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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 existence 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.

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 (da Silva et al., 2005; Leite and 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 and 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 and Rogers, 2013).

Generally, two main mechanisms have been discussed as drivers of diversification in Neotropical lowlands. One suggests that

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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 and Clutton-Brock, 1992; Brumfield and 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 and 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; Nogueira et al., 2013; Rossetti et al., 2015); 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 and 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 and 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 and 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). Recent molecular phylogenetic analyzes have indicated that the genus is not monophyletic, and that a species from another genus, *Hyloctistes subulatus*, is nested within it (Derryberry et al., 2011; Claramunt et al., 2013). Also, the Amazonian species *A. infuscatus* clearly isn't monophyletic, 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 combined phylogenetic and phylogeographic framework to investigate the species complex comprising *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 in order to provide new information concerning the different processes that might have contributed to the origin of the great Amazonian biodiversity.

The currently recognized taxa within this complex have high levels of sympatry throughout the Neotropical lowland forests, specially in Amazonia, where many of them co-occur at the main Areas of Endemism (AoE) proposed for the region (Cracraft, 1985). Largely, the described distribution limits of those taxa are geographical elements such as rivers and the Andes, well-known barriers to several lowland understory upland forest bird lineages (Smith et al., 2014). Thus, if major landscape changes and climatic oscillations were responsible for the diversification within the group, then the question arises whether different lineages dwelling the same areas 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 with similar habitat affinities, which might indicate a common effect of landscape history on diversification.

2. Materials and methods

2.1. Sampling and data

We sampled a total of 318 individuals distributed over most of the known distribution of each species within the *A. infuscatus* complex. *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* (cytb). For a subset of samples, including all species and most described subspecies, at least one, and up to three nuclear introns were also sequenced: b-fibrinogen intron 7 (Fib7, 159 individuals), glyceraldehyde-3-phos phodehydrogenase gene (G3PDH, 104 individuals), and the sex-linked gene for aconitase (ACO, 132 individuals). Details on sampling, primers information and PCR and sequencing methods are detailed in Appendices A and B.

Sequences were assembled, edited and aligned in Geneious v7.1.9 (Kearse et al., 2012). For the nuclear sequences, alleles from heterozygous individuals were inferred through the Bayesian algorithm implemented in PHASE v2.1.1 (Stephens et al., 2001), using SeqPHASE (Flot, 2010) to format the input files. A few individuals exhibited insertion-deletions (indels) in Fib 7 sequences, which were treated as missing data.

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 cytb) 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 Parcimony (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 analysis 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. Additionally, mean pairwise p-distances within and among popu-

lations were calculated using the concatenated mtDNA dataset in MEGA 7.0 (Kumar et al., 2016).

2.3. Genetic structure of nuclear markers

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 and 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 and 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 analyses 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. Additionally, to visualize genealogical relationships among sequenced alleles for each nuclear intron, haplotype networks were constructed in HaploViewer (Salzburger et al., 2011) based on the topologies recovered in the species tree analysis.

2.4. Species delimitation

To test if the well-supported mitochondrial clades could be treated as independent evolutionary lineages when also considering nuclear DNA variation, we analyzed all sequenced markers (3 introns plus mtDNA) using two distinct species delimitation methodologies: (i) BPP program (Yang and Rannala, 2010) and (ii) BEAST2 package STACEY (Bouckaert et al., 2014; Jones, 2014). Both methods are Bayesian approaches based on the multispecies coalescent model and estimate the probability of distinct species delimitation hypotheses given multilocus data. Since both methods allow merging but never splitting a given taxa, well-supported mitochondrial clades and diagnosable species were provided as initial hypothesized species.

On BPP, different scenarios were tested in order to check for convergence in the results. First, we used the topology resulting from the mitochondrial ML tree (Appendix A) as guide tree and tested three different combinations of priors (Table 1, a–c). Additionally, seeking to inquire into bias resulting from the given guide tree (Leaché and Fujita, 2010) we tested different guide trees with different prior combinations (Table 1, d–f). Grossly wrong guide trees, as generated by random permutations of populations, are more likely to result in spurious species delimitation (Zhang et al., 2014). Thus, since the main source of uncertainty in our phylogenetic analysis are the basal relationships between the four main clades recovered (see results), the different guide trees tested had random combinations of the relationship among those clades, with internal nodes maintained (Table 1). Each test was performed for 200,000 generations with a burn-in of 50,000.

On STACEY no guide tree is required and, therefore, errors resulting from a priori phylogenetic assumptions are avoided. The input file was created in BEAUti. 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. Four independent runs of 10^8 generations were performed sampling trees every 10,000 generations. A 10% burn-in was applied after checking for convergence and stationarity using Tracer v1.6 (Rambaut et al., 2014).

2.5. Multilocus phylogenetics and molecular dating

Divergence times were estimated in a species tree framework (*BEAST, Heled and Drummond, 2010) using the species limits defined in the species delimitation 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 four loci, when available. This last approach was employed to maximize support for basal nodes in the phylogeny. A Yule tree prior and an uncorrelated lognormal relaxed-clock were implemented on both analyzes. Calibration was based on two prior distributions: (i) The widely used cytb substitution rate of 2.1% (Weir and 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; (ii) 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 followed as on STACEY analysis. Two independent runs of 50 million generations were performed sampling trees every 1000 generations. A 10% burn-in was applied and convergence and stationarity were verified in Tracer v1.6 (Rambaut et al., 2014).

2.6. 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

Table 1
Results of BPP analyses showing posterior probabilities (PP) 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. Guide tree shows the relationship between the four main clades in the guide tree in each analysis.

Algorítmo	SMP	θ	τ	Guide tree	Lineages recovered	PP
(a) 0 (ε = 5)	3	G(21,000)	G(21,000)	(I,(II,(III,IV)))	19	0.9988
(b) 1 (α = 2, m = 1)	2	G(21,000)	G(21,000)	(I,(II,(III,IV)))	19	0.9991
(c) 1 (α = 2, m = 1)	3	G(21,000)	G(21,000)	(I,(II,(III,IV)))	19	0.9997
(d) 0 (ε = 5)	3	G(21,000)	G(21,000)	((III, II),(I,IV))	15	1
(e) 1 (α = 2, m = 1)	2	G(21,000)	G(21,000)	((III, II),(I,IV))	19	0.9992
(f) 1 (α = 2, m = 1)	3	G(21,000)	G(21,000)	((I,II),(III,IV))	19	1

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 et al., 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 and 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 Fig. 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.

As additional reinforcement to the biogeographic reconstruction we have also applied the DEC model, as implemented in Lagrange (Ree and Smith, 2008) to our data (see Appendix B for details). The DEC models implemented in Lagrange and BioGeoBEARS work slightly different but are expected to produce similar results (Matzke, 2014).

2.7. Historical demography

The main assumption of the refuge hypothesis implies drastic population size changes of Amazonian species during the Pleis-

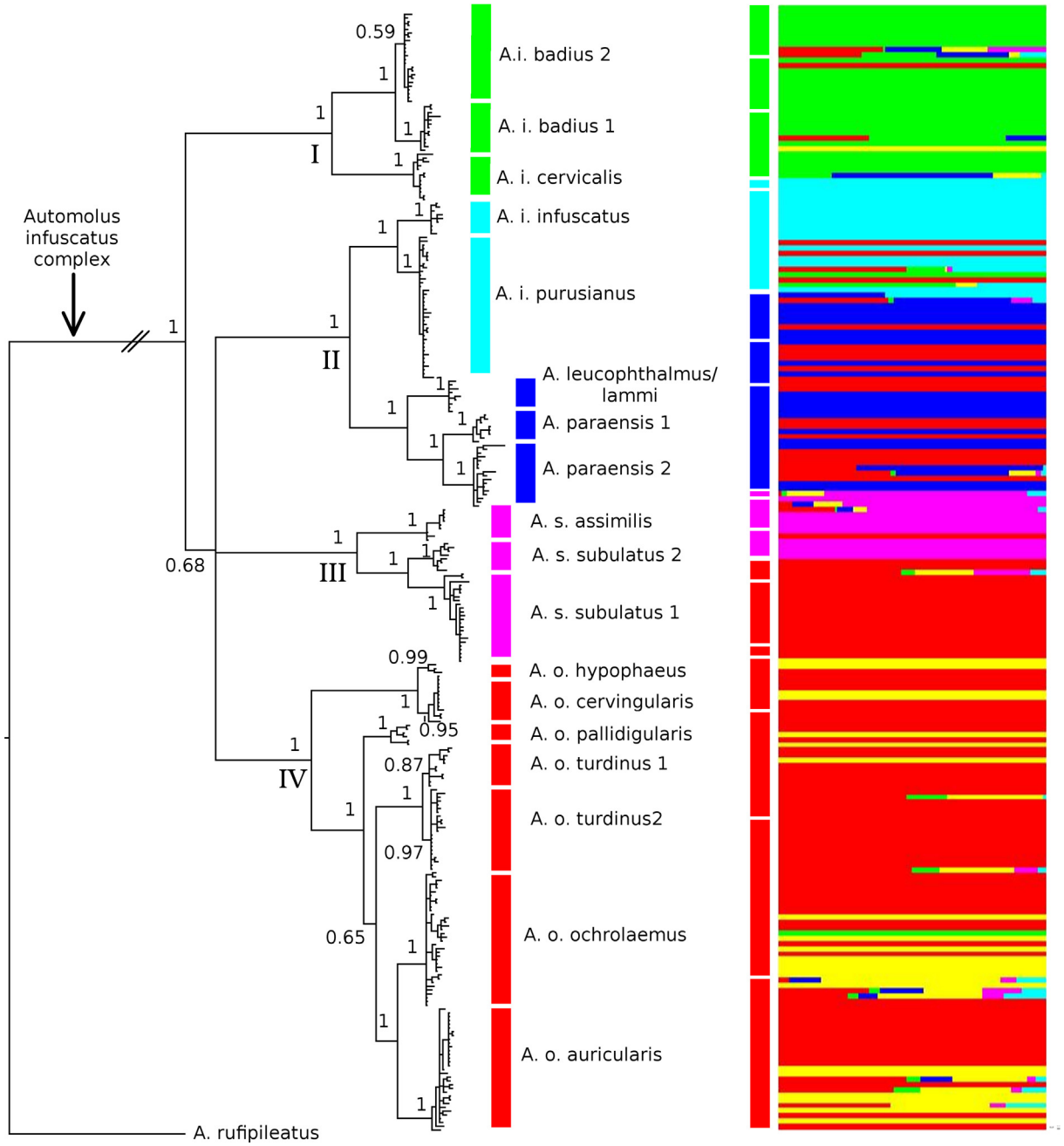

Fig. 1. Mitochondrial gene tree (ND2 and cytb) obtained using Bayesian inference. BAPS nuclear admixture clusters shown to the right. Colored bars indicate corresponding clades in the same order in both analyzes, although the number of individuals in each analysis vary. Values at the nodes represent posterior probabilities. Roman numerals indicate the four main clades recovered by all analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

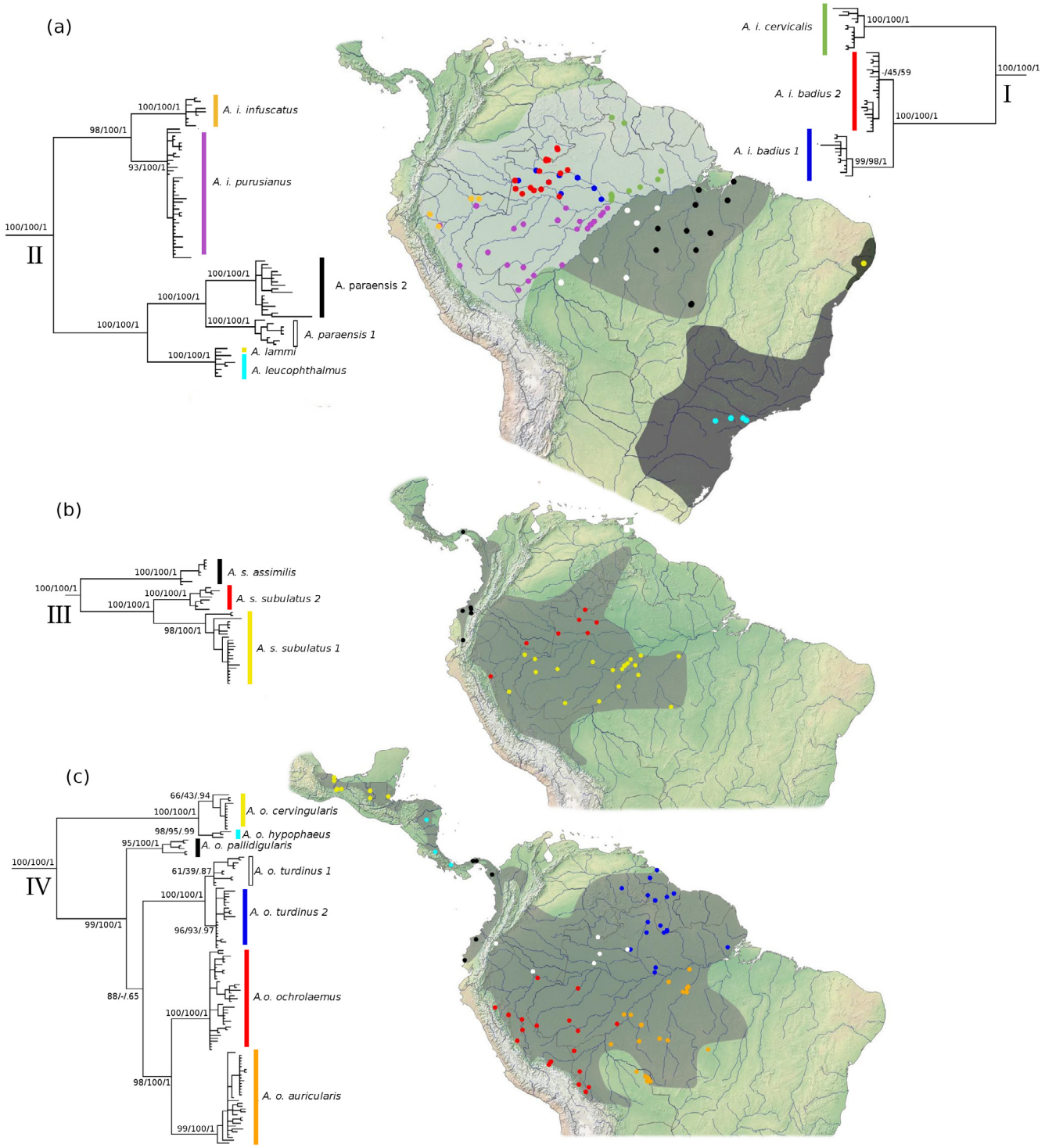

Fig. 2. Geographical distribution and sampling of *A. infuscatus* complex. Insets are mtDNA Bayesian tree with ML bootstrap/MP bootstrap/BI posterior probabilities as support values. "-" represents the absence of that node in specific analysis. Shaded areas illustrate described ranges for: (a) *A. infuscatus*,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

tocene. Thereby, variations on population size through time for each Amazonian lineage were estimated through Extended Bayesian Skyline Plots (EBSP; Heled and 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 2.

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 (Fig. 1 and Appendix C Figs. C.1,

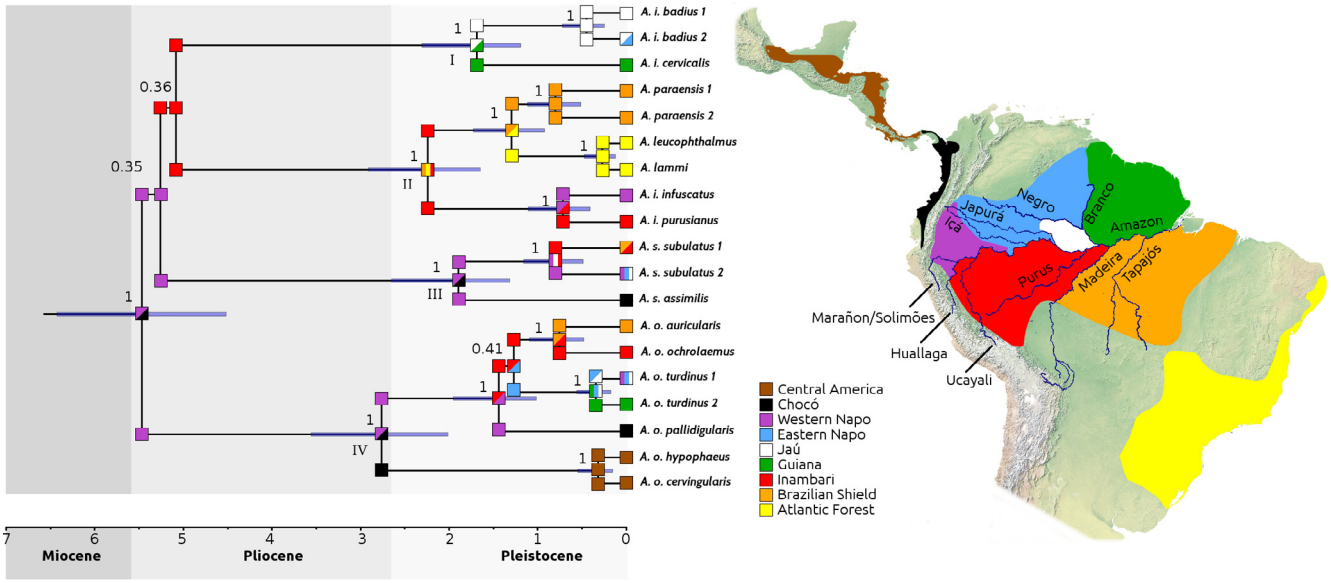

Fig. 3. BEAST tree with the results of BioGeoBEARS. Coloured squares represents most likely ancestor areas estimated by the DIVALIKE model. Up to three areas can be occupied by each taxa as maximum range was set to three in the analysis. Biogeographic areas delimited from the *Automolus infuscatus* complex 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), Brasilian 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. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
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	cytb	ACO	G3PDH	Fib7	Median	Mean	95% HPD interval	
A. i. badius 1	13	13	18	16	10	1	1.1574	[0,2]	2 × 10e8
A. i. badius 2	24	24	18	20	12	1	1.2751	[1,2]	1.5 × 10e8
A. i. cervicalis	13	13	22	18	6	0	0.2532	[0,1]	1 × 10e8
A. i. infuscatus	9	9	–	–	6	1	1.3142	[1,3]	2 × 10e8
A. i. paraensis 1	17	17	40	28	20	1	1.1461	[1,2]	2 × 10e8
A. i. paraensis 2	8	8	12	8	6	1	1.0405	[0,3]	2 × 10e8
A. i. purusianus	38	38	38	26	24	1	1.3666	[1,3]	2 × 10e8
A. o. auricularis	33	33	26	14	44	1	1.5933	[1,3]	2 × 10e8
A. o. ochrolaemus	36	36	14	12	56	1	1.4185	[1,3]	2 × 10e8
A. o. turdinus 1	12	12	–	–	22	0	0.3858	[0,2]	1 × 10e8
A. o. Turdinus 2	21	21	8	6	18	1	1.2585	[1,2]	2 × 10e8
A. s. subulatus1	24	24	–	10	12	1	1.2522	[0,2]	2 × 10e8
A. s. subulatus 2	8	8	12	12	6	1	1.0017	[0,2]	4 × 10e8

C.2 and C.3). Four major well-supported clades were recovered in all three approaches (Clades I–IV in Fig. 1), but there was no support for the relationships among them. BAPS analysis grouped nuclear sequences in six clusters. The clusters substantially match clades from the mitochondrial phylogeny. Nonetheless, high admixture was found between populations (Fig. 1).

Haplotype networks from the nuclear data also show many haplotypes being shared by different populations, often from a different clade (Appendix C, Fig. C.8).

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 species delimitation tests. Hence, based on the eighteen monophyletic lineages recognized by our mitochondrial analysis, we supplied nineteen taxa for the species delimitation tests.

On BPP, from the six models tested, five recognized the nineteen taxa supplied as independent lineages, with high posterior probabilities (Table 1). Nonetheless, one model recognized 15 lineages as the more likely, also with high support, merging five *A. ochrolaemus* lineages into a single species: the four amazonian (*A. o. ochrolaemus*, *A. o. auricularis*, *A. o. turdinus1* and *A. o. turdinus2*) and the chocoan (*A. o. pallidigularis*). STACEY results are in accordance with the ones from BPP, also recognizing the nineteen lineages supplied as independent species with high support (posterior probability >0.99). Considering the congruence between the two analyses, the nineteen lineages recognized by most tests were treated as independent lineages for the ensuing analysis.

Paraphyly of *A. infuscatus* as currently recognized was confirmed, as the 5 independent lineages recognized by the species delimitation analysis within this "species" are included in clades I and II (Fig. 1), the later also including *A. leucophthalmus*, *A. lammi*, and *A. paraensis* (Figs. 1 and 2a). Nuclear population structure also suggests two distinct groups within "*A. infuscatus*" (green and light blue, Fig. 1). Within *A. subulatus*, three lineages were recovered, one in Chocó, sister to two in Amazonia, separated by the upper Amazon/Solimões river (Fig. 1, Clade III; Fig. 2b). All 7 independent lineages recognized by our species delimitation analysis that are currently included in *A. ochrolaemus* were recovered as a mono-

phyletic clade. Within this clade, two lineages from Central America and one from Chocó are sister to the four Amazonian lineages (Fig. 1, Clade IV; Fig. 2c). The mean pairwise genetic p-distances of mtDNA between the nineteen lineages recovered by our analysis ranged from 0.2% to 8.9%, while the mean genetic p-distance within these clades varied between 0.1% and 0.4% (Appendix C, Table C.2).

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 (Fig. 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 and Carstens, 2013; Gatesy and Springer, 2013; Xi et al., 2015). Although some authors suggest that coalescent based methods produce more accurate results (Heled and 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 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. 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 (Fig. 3). The subsequent diversification of these lineages occurred at the Late Pliocene-Pleistocene with extant species aging less than 2 Ma (Fig. 3). The best-fitting model for our BioGeoBEARS analysis was DIVALIKE (Table 3). 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 other areas in South and Central America (Fig. 3). By the end of the Pleistocene clades I and II began diversifying eastward over Northern and Southern Amazonia respectively, while clades III and IV were still restricted to western Amazonia and Chocó. On clade II, our analysis also indicates a southeast Amazonia and Atlantic Forest distribution to the late Pleistocene ancestor of *A. paraensis* and *A. leucophthalmus/lammi* which suggests a connection between those forests at that time.

The DEC implementation in Lagrange also shows an early evolution of the group in western Amazonia, with posterior occupation of eastern Amazonia. In general, DIVALIKE model in BioGeoBEARS and DEC model in Lagrange yielded similar results (see Lagrange results in the Appendix B). Curiously, DEC models on both analysis contrast significantly.

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 and Prum, 1999; Remsen, 2016a). Nonetheless, many species are cryptic (Ridgely and 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.

The four main clades recovered by our analysis represent: (I) Northeastern *A. infuscatus* lineages, (II) Southwestern *A. infuscatus*, *A. paraensis* and Atlantic Forest's lineages, (III) *A. subulatus* lineages and (IV) *A. ochrolaemus* lineages. Despite the recovery of those

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

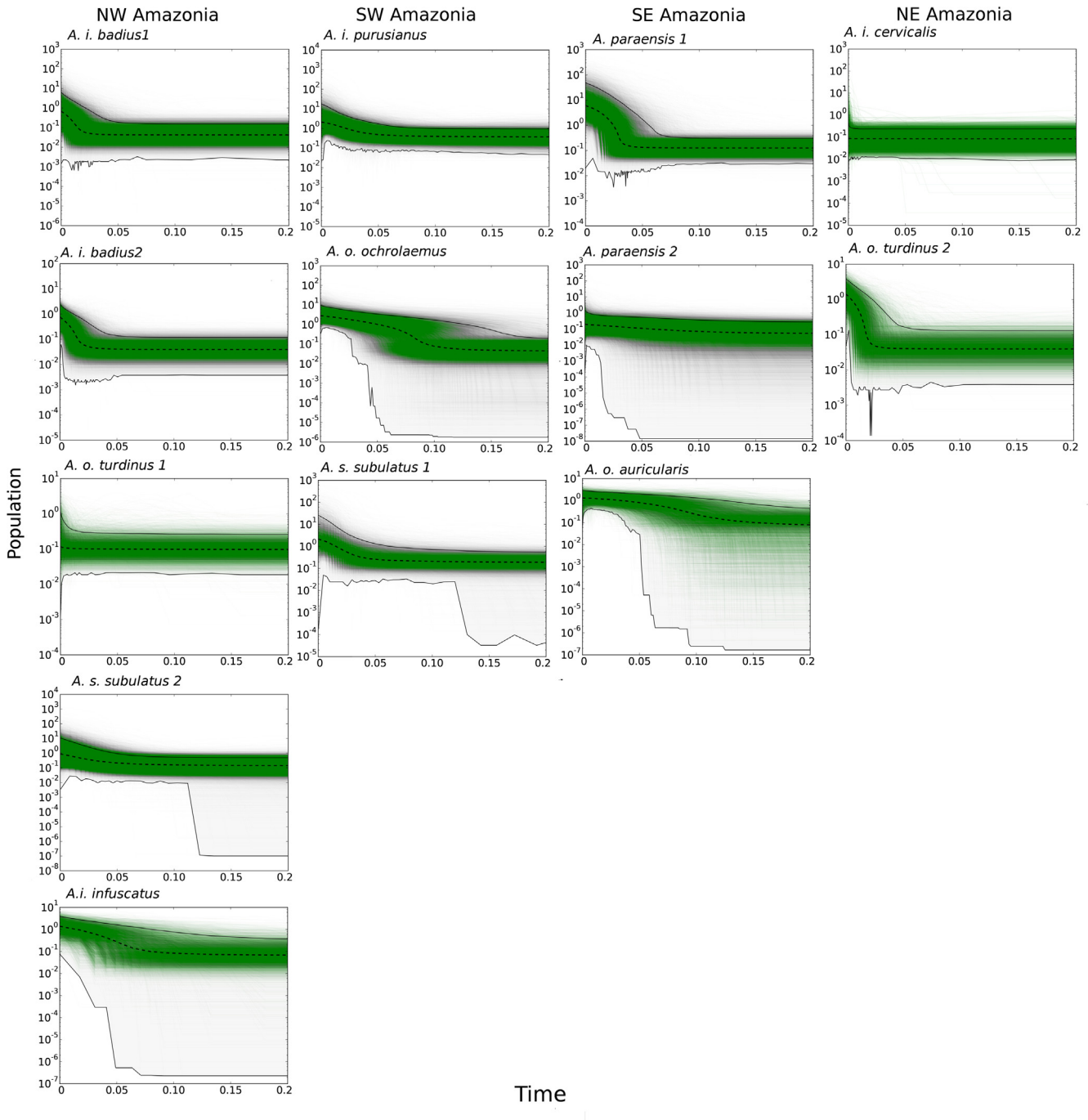

Fig. 4. Extended Bayesian Skyline Plots from Amazonian lineages, sorted by Amazonian region. Dashed lines depict the median population size and the lightly shaded grey the 95% HPD. Green lines show the samples that are summarized by the median and credible intervals lines. The Y axis represents the effective population size (Ne) x mutation rate in a logarithmic scale and X axis is time in million years. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

clades in all of our phylogenetics analyses, the relationship between them could not be solved. 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. The high admixture between populations and the high proportion of haplotypes being shared by distantly related species might be a consequence of the fast splitting of the four main clades recovered by our analysis, combined with the slow mutation rate of the nuclear genes, leading to incomplete lineage sorting.

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 evidences (Figs. 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 and 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 Branco and lower Negro rivers are known to be barriers delimiting the distribution of many lineages in Northern Amazonia, as is the case of *A. i. cervicallis* (Naka et al., 2012). *A. i. badius 1* and *A. i. badius 2* show a more intricate pattern with *A. i. badius 1* seemingly restricted to the Negro/Japurá interfluvium, while *A. i. baidus 2* trespasses the upper portions of those rivers. 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, Napo and Imeri AoEs (Borges and 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 and 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 AoE (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 Mar añon/Solimões rivers, and possibly with the Içа 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. The past connection between Amazon and Atlantic forests is well-supported and many related taxa have disjunct distributions across them. Batalha-Filho et al. (2013) suggested that two distinct spatiotemporal pathways took place between those rainforests nowadays divided by the South American dry diagonal: (i) an older Miocene connection through the current south Cerrado and palm savannas of Bolivia and Paraguay and (ii) a younger Plio-Pleistocene connection through the Cerrado and Caatinga in northeastern Brazil. The relationship between eastern Amazonian *A. paraensis* lineages and Atlantic forest clade, and the Pleistocene age of their divergence seem to agree with a recent northeastern pathway connecting the two rainforests.

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 Huallaga 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 (Fig. 2c). 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. For both clades, the first diversification event within Amazonia corresponds 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 flooded forests and riparian areas, whereas species within the *A. infuscatus* complex in general prefer upland *terra firme* forests (Ridgely and 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). However, the age of cross-Andean diversification in different avian taxa are 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 and 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 within the *A. infuscatus* complex 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 and Brumfield, 2009; Smith et al., 2014).

Divergences across the Solimões are temporally congruent in three of the major clades (clades II, III and IV), with splits happening at around 0.5–1.5 Ma. The recent age of these splits may indicate a recent reorganization of the western Amazonian drainage system, related to the gradual establishment of upland terra firme forest in the region, coming as another evidence of the recent history of the landscape in the region (Rossetti et al., 2005, 2015; Nogueira et al., 2013). However, the same pattern could be a result of dispersal across the upper Solimões and its tributaries, mainly the Ucayali and Marañon, related to changes in climate affecting river discharge. Climate patterns in Amazonia have changed at about 1 Ma, with changes in the duration of climatic cycles and stronger glacial periods (Cheng et al., 2013). Nevertheless, these congruent patterns indicates that more detailed sampling and explicit tests of co-diversification for these pairs of taxa could give important clues about the recent evolution of this complex region .

In contrast, 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; Sousa-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 and Tudor, 2009; Remsen, 2016b, 2016c, 2016d). Moreover, although the whole complex forages by gleaning arthropods from dead leafs 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 (Remsen and Parker, 1984; Robinson and 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 and 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, 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, Fernandes et al., 2014; d'Horta et al., 2013; Lutz et al., 2013; Sousa-Neves et al., 2013; Thom and 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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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.12.023.

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