a falta de recursos e infraestrutura tem gerado um déficit de informação comparadas com regiões temperadas onde, em geral, estão os países mais ricos do mundo (Beheregaray, 2008). Contrastantemente, essas regiões sub-representadas apresentam, em geral, as maiores biodiversidades do planeta (Jenkins et al., 2013).

As florestas de terras baixas da América do Sul e Central são um desses casos. Essas florestas abrigam as maiores diversidades em plantas e vertebrados da Terra (Myers et al., 2000; Mittermeyers et al., 2003, Jenkins et al., 2013). Embora as aves sejam provavelmente o grupo melhor conhecido no Neotrópico, a constante descrição de novas espécies ilustra o parco conhecimento taxonômico que temos da região (Brumfield, 2012; Whitney & Cohn-Haft, 2013) e, apesar de grande parte dos estudos biogeográficos pra região ter se baseado em linhagens de aves, a falta de uma taxonomia refinada retém o avanço do conhecimento da história evolutiva da região (Ribas *et al.*, 2012).

Nesse contexto, estudei filogenética e filogeograficamente um grupo de aves com ampla distribuição pelas florestas de terras baixas na região Neotropical, o complexo *Automolus infuscatus* (Aves: Furnariidae). Como muitos táxons de aves Neotropicais, as espécies dentro deste complexo apresentam taxonomia e sistemática confusas, com indícios prévios de uma ampla diversidade críptica envolvida (Zimmer, 2002; 2008; Derryberry et al., 2011; Claramunt et al., 2013). Para isso, realizei uma ampla amostragem pela distribuição conhecida das espécies descritas, com amostras provenientes de diferentes coleções nacionais e internacionais. Para acessar a diversidade genética, a relação entre as linhagens e suas respectivas histórias demográficas, trabalhei com dois marcadores mitocondriais e três nucleares. Baseado nos padrões encontrados, avaliei para as linhagens do complexo as principais hipóteses biogeográficas propostas para a biota das florestas de terras baixas neotropicais.

Objetivos

Objetivo geral

Desvendar a diversidade críptica dentro do complexo *Automolus infuscatus* e sua história evolutiva nas florestas de terras baixas na região Neotropical

Objetivos específicos

- Identificar quais são as linhagens evolutivas dentro do complexo e quais os limites de suas distribuições;
- Estimar as relações filogenéticas entre as linhagens e os tempos de divergência entre elas;
- Investigar a dinâmica demográfica de cada linhagem ao longo do tempo;
- Relacionar os padrões encontrados aos eventos biogeográficos que afetaram a Amazônia e a região Neotropical durante o Neogeno e o Quaternário.

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Capítulo 1

Schultz, E. D., Burney, C. W., Brumfield, R. T., Cracraft, J., Polo, E.,
Ribas C. C. **Systematics and Biogeography of the *Automolus infuscatus*
complex (Aves:Furnariidae): cryptic diversity reveals western Amazonia
as the origin of a transcontinental radiation.** Manuscrito em revisão –
Molecular Phylogenetics and Evolution

Systematics and biogeography of the *Automolus infuscatus* complex (Aves; Furnariidae): cryptic diversity reveals western Amazonia as the origin of a transcontinental radiation.

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ABSTRACT

A revision of the avian Neotropical genus *Automolus* and the Furnariidae family points to the paraphyly of *A. infuscatus* and reveals a species complex comprising *A. infuscatus*, *A. ochrolaemus*, *A. paraensis*, *A. leucophthalmus*, *A. lammi* and *A. subulatus*, the latter historically classified in the genus *Hyloctistes*. Detailed knowledge of the taxonomy, geographic distribution, phylogenetic relationship and divergence times of a taxon allows exploration of its evolutionary history and the testing of different scenarios of diversification. In this context, we studied the *A. infuscatus* complex using molecular data in order to unveil its cryptic diversity and reveal its evolutionary history. For that we sequenced two mitochondrial (ND2 and cytb) and three nuclear markers (G3PDH, ACO, Fib7) for 302 individuals belonging to all species in the complex and most described subspecies. Our analysis supports the paraphyly of *A. infuscatus*, indicating the existing of at least two distinct clades not closely related. The remaining species were all recovered as monophyletic. Notwithstanding, a well-structured intraspecific diversity was found with 19 lineages suggesting substantial cryptic diversity within the described species. *A. subulatus* was recovered within the complex, corroborating its position inside the genus. In spite of the high congruence between distributions of different lineages, with several sister lineages currently separated by the same barriers, the temporal incongruence between divergences over the same barriers reveals a complex evolutionary history. While older events might be related to the emergence of barriers such as the Andes and major Amazonian rivers, younger events suggest dispersal after the consolidation of those barriers. Our analysis suggests that the complex had its origin around 6 million years (Ma) and inhabited Western Amazonia in Late Miocene-Early Pliocene. Considering the riparian habit of species in its sister clade, the rise and early diversifications of the complex may be related to the establishment of terra firme forests as it changed from a floodplain to a fluvial system. The late Amazonian colonization by *A. subulatus* and *A. ochrolaemus* lineages may have been hampered by the previous existence of well established *A. infuscatus* lineages in the region.

KEYWORDS

Molecular systematics, cryptic diversity, molecular clock, Neotropical lowlands, historical biogeography

1. INTRODUCTION

The Neotropical lowlands harbor one of the most diverse biotas in the world (Pearson, 1977; Mittermeier *et al.*, 2003, Jenkins *et al.*, 2013). Nonetheless, the taxonomic knowledge of this diversity, along with the phylogenetic relationships and the patterns of distribution of its taxa are still in early stages (Silva *et al.*, 2005; Leite & Rogers, 2013). Despite this incipient knowledge, the origin of this astonishing diversity has been a central issue in biogeographers' minds for over a century (e.g. Wallace, 1852; Chapman, 1917, Haffer, 1969; Cracraft & Prum, 1988; Smith *et al.*, 2014). Different hypotheses under distinct approaches have been proposed seeking to elucidate the main drivers of diversification in the region (revisions in Haffer, 1997; Moritz *et al.* 2000; Leite & Rogers 2013).

Generally, two main mechanisms have been discussed as drivers of diversification in Neotropical lowlands. One suggests that the geological evolution of the region, particularly the Andean uplift and the consequent evolution of major drainage systems, was responsible for isolating populations on opposite sides of both rivers and the Andes, leading to speciation (Chapman, 1917; Sick, 1967; Capparella, 1991; Ayres & Clutton-Brock, 1992; Brumfield & Capparella, 1996; Hoorn *et al.*, 2010). The second proposes that climatic oscillations that were stronger during the Pleistocene (< 2.6 million years, Ma hereafter) caused cycles of forest retraction and expansion resulting in isolated populations in forest patches (refuges), thus leading to speciation (Haffer, 1969; Prance, 1982; Haffer & Prance 2001; Garzón-Orduña *et al.*, 2014).

Over the last decades many studies have associated Pleistocene and Neogene diversification with the geological evolution of the region (Rull, 2013). However, emerging evidence suggests that: (i) climate did not vary uniformly across South America over the Pleistocene glacial cycles (Cheng *et al.*, 2013); (ii) current configuration of the drainage system might be younger than previously thought, with a dynamic Plio-Pleistocene history (Campbell *et al.*, 2006; Latrubesse *et al.*, 2010); and (iii) current biogeographic patterns seem to be the result of an amalgam of processes that may have happened concomitantly (Ribas *et al.*, 2012; d'Horta *et al.*, 2013; Batalha-Filho *et al.*, 2014). Furthermore a recent comparison of multiple Neotropical bird lineages co-

separated by the same barriers has proposed that diversification in the tropics is mainly driven by the amount of time a lineage has persisted in the landscape and each lineage's specific dispersal abilities (Smith *et al.*, 2014), a hypothesis that minimizes the link between Earth history and biotic diversification. Spatio-temporal comparison of co-distributed lineages and landscape evolutionary histories can be especially revealing as it allows testing hypotheses of the effects of different events and processes across those lineages (Donoghue & Moore, 2003; Crisp *et al.*, 2011). However, our poor knowledge of Neotropical diversity and the absence of comprehensive distributional data remain as major challenges to Neotropical biogeography (Leite & Rogers, 2013).

Birds are arguably the best-known taxon in the Neotropics, even though new species are frequently described (Brumfield, 2012), sometimes by discovering species completely new to science (Cohn-Haft *et al.*, 2013), but mainly through systematic revision of widespread species, uncovered as several distinct lineages (Ribas *et al.*, 2012; Fernandes *et al.*, 2012, 2014; d'Horta *et al.*, 2013, Whitney & Cohn-Haft, 2013). The genus *Automolus* is one such case. Several subspecies have been described in each species (Vaurie, 1980; Remsen, 2016a, 2016b, 2016c, 2016d), and analysis of vocalization and morphology support the elevation of some of these taxa to species-level (Zimmer, 2002; 2008). Lately, molecular phylogenies have indicated that the genus is not monophyletic, with some species more closely related to species in other genera, as well as *Hyloctistes subulatus* nested within the *Automolus* clade (Derryberry *et al.*, 2011; Claramunt *et al.*, 2013). Also, the Amazonian *A. infuscatus* clearly isn't a monophyletic species, but the need for a denser sampling has resulted in authors keeping the species name awaiting a more detailed review (Claramunt *et al.*, 2013). Here, we employ a phylogenetic and phylogeographic framework to investigate the species complex containing *A. infuscatus*, *A. paraensis*, *A. leucophthalmus*, *A. lammi*, *A. ochrolaemus* and *A. subulatus*, hereafter called the *A. infuscatus* complex. Our goal is to reveal the hidden diversity within the complex and disentangle its evolutionary history. Considering the morphological and ecological similarity among these species (Zimmer, 2002; Claramunt *et al.*, 2013) it is reasonable to expect that if major landscape changes and climatic oscillations were responsible for the diversification within the group, then the question

arises whether different lineages show similar patterns of speciation and demographic histories. This prediction allows testing whether temporal co-divergence over the same barriers promoted co-speciation and congruent demographic histories of sympatric lineages, which might indicate a common effect of landscape history on diversification.

2. MATERIALS AND METHODS

2.1. Sampling

We sampled a total of 318 individuals distributed over most of the known distribution of each species within the *A. infuscatus* complex. One additional individual of *A. rufipileatus* was used as outgroup (Claramunt *et al.*, 2013). DNA was extracted from tissues obtained from several museum collections (Appendix A). Two mitochondrial markers were sequenced for 295 individuals: NADH dehydrogenase subunits 2 (ND2) and cytochrome b (cyt b). For a subset of samples, including all species and most described subspecies, at least one, and up to three nuclear loci were also sequenced: b-fibrinogen intron 7 (Fib7, 159 individuals), glyceraldehyde-3-phosphodehydrogenase gene (G3PDH, 104 individuals), and the sex-linked gene for aconitase (ACO, 132 individuals). For details on sampling and laboratorial procedures see Appendix B.

2.2. Phylogenetic analysis based on mtDNA data

To access the phylogenetic relationships among individuals, a concatenated matrix with the two mitochondrial markers (ND2 and Cyt b) was analyzed under three different approaches: Bayesian Inference (BI) in MrBayes 3.2.3 (Ronquist *et al.*, 2012), Maximum Likelihood (ML) in RaxML V.8.2 (Stamatakis, 2014) and Maximum Parsimony (MP) in TNT V. 1.5 (Goloboff *et al.*, 2008). The best-fitting model of evolution for each gene for BI analysis was estimated using the Bayesian Information Criterion (BIC) in jModelTest 2.1.7 (Darriba *et al.*, 2012). HKY+G for ND2 and HKY+I+G for Cytb were selected as the best fitting models. Two parallel runs were performed with four chains each with 10 million generations, sampling parameters and trees every 1000 generation. For the ML analyses a GTR + Gamma model was used, with 100 independent searches. Nodal supports were accessed through 1000 nonparametric bootstrap replicates. For the MP

analysis a heuristic search was implemented with branch-swapping using tree bisection and reconnection (TBR). The “xmult” option was used to run multiple replications using sectorial searching, ratcheting, and tree fusing, with 10 hits and 10 replications for each hit. Support values were accessed through bootstrap resampling with 100 replications.

2.3. Genetic structure of nuclear markers

Since we are dealing with recent events and considering the impact of the higher effective population size of nuclear markers compared to mitochondrial markers in the process of lineage sorting, a population structure analysis for the whole *A. infuscatus* complex was performed for the nuclear markers. BAPS 6.0 was used to define the most likely number of subpopulations present in the sample, as well as designating individuals for each (mixture), taking into account the linkage between sites belonging to the same gene (Corander & Tang, 2007). Then, an ancestral genotype mixture analysis (admixture) has been performed in which, based on the partition obtained in the previous mixture analysis, each individual is characterized by the assignment of portions of its genotype for each of the subpopulations found, allowing to identify possible plesiomorphies or gene flow (Corander & Marttinen, 2006). In the mixture analysis likelihood values for each number K of subpopulations, ranging from 1 to 20, was calculated three times, accepting the partition with K value with higher likelihood. Further analysis with the K value limited to numbers lower than the optimum were performed to get an idea of genetic relative distance between each defined subpopulation. The Admixture analysis started with 50 iterations, 50 individuals of reference for each subpopulation and 10 iterations for each individual, which were doubled until achieving convergence in results. The admixture result was plotted with the mtDNA gene tree, to facilitate cross-visualization (Fig. 1).

2.4. Species delimitation, multilocus phylogenetics and molecular dating

To test if the well-supported mitochondrial clades could be treated as independent evolutionary lineages, we employed a multilocus coalescent framework implemented in BPP (Yang & Rannala, 2010). The method is a bayesian approach that considers the

uncertainty of gene trees to calculate the posterior probability of potential species delimitations. The lineages recognized in the BPP analysis were used as independent evolutionary units for the demographic analysis, species tree reconstruction, and biogeographic analysis.

Divergence times were estimated in a species tree framework (*BEAST, Heled and Drummond 2010) using the species limits defined in the BPP analysis. In addition, molecular dating was also performed in BEAST (Drummond *et al.*, 2012) using a dataset with one individual representing each recognized lineage and the five loci, when available. This last approach was employed to maximize support for basal nodes in the phylogeny. An uncorrelated lognormal relaxed-clock was implemented on both analyzes. Calibration was based on two prior distributions: 1- The widely used Cyt B substitution rate of 2.1% (Weir & Schluter, 2008). The rate prior was set to follow a normal distribution with a mean of 0.0105 substitutions/site/lineage/million years and a standard deviation of 0.0034.

2- The dating and confidence interval obtained by Derryberry et al (2011) for the basal node within the complex. A normal distribution was used, with mean at 5.57 Ma and standard deviation of 0.5 (4.7 - 6.3 Ma).

Substitution models were unlinked. For the mitochondrial markers the same models selected for the phylogenetic analyses in MrBayes were used, and for the nuclear markers the models estimated in jModeltest were HKY+I+G for G3PDH and Fib7 and HKY for ACO. Two independent runs of 50 million generations were performed sampling trees every 1000 generations. A 10% burn-in was applied after checking for convergence and stationarity using Tracer v1.6 (Rambaut *et al.*, 2014).

2.5. Biogeographical analysis

The R package BioGeoBEARS (Matzke, 2014) was used to reconstruct ancestral distributions and diversification patterns within *A. infuscatus* complex. Based on a given phylogeny and the occurrence areas from each taxon, the method compares in a likelihood framework several ancestral area reconstruction models. Twelve different models were tested, namely: the Dispersal-Extinction Cladogenesis Model (DEC), a

likelihood version of the Dispersal-Vicariance Analysis (DIVALIKE) and a of the range-evolution model of the Bayesian binary model (BAYAREALIKE). The others consists of modifications of the aforementioned models adding parameters to include founder-event speciation (+J) and to improve estimation of extinction rates by prohibiting observed species to transition into being present in no areas (“*”; Massana, 2015). BioGeoBEARS was run using the tree from the molecular dating analysis in BEAST. Based on the distributions of the lineages (Fig. 2) and described Neotropical lowland areas of endemism (Cracraft, 1985; Borges & Da Silva, 2012), nine areas were considered for the analysis: Central America, Chocó, Western Napo, Eastern Napo, Jaú, Guiana Shield, Inambari, Brazilian Shield and Atlantic Forest (see Figure 3). An adjacency matrix was created wherein areas that cannot be connected without trespassing another area are assigned as non-adjacent. The maximum number of areas allowed was set to 3.

2.6. Historical demography

The main assumption of the refuge hypothesis implies drastic population size changes of Amazonian species during the Pleistocene. Thereby, variations on population size through time for each Amazonian lineage were estimated through Extended Bayesian Skyline Plots (EBSP; Heled & Drummond, 2008). Substitution and clock models were unlinked for all the loci and trees were linked for the mitochondrial loci only. Mutational rates and substitution models were used as in the molecular dating. Details on number of individuals and loci as well as number of generations used for each lineage are in Table 1.

3. RESULTS

3.1. mtDNA phylogenetic analysis, nuclear DNA structure and species delimitation

Based on the mtDNA markers, 18 reciprocally monophyletic lineages were recognized with the BI and MP analysis, while seventeen were recognized with ML (Figure 1 and Appendix C Figs. C.1, C.2 and C.3). Four major well-supported clades were recovered in all three approaches (Clades I - IV in Figure 1), but there was no support for the relationships among them. Previous phylogenies of the Furnariidae (Derryberry *et al.*, 2011) and of *Automolus* and closely related genera (Claramunt *et al.*,

2013) were likewise not able to achieve good support for the basal relationships within the complex, even using several different genetic markers. BAPS analysis grouped nuclear sequences in six clusters. The clusters substantially match clades from the mitochondrial phylogeny (Figure 1). Nonetheless, high admixture was found between populations, which could be due to incomplete lineage sorting, or introgression, but no signal of introgression was found in the mtDNA dataset (Figure 1).

The northern Atlantic Forest species *A. lammi*, formerly separated from the southern form *A. leucophthalmus* based on vocal characters was not recognized in our mitochondrial trees. However, because of its clear diagnosis (Zimmer, 2008) we included the species as a hypothesis for our BPP species delimitation test. Hence, based on the eighteen monophyletic lineages recognized by our mitochondrial analysis, we supplied nineteen taxa for the species delimitation test. All three different schemes tested by the BPP analysis recognized nineteen lineages as separated taxa ($p > 0.99$ for all schemes), strongly supporting their validation as independent evolutionary lineages (Table 2).

Paraphyly of *A. infuscatus* as currently recognized was confirmed, as the 5 independent lineages recognized by the BPP analysis within this species are included by the phylogenetic analysis in clades I and II (Figure 1), the later also including *A. leucophthalmus*, *A. lammi*, and *A. paraensis* (Figures 1 and 2a). Nuclear population structure also suggests two distinct groups within "*A. infuscatus*" (green and light blue, Figure 1). Within *A. subulatus*, three lineages were recovered, one in Chocó, sister to two in Amazonia, separated by the upper Amazonas/Solimões river (Figure 1, Clade III; Fig. 2b). All 7 independent lineages recognized by our BPP analysis that are currently included in the species *A. ochrolaemus* were recovered as a monophyletic clade. Within this clade, two lineages from Central America and one from Chocó are sister to the four Amazonian lineages (Figure 1, Clade IV; Fig. 2c).

3.2. Multilocus phylogenetics, divergence dating and biogeographic history

The species tree based on all 5 gene regions had low support for most nodes (Appendix C, Fig. C.4). Although the four main clades were recovered with high support, relationships among them as well as within these main clades were not recovered in the

species tree, despite the high support found for these recent relationships in the mtDNA analysis (Figure 1). There is a vast discussion in the literature about the efficacy of coalescent based versus concatenation methods for relationship and dating estimations (eg. Edwards, 2009; McVay & Carstens, 2013; Gatesy & Springer, 2013; Xi *et al.*, 2015). Although some authors suggest that coalescent based methods produce more accurate results (Heled & Drummond, 2010; Song *et al.*, 2012; Xi *et al.*, 2014), others defend that concatenation remain a useful tool and can perform as well as species tree methods (Pyron *et al.*, 2014; Thompson *et al.*, 2014; Tonini *et al.*, 2015). The tree generated by BEAST based on all 5 gene regions and a reduced sampling of one individual per BPP lineage also recovered the four major clades found in the mitochondrial trees. The relationships among the four clades were not well supported, but most relationships within each of them had high support and agreed with the mtDNA topology. Therefore, we used the BEAST tree for inferring the biogeographic history of the *A. infuscatus* complex (Fig. 3 and Appendix C Figs. C.5 and C.6).

Diversification of the *A. infuscatus* complex started at about 5.5 Ma (Derryberry *et al.* 2011). Although it's not possible to infer the exact order of the first splits within the complex, our data suggest a rapid radiation generating the four main lineages around 5 Ma (Figure 3). The subsequent diversification of these lineages occurred at the Late Pliocene-Pleistocene with extant species aging less than 2 Ma (Figure 3). The best-fitting model for our BioGeoBEARS analysis was DIVALIKE (Table 2). According to this model the ancestral distribution of the complex maps to western South America, with a slightly higher probability of including both Western Napo and Chocó than of including only Western Napo, but with high uncertainty (Appendix C Fig. C.5). The following diversification events related to the origin of the four main lineages are centered in Western Amazonia, with these lineages later occupying the other areas in South and Central America (Fig. 3).

3.3. Demographic history

Extended Bayesian Skyline Plots (EBSP) were produced for all thirteen Amazonian lineages (Fig 4). In seven populations constant population size could be

rejected, as the 95% HPD interval of the number of population size changes does not contain 0. Two lineages, *A. i. cervicalis* and *A. o. turdinus* 1, showed a median of 0, suggesting constant population sizes through time (Table 1). There is no clear spatial pattern related to population size changes, with lineages showing signs of population expansion throughout Amazonia.

4. DISCUSSION

4.1. Cryptic diversity and distribution patterns

The Furnariidae is among the most diverse Neotropical bird families, with high diversity of phenotypes and habitat use (Zyskowski & Prum, 1999; Remsen, 2016a). Nonetheless, many species are cryptic (Ridgley & Tudor, 2009) and several morphologically recognized genera have recently been shown to be paraphyletic (Derryberry *et al.*, 2011). Hence it is interesting to note the partial congruence between genetic diversity and recognized subspecies within the *Automolus infuscatus* complex: thirteen of the nineteen lineages found here match previously described subspecies. The remaining six represent subdivisions of three other subspecies. This indicates that phenotypic data is a good proxy, but at least within this complex, still underrepresents genetic diversity. Similarly, molecular lineages can also shed light on overlooked phenotypic variation. This reciprocal illumination might greatly improve our taxonomic knowledge of Neotropical birds, a basic information for any biogeographic or evolutionary inference.

Historically, five subspecies were recognized for *A. infuscatus*. Over the last years, vocal, morphological and molecular evidence have been indicating that different species might be involved (Zimmer, 2002; Derryberry *et al.*, 2011; Claramunt *et al.* 2013). Our results, including for the first time samples of all the five subspecies, confirm the paraphyly of *A. infuscatus*, corroborating those evidence (Fig. 1 and 3).

The distributions of most lineages within the *A. infuscatus* complex coincide with the main areas of endemism (AoE) described for the Neotropical lowland forests (Cracraft, 1985; Borges & Da Silva, 2012). Within Clade I three taxa were identified in our analyses (Fig. 2a): *A. i. cervicalis* in the Guiana AoE, plus two partially sympatric

lineages within *A. i. badius*, one occupying the Jaú AoE (*badius* 1) and the other (*badius* 2) the eastern Napo AoE as well as the Jaú. The lower Negro River is the barrier currently separating *A. i. cervicalis* and *A. i. badius* 1, whereas *A. i. badius* 2 was found on both margins of the upper Rio Negro (Fig. 2a). The contact between the two *A. i. badius* lineages is in a complex biogeographic region, which is reflected in an uncertainty about the limits between the Jau and Napo AoEs (Borges & da Silva, 2012; Ribas *et al.*, 2012). This contact region is characterized by large areas of white sand soil, that support a distinct kind of forest, classified as white sand vegetation (Borges & da Silva, 2012).

Nominate *A. i. infuscatus* is attributed to western Amazonia, from southeastern Colombia to northern Bolivia being replaced by *A. i. purusianus* in western Brazil, south of the Solimões and west of the Madeira river (Zimmer, 2002; Remsen, 2016d). Our data, in contrast, indicate there is a single lineage occupying the Inambari area of endemism (Fig. 2a): *A. i. purusianus* occurs from the western bank of the Madeira river to the foothills of the Andes. On the other hand, *A. i. infuscatus* is actually restricted to western Napo, north of the Marañon/Solimões rivers, and possibly with the Iça river as the eastern limit to its distribution. Nonetheless, at the base of the Andes one individual was found south of Marañon/Solimões on the left bank of the Huallaga river, at the same locality (Jeberos, Peru) as an individual assigned to *A. i. purusianus* (Fig. 2a). Within Clade II, the *infuscatus/purusianus* clade is sister to the Brazilian Shield and Atlantic Forest (AF) lineages (Fig. 2a). The Brazilian shield *A. paraensis* is a former subspecies of *A. infuscatus* that has been described as a distinct species based on phenotypic and molecular data (Zimmer, 2002; Derryberry *et al.* 2011; Claramunt *et al.*, 2013). In our analysis two reciprocally monophyletic lineages of *A. paraensis* were found, having their distributions divided by the Tapajós river (Fig. 2a). Atlantic Forest species, *A. leucophthalmus* and *A. lammi* were recovered as a monophyletic clade. A close relationship between the northern Atlantic Forest species, *A. lammi*, and the SE Amazonian *A. paraensis* has been proposed based on vocal similarity (Zimmer, 2008). However, like previous phylogenies (Derryberry *et al.* 2011; Claramunt *et al.*, 2013), our results suggest that these lineages are not each other's sister-taxon.

Among the six recognized subspecies of *A. subulatus* we were not able to sample

four, which have very narrow distributions (Remsen, 2016c). They are: *A. s. nicaraguae* in eastern Nicaragua, *A. s. virgatus* in Costa Rica and western Panama, *A. s. cordobae* in northern Colombia and *A. s. lemae* in Sierra de Lema, Venezuela. Nevertheless, we have a broad sampling throughout the species distribution in South America (Fig. 2b). Three lineages were identified in our analysis: one in Chocó, which corresponds to *A. s. assimilis* and two Amazonian lineages mostly separated by the Marañon/Solimões and Hullaga or Ucayali rivers at the foothills of the Andes, both traditionally classified in the subspecies *A. s. subulatus* (Fig. 2b).

According to Remsen (2016b) there are seven subspecies of *A. ochrolaemus*, most of which are in agreement with our results (Fig. 2c). The two lineages found in Central America correspond to *A. o. cervingularis* from southern Mexico to Guatemala and *A. o. hypophaeus* from Honduras to western Panama. There is a third described subspecies for CA, *A. o. exsertus* from the Pacific slope of Costa Rica and Panama for which we had no samples. The Chocoan lineage from eastern Panama to western Ecuador corresponds to *A. o. palidigularis*. Four phylogeographic lineages were found in Amazonia, in contrast to only three described subspecies. Within the northern Amazonian subspecies, *A. o. turdinus*, our analysis recovered two distinct lineages, one of them from the Napo and the other from the Guiana AoE, but apparently co-occurring in the Negro-Branco interfluvium and with low support for the Napo lineage. The two southern Amazonian subspecies, *A. o. ochrolaemus* from Inambari and *A. o. auricularis* from the Brazilian Shield, correspond to genetic lineages identified in our analysis. The barrier for their described distribution is the Purus river but our data show that they co-occur in the upper Madeira-Purus interfluvium. It is interesting to note that despite extensive sampling in the lower portion of this interfluvium, *A. ochrolaemus* was not sampled there, whereas *A. subulatus* 1 and *A. i. purusianus* are quite common in this same region (Fig. 2 a and b).

Both *A. subulatus* and *A. ochrolaemus* (Clades III and IV) have representatives on both sides of the Andes, with Amazonian lineages forming a monophyletic clade, and the first diversification event within this clade corresponding to the Amazonas/Solimões river. Other main Amazonian rivers also delimit lineage distributions, including Madeira, Tapajós, Japurá and Negro. However, the limits of some distributions cannot be clearly

related to any physical barrier, as is the case of the two lineages within *A. i. badius* (Fig. 2a) and within *A. o. turdinus* (Fig. 2c). Also, in western Amazonia, limits between *A. i. infuscatus* and *A. i. purusianus* distributions are fuzzy due to one common locality found for the two taxa (Fig. 2a). The crossing of individuals in the headwaters of Amazonian rivers has already been documented (Harvey *et al.*, 2014; Weir *et al.*, 2015), but this does not seem to disrupt the general pattern of large rivers as the main barriers delimiting current distributions in Amazonia.

4.2. Diversification in Neotropical lowlands

The sister species to the *A. infuscatus* complex (*A. rufipileatus* and *A. melanopezus*), (Derryberry *et al.* 2011, Claramunt *et al.* 2013) are associated with varzea and riparian areas, whereas species within the *A. infuscatus* complex in general prefer *terra firme* forests (Ridgely & Tudor, 2009). According to Derryberry *et al.* (2011) the split between (*A. rufipileatus* and *A. melanopezus*) and the *A. infuscatus* complex occurred at about 8.3 Ma (CI 7.1 - 9.7 Ma). Geological evidence suggests that at this time this region was changing from a floodplain to a fluvial system oriented to the East (Campbell *et al.*, 2006; Figueiredo *et al.*, 2009; Latrubesse *et al.*, 2010; Hoorn *et al.*, 2010). Thus, the origin and early diversification of the complex may be related to the establishment of *terra firme* forests in western Amazon as their ancestor shifted from flooded to more terrestrial habitats. Accordingly, our biogeographic reconstruction suggests that the ancestral distribution of the complex was in western South America, and that around 5 Ma a rapid radiation centered in western Amazonia gave rise to the four main lineages within the complex (Fig. 4).

Long branches lead to diversification within each of the four clades, in the Plio-Pleistocene. Within clades III and IV (*A. subulatus* and *A. ochrolaemus*) the first split corresponds to the separation of cis and trans-Andean lineages. In *A. ochrolaemus* our results suggest it happened twice, first separating Central America lineages and afterward isolating Chocoan from Amazonian lineages. Several phylogeographic studies have proposed that the final uplift of the Andes was responsible for isolating populations, causing allopatric speciation (Ribas *et al.*, 2005; d'Horta *et al.* 2013; Fernandes *et al.*,

2014). When put together, the age of cross-Andean diversification in different avian taxa are however highly variable, diverging in millions of years (Smith *et al.*, 2014) and some studies encountered multiple splitting events in single clades, suggesting a combination of vicariant and dispersal events (e.g. Miller *et al.*, 2008; Weir & Price, 2011). The age of the current configuration of the Andes is also a matter of discussion, being associated from Late Miocene to Plio-Pleistocene (Baker *et al.*, 2014).

According to our biogeographic reconstruction, the first cross-Andean split within *A. subulatus* and *A. ochrolaemus* are associated with vicariant events whereas the second event in *A. ochrolaemus* clade would correspond to dispersal. Although not totally congruent, the estimated time of the two cross-Andean vicariant events (basal split of clades III and IV, Fig. 4) overlap around 2 and 3 Ma, which may indicate a single event related to a relatively recent establishment of the modern configuration of the Andes. The more recent cross-Andean dispersal in the *A. ochrolaemus* clade occurred after the Andes had already reached high elevations. Haffer (1967) proposed that during warmer Pleistocene interglacial periods forests expanded northwards to Caribbean lowlands and upwards to low passes, connecting cis and trans-Andean biotas. Our data, however, do not allow testing the proposed pathways.

Several Amazonian lineages are sympatric with distributions delimited by the same barriers that often correspond to main Amazonian rivers, thus they are suitable models to test hypotheses of diversification in Amazonian lowlands (Figs. 2 and 4). Rivers dividing more than one pair of taxa within the complex include the Madeira, Negro and Solimões, which are among the largest rivers in the region (Latrubesse *et al.*, 2005) and often are credited as major geographical barriers in Amazonia (Wallace, 1852; Burney & Brumfield, 2009; Smith *et al.*, 2014).

Divergences across the Solimões are temporally congruent in three of the major clades (clades 2, 3 and 4), with splits happening at around 0.5 - 1.5 Ma. Despite the congruence, the recent age of these splits makes it unlikely they were caused by the origin of the drainage system (Latrubesse *et al.*, 2010; Hoorn *et al.* 2010). Eventual dispersal across the upper Solimões and its tributaries, mainly the Ucayali and Marañón, could be related to changes in climate affecting river discharge. The complexity of this

region is evidenced by the current patterns of distribution in far western Amazonia (Fig. 2).

On the other hand, divergences across the Negro River on Clades I and IV are temporally discordant, suggesting that different processes are probably involved. The split within Clade I dates to about 1.7 Ma (95% HPD: 2.31-1.19 Ma) in temporal agreement with several other taxa (Ribas *et al.*, 2012; d'Horta *et al.*, 2013; Souza-Neves *et al.*, 2013; Fernandes *et al.*, 2014). However, a much younger divergence time (0.3 Ma, HPD: 0.56-0.17) was encountered in Clade IV over the same barrier (Fig. 4). Within this clade, the demographically stable western, and expanding eastern population, suggest an eastward expansion to the Guiana Shield (Fig. 3). The split across the Madeira river in Clade II dates to about 2.2 Ma (95% HPD: 2.91-1.65), coinciding with estimates from other bird taxa that have been associated to the origin of this river (Ribas *et al.*, 2012; Fernandes *et al.*, 2012, 2014; d'Horta *et al.*, 2013; Lutz *et al.*, 2013). A more recent divergence in the same region is found in Clade IV (0.76 Ma, 95% HPD: 1.1-0.48 Ma), however our sampling shows that the lineages are not clearly delimited by the current course of the Madeira river (Fig. 2c), with lineages from the Brazilian shield and Inambari co-occurring in the headwaters of the Madeira and *A. ochrolaemus* being absent of the lower Madeira-Purus interfluvium despite dense sampling in the region.

According to our dating and biogeographic reconstruction, Clades III and IV (*A. subulatus* and *A. ochrolaemus*, respectively) have a much younger history within the Amazonian lowlands than Clades I and II. *A. subulatus* and *A. ochrolaemus* apparently gradually occupied the region from west to east, and only diversified in central and eastern Amazonia in the last 1.5 Ma (Fig. 4). Cis-Andean *A. subulatus* lineages are still mostly restricted to Western Amazonia (Fig. 2). Within *A. ochrolaemus*, diversification events corresponding to the Madeira and Negro rivers are younger than events corresponding to these same barriers in Clades I and II, which were already broadly distributed in Amazonia at the time. It is possible that the occupation of Amazonia by ancestors of Clades III and IV was affected by the pre-existence of close relatives in the region. Nowadays, Amazonian lineages in Clades I and II are mainly associated to *terra firme* forests, whereas Clades III and particularly IV occupy mainly second growth,

transitional and seasonally flooded forests (Ridgely & Tudor, 2009; Ramsen, 2016b; 2016c; 2016d). Moreover, although the whole complex forages by gleaning arthropods from dead leaves and debris in mixed-species flocks, different lineages rarely occur together in the same flock and, if they do, they vary strikingly in foraging height and are often physically aggressive with each other (Ramsen & Parker, 1984; Robinson & Terborgh, 1995; Rosenberg, 1997). Therefore, interspecific competition possibly played an important role in the evolution of patterns of landscape use in Amazonian *A. subulatus* and *A. ochrolaemus* lineages, and in recent diversification of this whole complex within Amazonia.

4.3. Drivers of diversification in Amazonia

Estimates of divergence times of different lineages across the same barriers often vary, indicating that several processes have shaped the modern distribution of Neotropical birds (Miller *et al.*, 2008; Weir & Price, 2011; d'Horta *et al.*, 2013; Fernandes *et al.*, 2014; Smith *et al.*, 2014). Within the *A. infuscatus* complex several distributional limits coincide, but temporal congruence is limited to splits associated to the Andes and the Solimões river. Therefore, the hypothesis of a single set of biogeographic events, either related to geology or climate, generating the congruent distributions is unlikely. However, we have shown that temporal incongruence may be related to the history of each lineage and the way it uses the habitat. Within the complex, lineages that occupied Amazonia earlier (Clades I and II) have divergences across the Negro and Madeira rivers that are temporally congruent with other Amazonian upland forest groups. Amazonian species within these clades currently occupy denser upland forest, and have formerly been included in the species "*A. infuscatus*". On the other hand, clades III and IV (*A. subulatus* and *A. ochrolaemus*) seem to have been restricted to western South America for a long time, occupying eastern Amazonia more recently, when clades I and II were already there. This may explain the more recent splits, the current distribution patterns, and the use of marginal upland forest habitats by these taxa.

In our demographic analysis, most Amazonian lineages show signs of recent expansion as predicted by the refuge hypothesis. Nonetheless, signal of population

expansion for lineages from all Amazonian areas of endemism argues against the evidence of unequal paleoclimate evolution in eastern and western Amazonia during the Pleistocene (Cheng et al., 2013). Moreover, lineages from the same areas sometimes have contrasting demographic patterns. Thus, although the refuge theory cannot be discarded due to demographic patterns and young diversification events, it is clear that the great diversity in the complex cannot be explained by this mechanism alone.

Ribas et al. (2012) presented a detailed paleobiogeographical model for Amazonian lowland avian diversification, but further phylogeographical studies have also revealed different diversification patterns in Neotropical birds (e.g. Patel et al., 2011; Fernandes et al., 2012, 2013, 2014; d'Horta et al., 2012; Lutz et al., 2013; Souza-Neves et al., 2013; Thom & Aleixo, 2015). Although several instances of temporal coincidence for splits among lineages isolated by the same barriers are found, the order of the events varies greatly. This discrepancy can be related in part to the ancestral distribution of each group, which seems to greatly influence the way landscape changes affected its evolutionary history. Thus, ancestral distribution and ecological features like landscape usage and interspecific competition seem to have greatly influenced the diversification within the complex studied here, and should be incorporated into models that try to explain the great and extremely complex Amazonian diversity.

5. CONCLUSIONS

Our broad sampling has allowed the delimitation of taxonomic units and recognition of complex distribution patterns within the *A. infuscatus* complex. Although many similar distribution patterns are found for the lineages within the complex, differences in age of diversification events and demographic histories suggest a complex evolutionary history, with different historical events and ecological patterns influencing the diversification of the group. The earliest events seem to be related to the formation of the Andes and the consequent evolution of the drainage system, isolating populations at opposite margins of major Amazonian rivers. On the other hand, more recent events seem to be associated with rearrangements of river courses and dispersal across those rivers, possibly associated to Pleistocene climatic fluctuations. The western Amazonian origin of

the complex in the Early Pliocene and the water-association of close relatives in contrast to more terrestrial lineages within the complex may indicate that the early diversification events were associated with the establishment of *terra firme* forests in the region. The ancestral distribution and interspecific competition within the *A. infuscatus* complex seem to have played a crucial role in its evolutionary and biogeographical history.

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Table 1. Number of sequences per marker used, number of MCMC generations ran and Demographic Population Size Changes results for Amazonian lineages in EBSP.

Species	Markers					Demographic Population Size Changes			MCMC
	ND2	CitB	ACO	G3PDH	Fib7	Median	Mean	95% HPD interval	
<i>A. i. badius 1</i>	13	13	18	16	10	1	1.1574	[0,2]	2x10e8
<i>A. i. badius 2</i>	24	24	18	20	12	1	1.2751	[1,2]	1.5x10e8
<i>A. i. cervicalis</i>	13	13	22	18	6	0	0.2532	[0,1]	1x10e8
<i>A. i. infuscatus</i>	9	9	-	-	6	1	1.3142	[1,3]	2x10e8
<i>A. i. paraensis 1</i>	17	17	40	28	20	1	1.1461	[1,2]	2x10e8
<i>A. i. paraensis 2</i>	8	8	12	8	6	1	1.0405	[0,3]	2x10e8
<i>A.i. purusianus</i>	38	38	38	26	24	1	1.3666	[1,3]	2x10e8
<i>A. o. auricularis</i>	33	33	26	14	44	1	1.5933	[1,3]	2x10e8
<i>A. o. ochrolaemus</i>	36	36	14	12	56	1	1.4185	[1,3]	2x10e8
<i>A. o. turdinus 1</i>	12	12	-	-	22	0	0.3858	[0,2]	1x10e8
<i>A. o. Turdinus 2</i>	21	21	8	6	18	1	1.2585	[1,2]	2x10e8
<i>A. s. subulatus1</i>	24	24	-	10	12	1	1.2522	[0,2]	2x10e8
<i>A. s. subulatus 2</i>	8	8	12	12	6	1	1.0017	[0,2]	4x10e8

Table 2. Results of BPP analyses showing posterior probabilities (p) of different divergence schemes tested. ϵ , α and m are different values of fine-tune parameters. θ and τ are priors for the population size parameters and divergence time at the root of the species tree, respectively. SMP = Species Model Prior algorithm.

Algorithm	SMP	θ	τ	Results
0 ($\epsilon = 2$)	3	G(2,1000)	G(2,1000)	Supports all 19 lineages as distinct species (p >0.99)
1 ($\alpha=2, m=1$)	2	G(2,1000)	G(2,1000)	Supports all 19 lineages as distinct species (p >0.99)
1 ($\alpha=2, m=1$)	3	G(2,1000)	G(2,1000)	Supports all 19 lineages as distinct species (p >0.99)

Table 3. Biogeobears results. For each model implemented in the analysis are shown: dispersal (d), extinction (e), founder (j), and values of log-likelihood (LnL) and Akaike Information Criteria (AIC). Best-fitting model (DIVALIKE) and respective AIC value in bold.

Model	d	e	j	LnL	AICc
DEC	0.224	0.617	0.000	-71.898	148.547
DEC+J	0.061	0.000	0.285	-54.187	115.974
DIVALIKE	1.044	5.000	0.000	-49.147	103.043
DIVALIKE+J	0.951	4.991	0.013	-48.901	105.401
BAYAREALIKE	0.199	0.399	0.000	-66.470	137.689
BAYAREALIKE+J	0.091	0.000	0.101	-52.006	111.612
DECstar	0.976	4.892	0.000	-49.203	103.156
DEC+Jstar	0.914	4.858	0.013	-48.943	105.486

DIVALIKEstar	0.056	0.155	0.000	-100.625	206.000
DIVALIKE+Jstar	0.025	0.000	0.168	-50.863	109.326
BAYAREALIKEstar	0.253	0.354	0.000	-72.868	150.486
BAYAREALIKE+Jstar	0.931	5.000	0.013	-48.852	105.305

Fig 1. Mitochondrial gene tree (ND2 and cyt-b) obtained using Bayesian inference. BAPS nuclear admixture clusters shown to the right. Colored bars indicate corresponding clades in the same order in both analyzes, although the number of individuals in each analysis vary. Values at the nodes represent posterior probabilities. Roman numerals indicate the four main clades recovered by all analysis.

Fig 2. Geographical distribution and sampling of *A. infuscatus* complex. Insets are mtDNA Bayesian tree with ML bootstrap/MP bootstrap/BI posterior probabilities as support values. “-” represents the absence of that node in specific analysis. Shaded areas illustrate described ranges for: (a) *A. infuscatus*, *A. paraensis*, *A. leucophthalmus* and *A. lammi* (from lighter to darker grey); (b) *A. ochrolaemus*; (c) *A. subulatus*.

Fig 3. BEAST tree with the results of BioGeoBEARS. Coloured squares represents most likely ancestor areas estimated by the DIVALIKE model. Up to three areas can be occupied by each taxa as maximum range was set to three in the analysis. Biogeographic areas delimited from the *Automolus infuscatus* complex distribution are shown in the map and are: Central America (brown), Chocó (black), Western Napo (purple), Eastern Napo (light blue), Jaú (orange), Guianan Shield (green), Inambari (red), Brazilian Shield (dark blue) and Atlantic Forest (yellow). Nodes contain posterior probabilities of clades, associated confidence interval (95% HPD) for diversification time (blue bar). Roman numerals indicate the four main clades recovered by all analysis. Main rivers cited in the text are depicted in the map.

Fig 4. Extended Bayesian Skyline Plots from Amazonian lineages, sorted by area of endemism. Dashed lines depict the median population size and the lightly shaded grey the 95% HPD. Green lines show the samples that are summarized by the median and credible

intervals lines. The Y axis represents the effective population size (N_e) x mutation rate in a logarithmic scale and X axis is time in million years.

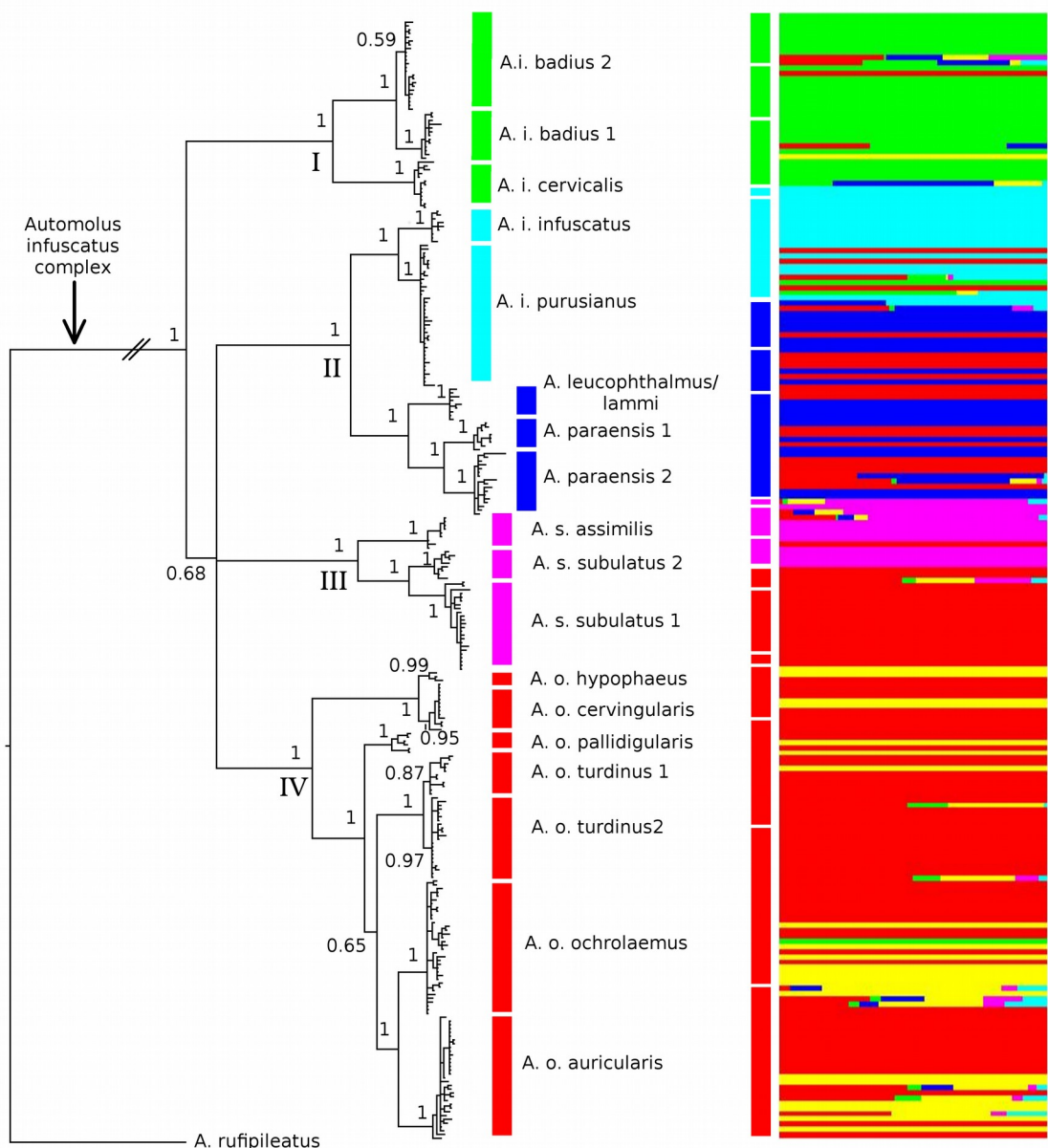


Figure 1.

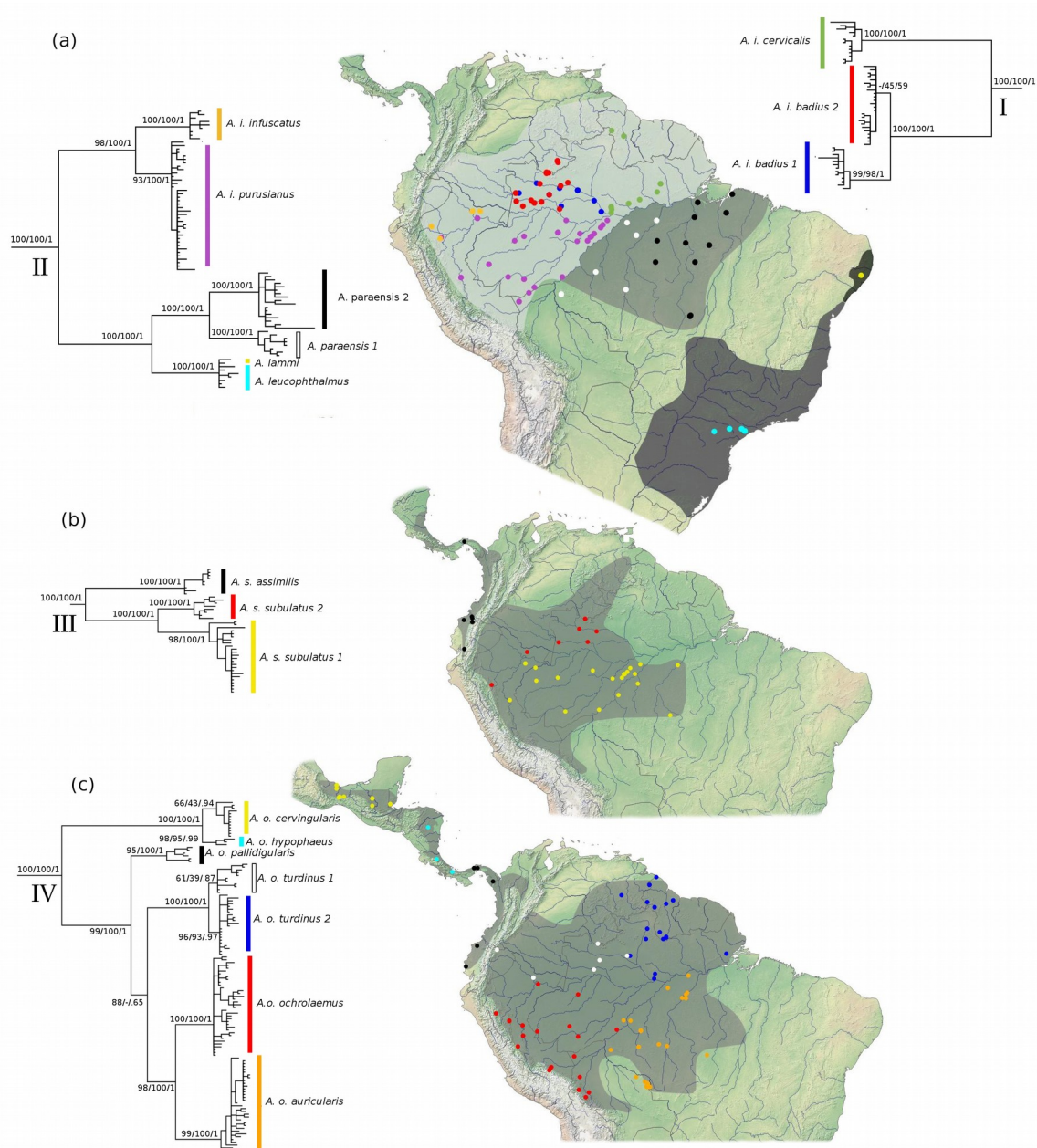


Figure 2.

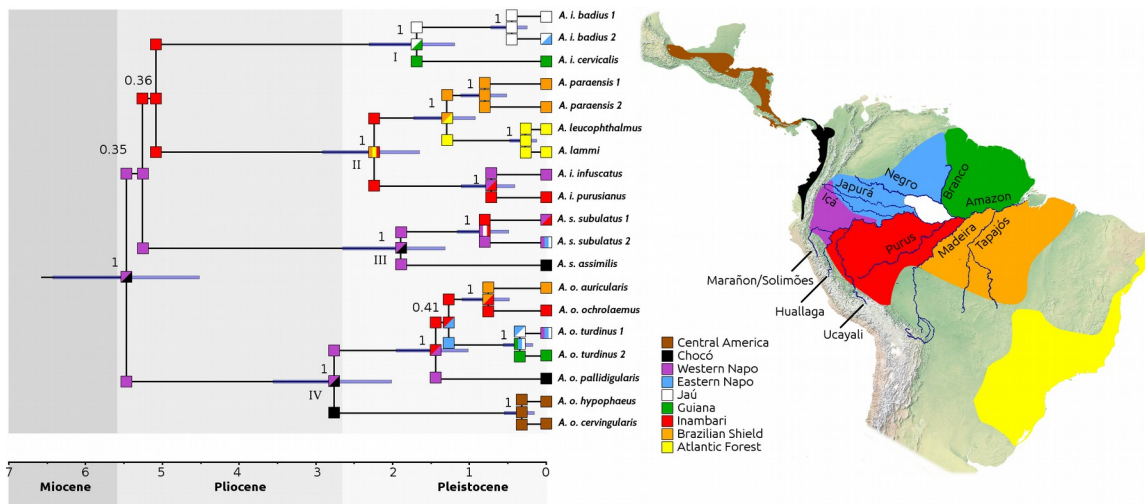
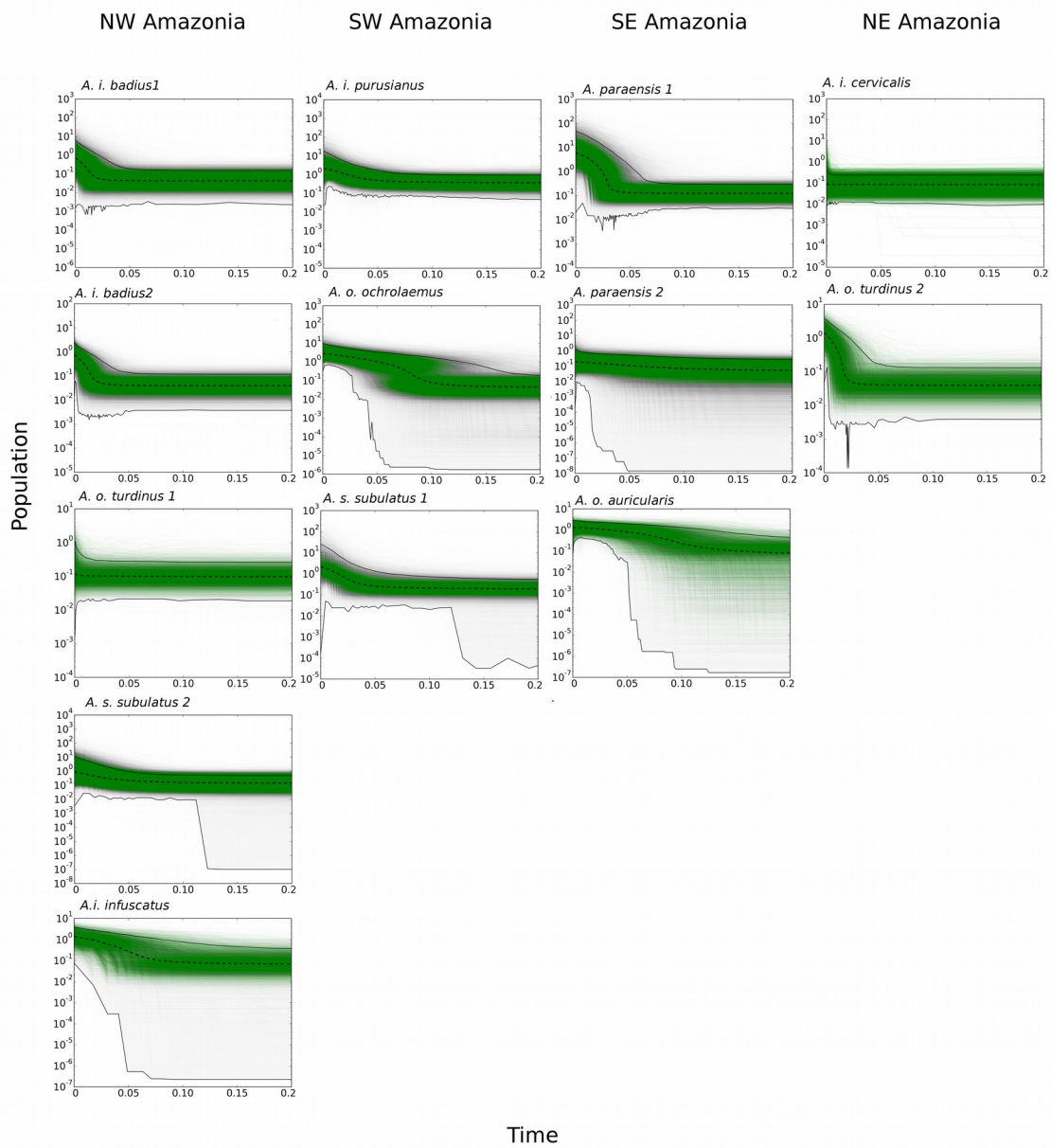


Figure 3.

Figure 4.



Appendix A

Institutions

All tissues were obtained from the following institutions: Academy of Natural Sciences of Drexel University, USA (ANSP); Field Museum of Natural History, Chicago, USA (FMNH); Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA); Laboratório de Genética e Evolução Molecular de Aves, São Paulo, Brazil (LGEMA); Louisiana State University Museum of Natural Science (LSUMZ); Museu Paraense Emílio Goeldi, Belém, Brazil (MPEG); American Museum of Natural History (AMNH);

DNA extraction, amplification and sequencing

Genomic DNA was extracted from muscle and blood tissue samples using a phenol-chloroform protocol (Brufford *et al.*, 1992). The thermal cycling was performed as follows: 95°C for 2 min, and 35 cycles of 94°C for 40 sec, annealing varying from 58C to 62C for 30sec, and 72°C for 1 min; followed by a final step of 72°C for 10 min. PCR products were purified with the PEG protocol. Sequencing of purified PCR products was performed with Big Dye terminator kit (Applied Biosystems, Foster City, California) in an ABI 3130/3130XL automated capillary sequencer (Applied Biosystems®). Sequences were edited and aligned in Genious R7 (Kearse *et al.*, 2012).

Table A.1. Code (as in Figures B.1, B.2 and B.3), Voucher number, Institute of origin, lineage (as recovered by mitochondrial trees) and locality information (presented coordinates are plotted in Fig. 2) associated with each sample of *A. infuscatus* complex studied.

Appendix B

Figure B.1. Maximum Likelihood mtDNA tree generated in RaxML. Values at node represent bootstrap values. Roman numerals indicate the four main clades recovered by all analysis.

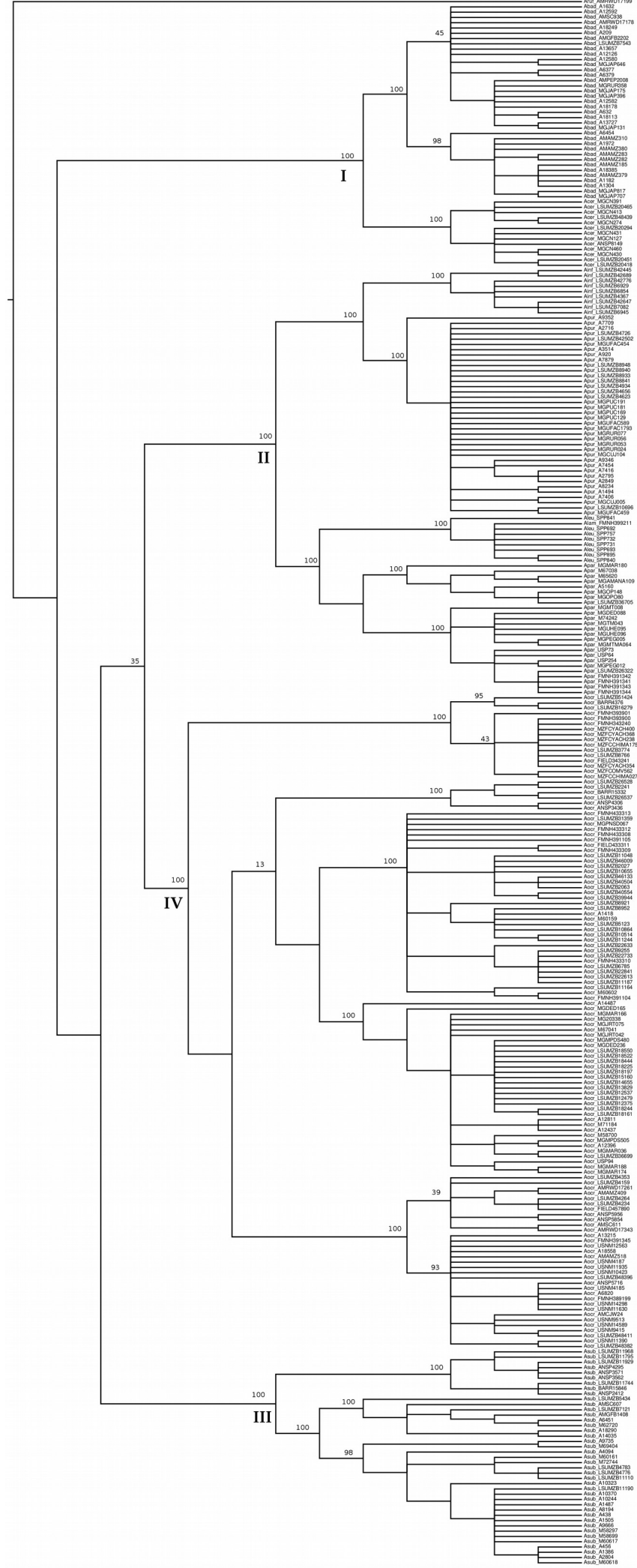
Figure B.2. Maximum Parsimony mtDNA tree generated in TNT. Values at node represent bootstrap values. Roman numerals indicate the four main clades recovered by all analysis.

Figure B.3. Bayesian inference mtDNA tree generated in MrBayes. Values at node represent posterior probabilities. Roman numerals indicate the four main clades recovered by all analysis.

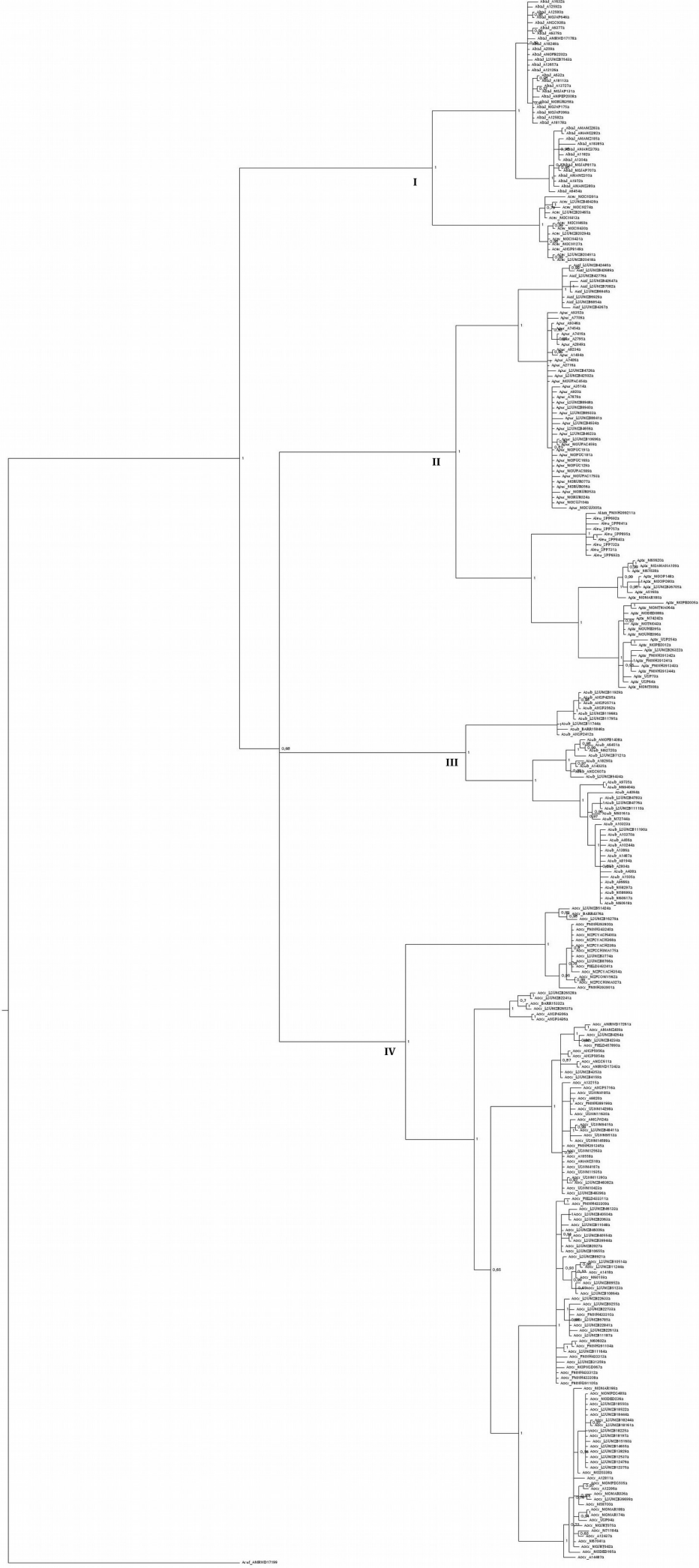
Figure B.4. Species tree generated in *BEAST. Values at node represent posterior probabilities. Roman numerals indicate the four main clades recovered by all analysis.

Figure B.5. BioGeoBEARS “pie” plot. Squares at the nodes represent most likely ancestor areas estimated by the DIVALIKE model. Areas follow. Delimited areas depicted in Fig. 3

Figure B.6. BioGeoBEARS “text” plot. Squares at the nodes represent most likely ancestor areas estimated by the DIVALIKE model. Areas follow. Delimited areas depicted in Fig. 3



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Abal_ABB0107190

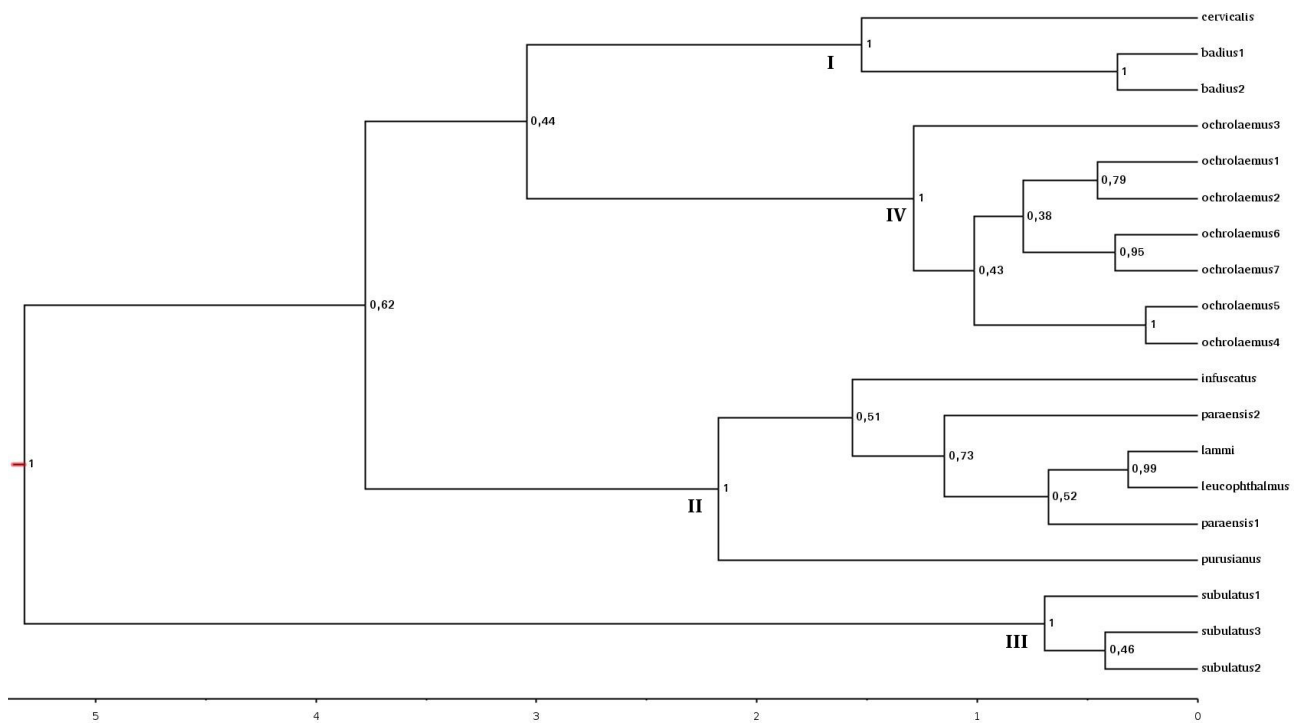


Figure B.4.

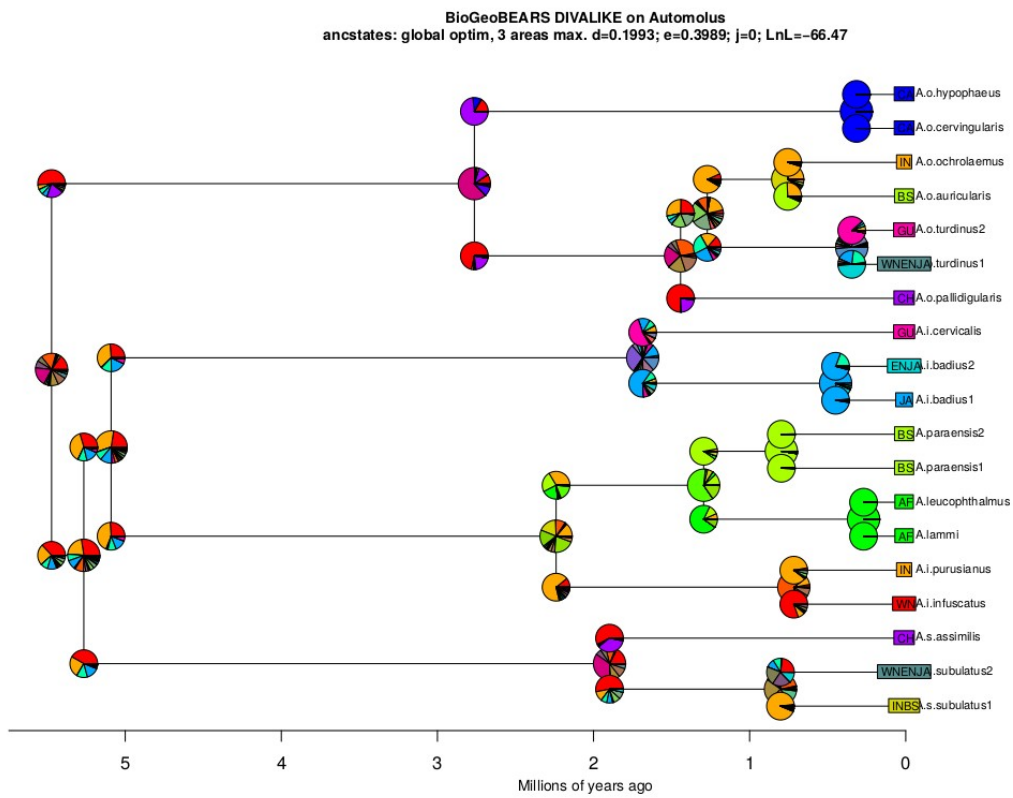
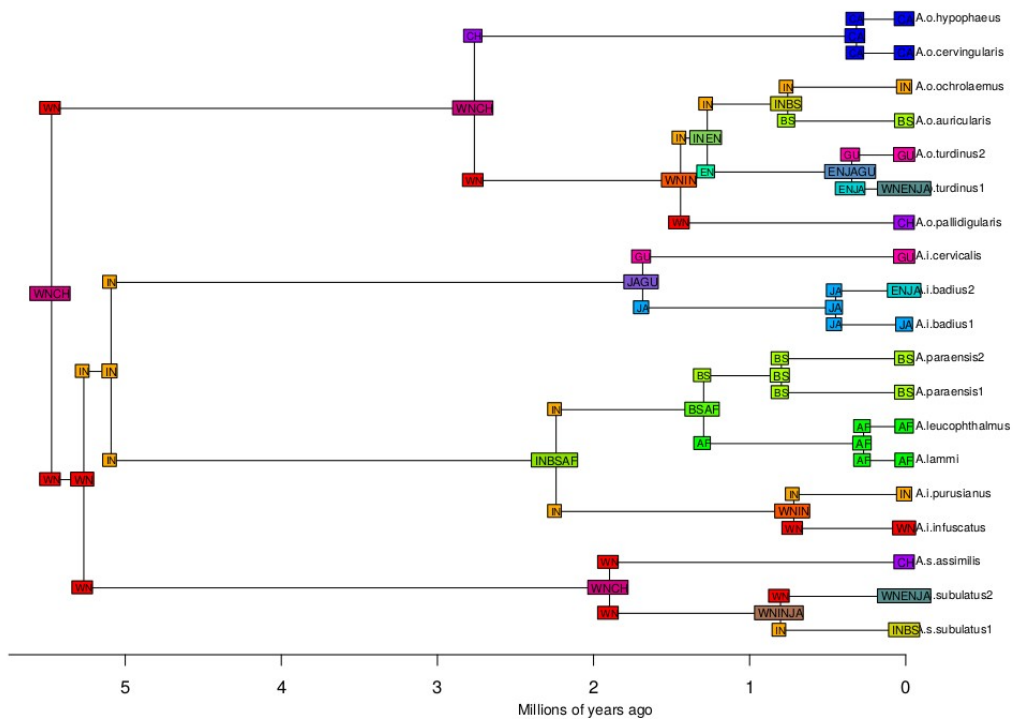


Figure B.5.

Figure B.6.

BioGeoBEARS DIVALIKE on Automolus
 ancstates: global optim, 3 areas max. d=0.1993; e=0.3989; j=0; LnL=-66.47



CONCLUSÕES

Nossa ampla amostragem permitiu a delimitação de unidades taxonômicas e determinação de seus padrões de distribuições no complexo *A. infuscatus*. Uma origem do complexo no oeste amazônico no Plioceno médio pode ser associada com uma mudança de habitats associados à água para terrestre, conforme o oeste amazônico alagado drenava para o leste. Embora muitos padrões comuns de distribuição sejam encontrados em linhagens dentro do clado, diferenças nas idades dos eventos de diversificação e nas histórias demográficas sugerem uma história evolutiva complexa, com diferentes eventos sendo responsáveis pela diversificação no grupo. Eventos mais antigos parecem estar relacionados com a formação dos Andes e a consequente evolução do sistema de drenagens, isolando populações em margens opostas de grandes rios amazônicos. Por outro lado, eventos mais recentes parecem estar associados com rearranjos dos cursos dos rios e dispersões cruzando os rios, possivelmente associado com flutuações climáticas do Pleistoceno.



Ministério da
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AULA DE QUALIFICAÇÃO

PARECER

Aluno(a): EDUARDO DE DEUS SCHULTZ
Curso: ECOLOGIA
Nível: Mestrado
Orientador(a): Dra. Camila Cherem Ribas (INPA)

Título:

“Filogeografia do complexo *Automolus infuscatus* (Aves:Furnariidae): testando hipóteses biogeográficas para o Neotrópico”

BANCA JULGADORA:

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RAFAEL DO NASCIMENTO LEITE	() Aprovado () Reprovado	_____
JANSEN ALFREDO SAMPAIO ZUANON	() Aprovado () Reprovado	_____

Manaus(AM), 25 de Março de 2015

OBS: _____

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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 30 dias do mês de junho do ano de 2016, às 09h00min, na Sala de Aula da Genética - INPA/Campus II/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 Pesquisa da Amazônia - INPA, o(a) Prof(a). Dr(a) **Sergio Henrique Borges**, do Instituto Nacional de Pesquisas da Amazônia - INPA e o(a) Prof(a). Dr(a) **Mario Eric Cohn Haft**, do Instituto Nacional de Pesquisas da Amazônia - INPA, tendo como suplentes o(a) Prof(a). Dr(a) Rafael do Nascimento Leite, do Instituto Nacional de Pesquisas da Amazônia - INPA e Prof(a). Dr(a) Albertina Pimentel Lima, do Instituto Nacional de Pesquisa da Amazônia, sob a presidência do(a) primeiro(a), a fim de proceder a arguição pública do trabalho de **DISSERTAÇÃO DE MESTRADO** de **EDUARDO DE DEUS SCHULTZ**, intitulado: "**Sistemática e biogeografia do complexo automolus infuscatus (Aves: Furnariidae): testando hipóteses de diversificação para o neotrópicos**" Orientado pelo(a) Prof(a) Dr(a). Camila Cherem Ribas, do Instituto Nacional de Pesquisas da Amazônia - INPA.

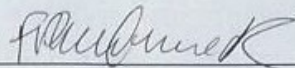
Após a exposição, o(a) discente foi arguido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

APROVADO(A) REPROVADO(A)

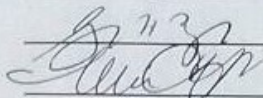
POR UNANIMIDADE POR MAIORIA

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



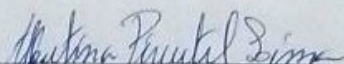
Prof(a). Dr(a). Sergio Henrique Borges



Prof(a). Dr(a). Mario Eric Cohn Haft

Prof(a). Dr(a). Rafael do Nascimento Leite

Prof(a). Dr(a). Albertina Pimentel Lima


Coordenação PPG-ECO/INPA