Rivers acting as barriers for bird dispersal in the Amazon

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ABSTRACT: Morphological, vocal and genetic studies have shown that the Madeira River and its right bank tributaries delimit populations of primates and birds. We sequenced the cytochrome *b* gene (approx. 950 bp) for individuals of three suboscine passerine bird species, *Glyphorynchus spirurus* (Furnariidae), *Willisornis poecilinotus* (Thamnophilidae) and *Schiffornis turdina* (Tityridae), on opposite banks of the Madeira River and two of its right-bank tributaries, the Aripuanã and Jiparaná rivers. Phylogenetic hypotheses (parsimony, maximum likelihood and Bayesian analysis) revealed clades that have over 3.1% genetic differentiation on opposite banks of the Madeira River for *G. spirurus*, *W. poecilinotus* and *S. turdina*, suggesting that this river restricts gene flow among populations of these three species. The Jiparaná and Aripuanã rivers apparently separate distinct populations of *G. spirurus*, the smallest species we examined, but not those of the other two heavier bodied species, *W. poecilinotus* and *S. turdina*. In *G. spirurus* four clades with high levels of genetic differentiation (3.2–5.5%) were found to be delimited by the three rivers evaluated, whereas in *W. poecilinotus* and *S. turdina* no genetic structure across the Jiparaná and Aripuanã rivers was detected. In general, birds that are known to show population structure across the Madeira tributaries (*Glyphorynchus spirurus, Hemitriccus minor, Hypocnemis rondoni, Herpsilochmus stotzi,* and *Hylophylax naevius*) have body masses smaller than those of both *Willisornis poecilinotus* and *Schiffornis turdina*, but some exceptions are discussed. Future studies controlling for several variables are necessary to determine the extent to which body mass is a useful predictor of genetic population structure in understory suboscine passerines.

KEY-WORDS: Areas of endemism, body mass, comparative phylogeography, conservation, dispersal rate, suboscine birds.

INTRODUCTION

Avian distribution patterns are reasonably well known and influential in studies of evolutionary processes. Jürgen Haffer was one of the first authors to compile bird distribution data to describe biogeographic patterns in South America (Haffer 1974). His work made a great contribution to evolutionary studies and to the formulation of speciation hypotheses in Amazonia. The "centres of species endemism" he described remain largely unchanged in analyses of distribution patterns for many avian groups. They have been generally accepted in subsequent works, and in the Amazon basin these regions are often delimited by large rivers (Haffer 1974; Cracraft 1985; da Silva & Oren 1996). Bird species are usually separated by the Amazon River and its major tributaries such as the Negro, Madeira, Tapajós and Tocantins rivers (Cohn-Haft 2000; Ribas *et al.* 2012; D'Horta *et al.* 2013; Fernandes *et al.* 2012, 2013, 2014). Similar patterns are also found in other Amazonian vertebrate taxa, including primates and butterflies (Wallace 1852; van Roosmalen *et al.* 1998; Hall & Harvey 2002), suggesting that rivers are important barriers to dispersal.

Recent studies of primates and birds in the Madeira-Tapajós interfluvium (M-T), also known as the Rondônia area of endemism (Cracraft 1985), have suggested that smaller rivers also limit the distributions of some taxa, thus forming smaller areas of endemism in what was referred to as "mini-interfluvia" (Cohn-Haft *et al.* 2007). Willis (1969), in a study of birds of the genus *Rhegmatorhina*, was one of the first to document complex patterns of bird distributions in this area. He discussed the parapatric occurrence of *Rhegmatorhina berlepschi* and *R. hoffmannsi* within the M-T and suggested that the Madeira and Tapajós rivers have occasionally changed their courses, resulting in the separation of populations and subsequent speciation. Van Roosmalen and collaborators (1998) described geographic substitutions of species in primates of the genera *Callithrix* and *Callicebus* on opposite banks of small rivers within this interfluvium and described a new species of marmoset, *Callithrix humilis*, that occurs only on the west bank of the Aripuanã River. Subsequently, several other bird species in this region have been found to contain vocally, morphologically or genetically distinct populations, with restricted distributions and geographic substitution

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on opposite banks of Madeira tributaries, such as the Aripuanã and Jiparaná (or Machado) rivers (Cohn-Haft *et al.* 2007; Isler *et al.* 2007; Tobias *et al.* 2008; Fernandes *et al.* 2012, 2013, 2014; Whitney *et al.* 2013a, b, c, d, e). Similar geographic patterns, with different races being separated by small Amazonian rivers, have also been found for butterflies (Hall & Harvey 2002).

Sardelli (2005) found genetic differentiation (cytochrome *b*, 500 bp) among morphologically indistinguishable populations of the Snethlage's Tody-Tyrant (*Hemitriccus minor*) apparently bounded by the Jiparaná and Aripuanã rivers. This study raised the possibility of the existence of cryptic endemism in mini-interfluvia, which was subsequently investigated for the other three species of passerine birds (*Myrmeciza hemimelaena*, *Glyphorynchus spirurus*, *Hylophylax naevius* (Fernandes *et al.* 2012, 2013, 2014). Fernandes (2013) cited in a review a number of publications corroborating the importance of the mini-interfluvia, highlighting that these diversity patterns are a key (and possibly unique) Amazonian feature and that despite the fact that this fine-scale endemism is well known and recognized among systematists working in the Amazon, it is not taken into account in conservation plans. Fernandes (2013) pointed out that many taxa in this region, including those yet to be given formal scientific names, may now be endangered or even extinct. Thus it is of utmost importance to consider species that present this kind of fine scale differentiation in future conservation proposals. The objective of our study was threefold: 1) describe phylogeographic patterns for three species of suboscine passerines across the Rondônia area of endemism; 2) compare these patterns to those of other species know to have populations delimited by the Madeira, Aripuanã and Jiparaná rivers; and 3) investigate the relationship between degree of phylogeographic structure and ecological attributes in the light of the riverine barrier hypothesis.

MATERIAL AND METHODS

Species studied and sampling design

We studied three species of passerine birds belonging to three different families: *Glyphorynchus spirurus* (Furnariidae), *Willisornis poecilinotus* (Thamnophilidae), and *Schiffornis turdina* (Tityridae). We sampled birds at 12 sites between the Tapajós and Madeira rivers and five sites on the left bank of the Madeira River (LM; Figure 1), with the final number of localities sampled per species differing among the three species (see Results). Individuals were collected along the Madeira, Aripuanã, Jiparaná and Roosevelt (the latter representing the largest tributary of the Aripuanã) rivers; the widths of these

rivers along their lower courses are roughly 3.0, 0.8, 0.4 and 0.3 km, respectively. Each collection point had a corresponding point located on the opposite bank and therefore in a different interfluvium. For purposes of sampling and analyses, we suggest the existence of three mini-interfluvia within the M-T: Madeira-Jiparaná (MJ), Aripuanã-Jiparaná (AJ) and Aripuanã-Tapajós (AT) (Fig. 1). A maximum of 5 individuals per species were collected at each sampling point, with the total sample as follows: *Willisornis poecilinotus* (*n* = 45), *Schiffornis turdina* (*n* = 23), and *Glyphorynchus spirurus* (*n* = 25) (see Appendix). Specimens were deposited in the bird collection of the National Institute for Amazonian Research (INPA), Manaus, Brazil, where tissue samples (muscle, heart and liver) were stored in liquid nitrogen for molecular analyses.
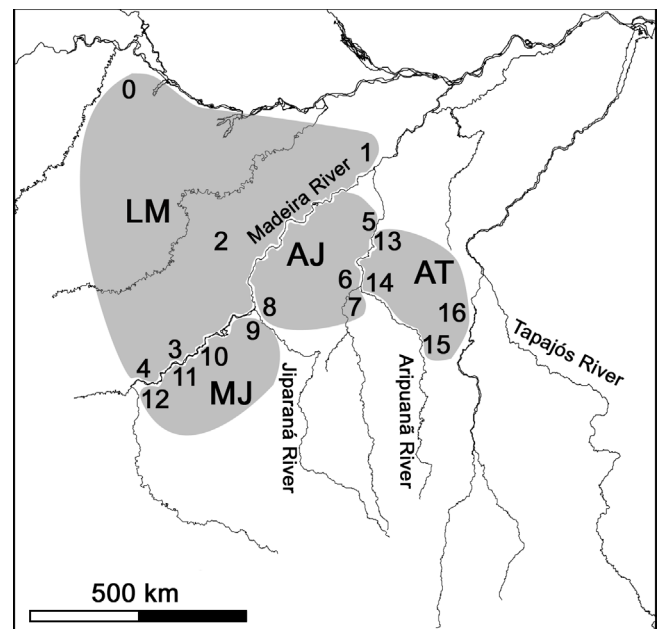

FIGURE 1. Collection points and the interfluvia sampled. Madeira-Jiparaná (MJ), Aripuanã-Jiparaná (AJ), Aripuanã-Tapajós (AT) and Left bank of the Madeira River (LM).

We chose to study these three target species primarily because they are common, easy to collect, and widely distributed across the entire Amazon basin. Although all three are suboscine passerines, they represent three distinct families and, as such, the results obtained in this study can be assumed to be instances of independent evolution, and, thus, support the generality of our conclusions. All three species can be found in the same habitat (*terra firme* forest) but they differ in a variety of ecological attributes:

Glyphorynchus spirurus – This is a polytypic species widely distributed in Neotropical lowland forests, occurring in Amazonia, Central America and along the Atlantic coast of Brazil (Ridgely & Tudor 1994). Marantz *et al.* (2003) recognized thirteen subspecies, six of which occur in the Brazilian Amazon. Three of these occur

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within or adjacent to the Madeira basin: *G. s. castelnaudii* (west of the Madeira River to the Andes), *G. s. albigularis* (south-eastern Bolivia and Peru), and *G. s. inornatus*, which occurs throughout the Brazilian portion of the Madeira-Tapajós interfluvium (Peters 1951; Marantz *et al.* 2003). With a body mass ranging from 10.5 to 21g (typically 12.6-14.8 g in central Amazonia; Bierregaard, 1988), this is the smallest woodcreeper (Marantz *et al.* 2003), and it is the smallest of the three species we studied. It occurs in both *terra firme* and seasonally flooded forests (*várzea* and *igapó*) (Marantz *et al.* 2003) and it is moderately sensitive to environmental perturbation (Ferraz *et al.* 2007). Recently, Fernandes *et al.* (2013) found that populations of *G. s. inornatus* are delimited by the Aripuanã and Jiparaná rivers.

Willisornis poecilinotus – A species endemic to the Amazon basin, with seven subspecies recognized (Peters 1951; Zimmer & Isler 2003). Only one subspecies (*W. p. griseiventris*) is recognized from the middle and upper Madeira River basin; and there is no evidence of vocal or morphological differentiation across the Madeira, Aripuanã and Jiparaná rivers (Isler & Whitney 2011), although Bates (2000) found genetic differentiation (based on analyses of isozymes) across the Madeira River. Occurs in the understory of *terra firme* forest, where it is a regular follower of army ant swarms (Zimmer & Isler 2003). It is larger on average than *Glyphorynchus*, with a body mass ranging from 15 to 19 g (Zimmer & Isler 2003).

Schiffornis turdina – Nyári (2007), with no samples from the middle or lower Madeira River basin described close geographic proximity in the upper Madeira of two genetically distinct forms, showing no obvious vocal or plumage differences; the author proposed recognizing them as distinct species (*S. amazona* and *S. turdina*), as adopted by the Brazilian Ornithological Records Committee (2014). *Schiffornis turdina* (in the polytypic sense used here) occurs in the understory of *terra firme* and sandy-belt *campinarana* forests. Body mass averages 31 g (Snow 2004). This species is sensitive to forest fragmentation, disappearing from small forest fragments (Ferraz *et al.* 2007).

Extraction, amplification and sequencing of DNA

DNA was extracted from breast muscle (approximately 0.2 g) using a standard phenol chloroform protocol (Sambrook *et al.* 1989). The mitochondrial cytochrome *b* was amplified via the polymerase chain reaction (PCR) using the primers: *forward* H16064 5′-ATCTCARCCTGATGAAAYTTYGG-3′, reverse L14993 5′-AAGTGGTAAGTCTTCAGTCTTTGGTT-3′, both of which were designed exclusively for this project. All amplification reactions were performed in 25 µl

volumes using a Thermo Hybaid PCR Express thermal cycler under the following conditions: (1) an initial denaturing step at 94°C for 5 min; (2) 35 cycles of the following: 1 min at 92°C, 1 min at 48°C, and 1 min at 72°C; (3) a 10-min extension step at 72°C. Following PCR, correct fragment size and the presence of a single amplification product was confirmed via electrophoresis on 1% agarose gel. After amplification, the PCR products were purified using a salt protocol (Sambrook *et al.* 1989). Sequencing was performed by the chain termination method (Sanger *et al.* 1977), using a Big Dye Termination Kit (Applied Biosystems) following the manufacturer's specifications. The products of the sequencing reaction were precipitated with Tris-HCl and alcohol, and resuspended in formamide and resolved by capillary electrophoresis in an ABI 3130xl automatic sequencer (Applied Biosystems). All sequences have been deposited in GenBank (accession numbers: HM164938 – HM165034).

Alignment

Sequences of DNA were visualized and edited using the Bioedit program (Hall, 1999). Alignments were performed in Clustal X within Bioedit (Hall, 1999). We used recommended precautions and are confident that all sequences represent mitochondrial DNA for the following reasons: (1) DNA was extracted only from tissue samples, which have high ratios of mitochondria to nuclei relative to blood or skin samples; (2) no stop codons occurred within the cytochrome *b* of any of the sequences; (3) sequences contain no insertions or deletions relative to one another or to other known avian cytochrome *b* sequences; (4) sequences in both DNA fragments from each individual were identical and unambiguous in their region of overlap; (5) in phylogenetic analyses, no samples appeared in unexpectedly basal portions of the tree or had exceptionally short or long branch lengths, both of which, if present, would indicate a fast evolving gene or an early diverged gene (a pseudogene, for example).

Phylogenetic analyses

Phylogenetic analysis of DNA sequence data was performed using maximum parsimony (MP) and maximum likelihood (ML) via PAUP* 4.0b10 (Swofford 2002) and Bayesian inference (BI) implemented in MRBAYES 3.0b4 (Hulsenbeck & Ronquist 2001). Maximum parsimony analysis was performed using a heuristic search with the following options: TBR branch-swapping with 10 trees held at each step. Support for nodes was assessed using 1000 bootstrap replicates. Maximum likelihood was performed using the model parameters determined in the program Modeltest

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(Posada & Crandall 1998). The support for nodes in the likelihood tree was assessed using 500 bootstrap iterations. For BI analyses, two independent runs of 8,000,000 generations each were performed; for each run four Markov chains were simulated. Trees were sampled every 500 generations and the first 4,000 samples were discarded as burn-in.

Because there is strong evidence that geographic distributions of Amazonian birds are bounded by large rivers that form areas of Neotropical endemism, we used individuals from populations from other interfluvia as outgroups for the three species studied. For the analysis of *Glyphorynchus spirurus* we used two individuals collected in the headwaters of the Negro River (Appendix) as the outgroup. For *Willisornis poecilinotus*, we used one individual collected in the Solimões-Negro River interfluvium and for *Schiffornis turdina*, we used as outgroups one individual collected north of Manaus and also one sequence of *Schiffornis virescens* from GenBank (accession number AF453816; Appendix).

Phylogenetic divergence analyses

Phylogenetic divergence was estimated in the program BEAST v1.6.1 (Drummond & Rambaut 2007) using the coalescent constant population size tree prior (Drummond *et al.* 2002), the uncorrelated lognormal relaxed molecular clock model (Drummond *et al.* 2006), and the HKY (Hasegawa *et al.* 1985) models of molecular evolution, including gamma-distributed rate heterogeneity among sites and invariant sites. After preliminary runs, we adjusted priors and MCMC operators to assure optimum performance. To assess the robustness of estimates and investigate the influence of the tree prior, we also performed analyses under the exponential (Drummond *et al.* 2002) and the Bayesian skyline (Drummond *et al.* 2005) tree priors. To convert divergence time estimates into units of millions of years, we used the mean substitution rate of 0.01105 substitutions/site/lineage/million years as proposed by Weir & Schluter (2008).

For each set of priors, two independent MCMC analyses were run for 100 million generations, sub-sampling every 100 thousand generations. After a 10% burn-in, convergence of parameter estimates was assessed using the Gelman-Rubin statistic implemented in the module *coda* in the statistical package R (R Development Core Team 2011). Independent chains were combined, and marginal posterior parameter means and their associated 90% highest probability density intervals (90% HPD) together with effective sample size (ESS) for each divergence time estimate were calculated in the statistical package R (R Development Core Team 2011).

RESULTS

We found significant phylogeographic structure among populations within all three study species. The Madeira River clearly separates genetically distinct populations in all of them. Within the Madeira-Tapajós interfluvium, the degree of structure varied among species (see below). In each species, tree topologies were identical for all four tree-building algorithms, thus we only show the tree resulting from the Bayesian inference analyses. The pair-wise genetic p-distance between individuals from opposite banks of the three rivers ranged from 3.1 to 5.5% but the variation within interfluvia was low (0.0–0.09%). Results for each species were as follows:

Glyphorynchus spirurus

We sequenced a total of 946 bp for 27 individuals of *G. spirurus*. Parsimony, maximum likelihood, and Bayesian inference analyses suggested a genetic structure in the form of monophyletic groups on opposite banks of the Madeira, Aripuanã and Jiparaná rivers, each supported by high bootstrap values (MP = 100, ML = 100, BI = 1.00). No barrier effect was found on opposite banks of the Roosevelt River. Parsimony analysis yielded two equally parsimonious trees (length = 170, CI = 0.8235, RI = 0.9504). From 130 variable sites, 110 were parsimony informative. Maximum likelihood (-ln L = 2012.00097) and Bayesian inference resulted in a topology very similar to that of the parsimony analysis. Levels of genetic divergence (uncorrected p-distance) between individuals of different clades ranged from 3.2% (populations of AJ versus MJ) to 5.5% (populations of LM versus AT) and levels of divergence between individuals within the same interfluvium ranged from 0.0–0.03% (Figure 2). Coalescent analyses in the program BEAST indicate a 6.5 mya (1.9 – 32.7, 90% HDP) divergence between populations on the left and right banks of the Madeira River.

Willisornis poecilinotus

We sequenced a total of 956 bp for 46 *Willisornis poecilinotus* individuals. The results of parsimony, maximum likelihood, and Bayesian inference analyses were concordant, thus indicating a strong phylogenetic signal supported by high bootstrap values (MP = 100, ML = 100, BI = 1.00) (Figure 3). Parsimony analysis yielded 100 equally parsimonious trees (length = 94, CI = 0.8723, RI = 0.9634). From 71 variable sites, 41 were parsimony informative. Maximum likelihood (-ln L = 1757.25307) and Bayesian inference resulted in a topology very similar to that of the parsimony analysis. The level of genetic divergence (uncorrected p-distance)

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between individuals of the two clades separated by the Madeira River, RM (right bank of Madeira River) versus LM (left bank of Madeira River), was 3.4% (Figure 3). Levels of divergence between individuals in the same interfluvium ranged from 0.0–0.09%. Coalescent analyses in the program BEAST indicate a 2.6 mya (0.8 – 13.8, 90% HDP) divergence between populations on the left and right banks of the Madeira River.
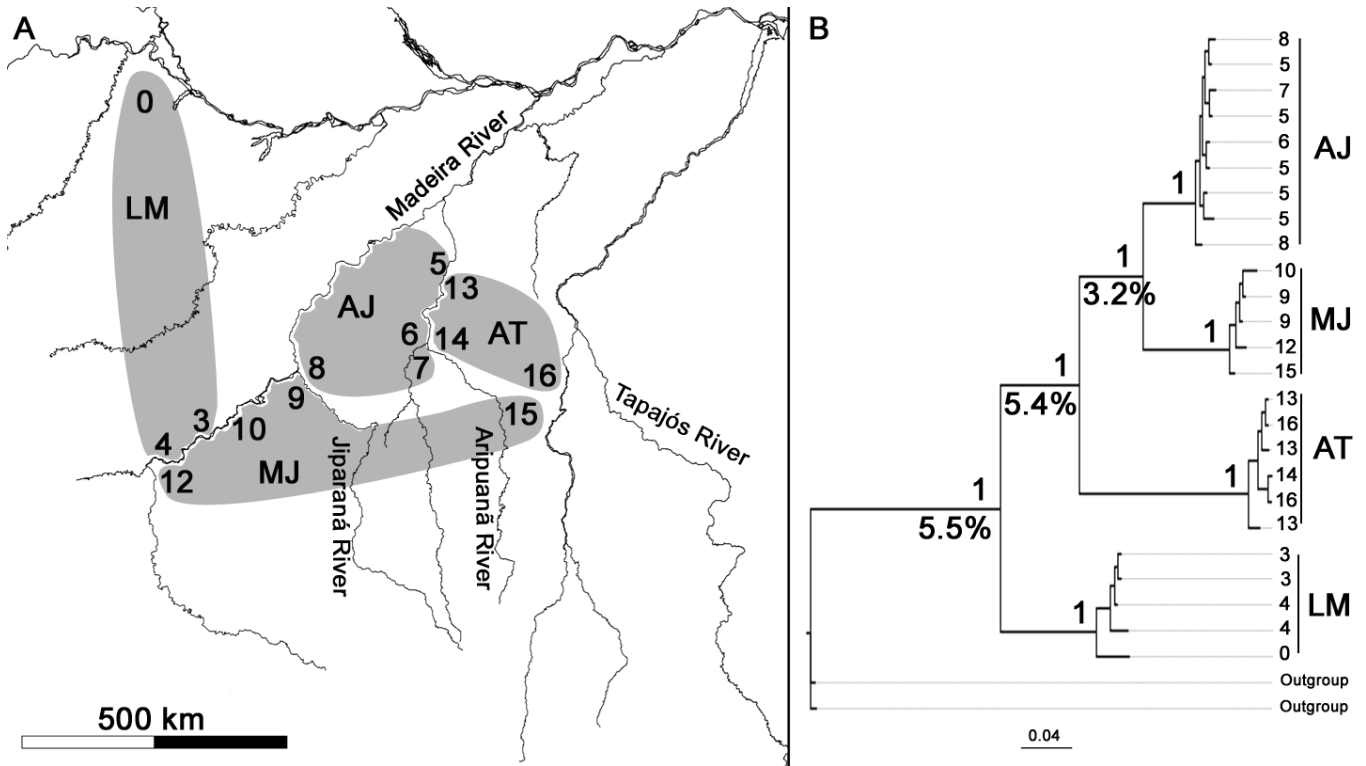

FIGURE 2. Species–area relationships (a) and Bayesian inference phylogeny (b) estimated for *Glyphorynchus spirurus*. Numbers at the tips of branches refer to localities where individuals were sampled. Bayesian inference (BI) posterior probabilities and genetic distance (uncorrected p-distance) values are indicated in the branches. Note grouping of a sample from the Aripuanã-Tapajós interfluvium (location 15) with those of the Madeira-Jiparaná interfluvium (see Discussion).
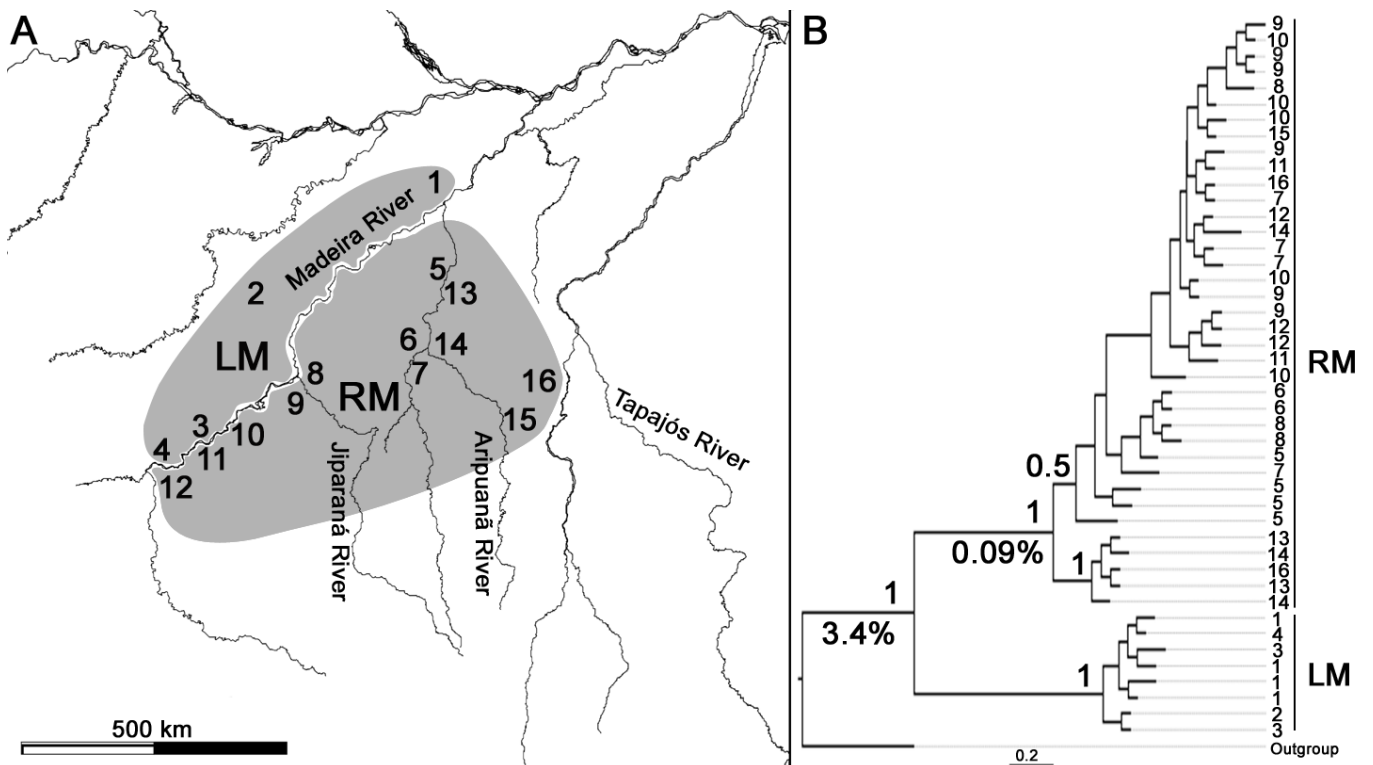

FIGURE 3. Species–area relationships (a) and Bayesian inference phylogeny (b) estimated for *Willisornis poecilinotus*. Numbers at the tips of branches refer to localities where individuals were sampled. Bayesian inference (BI) posterior probabilities and genetic distance (uncorrected p-distance) values are indicated in the branches.

Schiffornis turdina

We sequenced a total of 968 bp for 25 *Schiffornis turdina* individuals. Parsimony, maximum likelihood and Bayesian inference analyses suggested genetic structure on opposite banks of the Madeira River supported by high bootstrap values (MP = 100, ML = 98, BI = 1.00) (Figure 4). Parsimony analysis yielded 48 equally parsimonious trees (length = 175, CI = 0.9371, RI = 0.9214). From 159 variable sites, 67 were parsimony informative. Maximum

likelihood (-ln L = 2043.26577) and Bayesian inference resulted in a topology very similar to that of the parsimony analysis consensus topology. The maximum divergence (uncorrected p-distance) between individuals of the RM and LM clades was 3.1% (Figure 4). Levels of divergence among individuals of the same interfluvium ranged from 0.0–0.3%. Coalescent analyses in the program BEAST indicate a 3.1 mya (1.0 – 15.2, 90% HDP) divergence between populations on the left and right banks of the Madeira River.
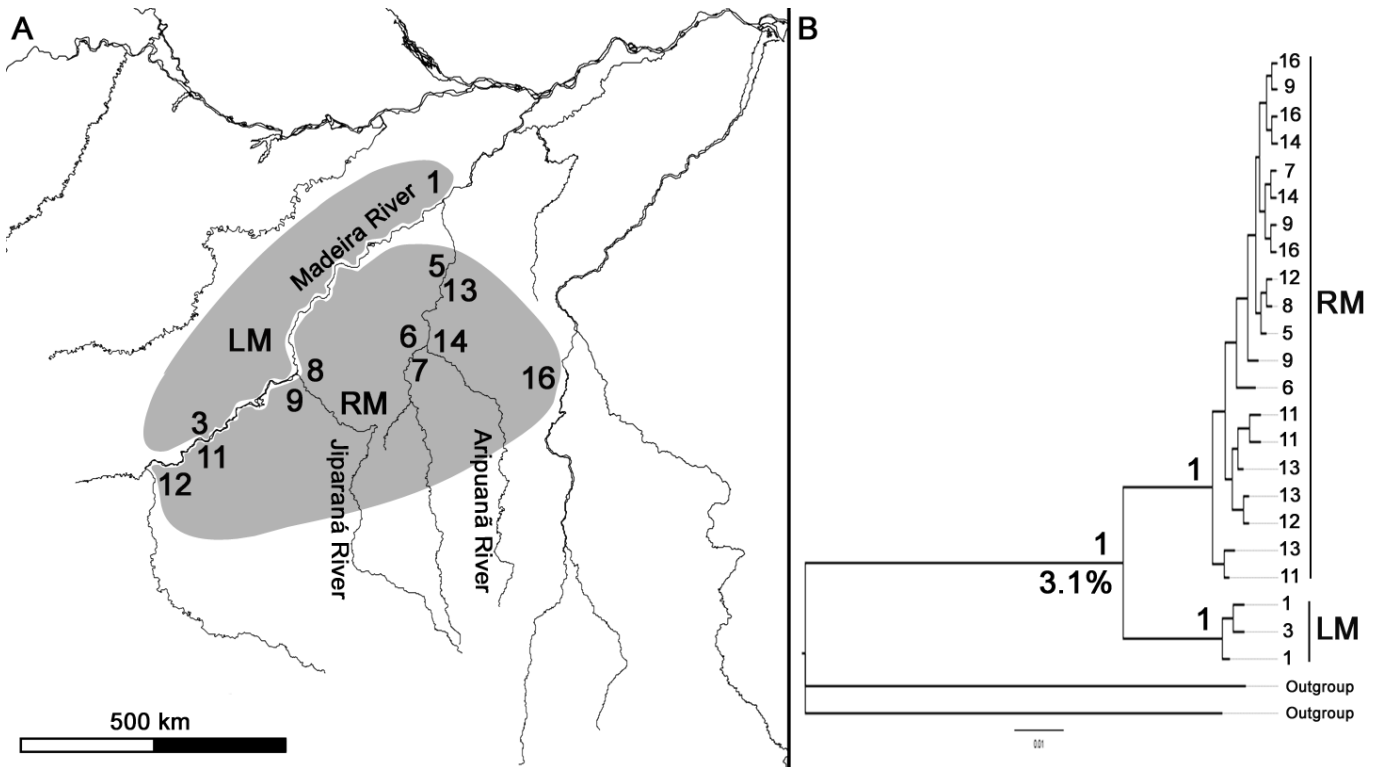

FIGURE 4. Species–area relationships (a) and Bayesian inference phylogeny (b) estimated for *Schiffornis turdina*. Numbers at the tips of branches refer to localities where individuals were sampled. Bayesian inference (BI) posterior probabilities and genetic distance (uncorrected p-distance) values are indicated in the branches.

DISCUSSION

Strong genetic differentiation in the face of highly conserved phenotype is at the heart of numerous descriptions of "cryptic species" in recent years (Whitney *et al.* 2013a, b, c, d, e) and appears to be a frequent phenomenon in the Amazon. In *S. turdina*, differentiation on opposite banks of the middle and lower reaches of the Madeira River is consistent with that detected earlier in the upper Madeira (Nyári 2007) and associated with species level taxa. In all three studied species, the observed molecular groups are monophyletic and parapatrically distributed, their geographic distributions are delimited by rivers, and the observed phylogenetic divergence between clades on opposite banks of the Madeira River (3.1–5.5%) is consistent with interspecific divergences in other avian

taxa separated by the same geographic barrier (Ribas *et al.* 2012). Based on coalescent analyses (see Methods), we estimated mean divergences of 6.5 mya, 2.6 mya and 3.1 mya between populations on left and right banks of the Madeira River for *G. spirurus*, *W. poecilinotus* and *S. turdina*, respectively. The separation of the lineages in all three species of passerines are clearly ancient, all lineages are diagnosable by multiple molecular synapomorphies, and all lineages are parapatrically distributed and likely represent phylogenetic species. However, it is also clear that a more detailed analysis evaluating species status and establishing species boundaries is necessary.

Irrespective of taxonomy, the pattern of geographic variation delimited by rivers is clear for all three taxa studied. Our results indicate genetically distinct populations on opposite banks of the Madeira River. For all three species analyzed in this study we found sister

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clades on opposite banks of the Madeira River, and for *G. spirurus*, as documented previously (Fernandes *et al.* 2013), the data further indicated sister clades on opposite banks of the smaller Aripuanã and Jiparaná rivers. Our data therefore reinforce the importance of rivers as geographic barriers, and suggest a hierarchical effect in which larger rivers divide older clades whereas smaller rivers are associated with more recent divergences. For *G. spirurus*, an individual collected on the right bank of the Aripuanã River that grouped in the clade Madeira/Jiparaná (MJ) provides evidence of upstream gene flow across both of the same rivers (Jiparaná and Aripuanã) that delimit differentiated populations in their lower reaches. Since rivers naturally tend to be narrower in the upper reaches, this result suggests that river width is important in determining a river's likelihood of delimiting distributions and further strengthens the hypothesis of a hierarchical effect of river width in structuring populations (Haffer 1974, 1997).

Assuming roughly equal rates of substitution, then *Willisornis poecilinotus* and *Schiffornis turdina* populations may have differentiated across the Madeira River at about the same time; however, *Glyphorynchus spirurus* would appear to have differentiated much earlier. This implies that not all sympatric bird taxa necessarily share the same evolutionary scenario. Although the Madeira River currently delimits the distributions of the left- and right-bank clades of all three species, the Madeira River might not necessarily be the primary agent that has driven the observed divergence. It may simply represent current limits of distribution for clades that have diverged due to other abiotic or biotic forces, independent of the formation of the Madeira River itself. Another non-exclusive possibility is that rates of molecular substitutions are 2–3 times faster in *G. spirurus* than in *W. poecilinotus* and *S. turdina*; however, such an elevated substitution rate appears to be a rare phenomenon in passerine birds, and has been suggested only for one case of an Old World species (*Nectarinia humbloti*; Warren *et al.* 2003). Finally, a third explanation suggested previously (Willis 1969, Fernandes *et al.* 2012, 2014) is that changes in the courses of rivers might confuse the phylogenetic pattern. There is evidence that the course of rivers in the Madeira basin changed throughout history, but remained stable for long periods of time (Latrubesse 2002). The period of stability could be enough to cause differentiation until their course was modified again and became stable for another long period of time thus causing both spatial and temporal incongruences among phylogenies of co-distributed species (Fernandes 2013). A comparative analysis including additional species and sampling nuclear markers is likely to shed more light on this issue, but at least three other studies have found populations separated by the Madeira River not to be reciprocally monophyletic (Aleixo 2004, Patané *et al.* 2009, Sousa-Neves *et al.* 2013), as recovered herein for *G. spirurus*, *W. poecilinotus*, and *S. turdina*, hence supporting a more complex scenario of differentiation and a broad range of phylogeographic patterns for the same region.

Despite the importance of rivers for avian differentiation, even the largest Amazonian rivers are not barriers for all species and smaller rivers are less likely to be barriers than larger rivers. There are several potential explanations for this phenomenon. Molecular studies suggest that populations of canopy species are less structured than those of understory birds (Capparella 1988; Burney & Brumfield 2009). The latter authors showed that genetic divergence is significantly smaller across the Andes and two Amazonian rivers (Amazon and Madeira rivers) in canopy birds than in understory species. Burney & Brumfield (2009) further suggested that there is a negative relationship between dispersal propensity and genetic structure. Species that occupy the understory are supposed to be less effective dispersers, which may be one reason why there are more species of understory birds, and that they are more locally distributed.

However, we found differences in genetic structure among understory species, suggesting that other factors may also influence the diversification of birds. One might also expect the degree of sensitivity to disturbance or habitat specialization on primary *terra firme* forest to predict the importance of rivers in driving or maintaining allopatric differentiation. Ferraz *et al.* (2007) analyzed thirteen years of capture/recapture data for birds in the reserves managed by the Biological Dynamics of Forest Fragmentation Project (BDFFP), located in the Brazilian state of Amazonas north of Manaus. These authors derived measures of the vulnerability of a species to isolation and sensitivity due to fragment size. These two measures reflect sensitivity to environmental change. Among the 54 species examined by Ferraz *et al.* (2007), *G. spirurus* was the least sensitive to the size of the fragment and one of the ten species least vulnerable to isolation. By contrast, *S. turdina* was among the most vulnerable and most sensitive species. *Willisornis poecilinotus* was not included in the analysis. One would therefore expect *G. spirurus*, the species least affected by isolation and fragmentation, to have lower genetic divergence across the rivers than the other two species; however, our results contradict the expected pattern. *Glyphorynchus spirurus*, although occurring in different types of forests and not being especially sensitive to disturbance, has populations that are much more strongly structured than are those of the other two species. In this case, sensitivity to disturbance and degree of specialization on primary *terra firme* forest were not good predictors of the degree of population genetic structure.

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The Jiparaná and Aripuaná rivers separate populations of *G. spirurus*, the smallest species we examined (average body mass 13.7 g), but not the populations of two other species, *W. poecilinotus* (15–19 g) and *S. turdina* (30–35.5 g). The Jiparaná and Aripuaná rivers also appear to limit the distributions of populations in other very small birds, including *Hemitriccus minor* (Sardelli 2005), *Hypocnemis rondoni* (Isler *et al.* 2007; Tobias *et al.* 2008; Whitney *et al.* 2013a), *Herpsilochmus stotzi* (Whitney *et al.* 2013b), *Hylophylax naevius* (Fernandes *et al.* 2014) and *Picumnus aurifrons* (Cohn-Haft *et al.* 2007), all of which weigh on average less than 13 g each. In other parts of the world, tiny birds make spectacular long-distance migrations and even in Amazonia, where most species tend to be sedentary (Stotz *et al.* 1996), certain species, such as those adapted to river islands (Remsen & Parker, 1983; Rosenberg, 1990), are likely to be excellent dispersers, independent of size. However, there is evidence that at least two other heavier bodied species have structured populations delimited by the Aripuaná and Jiparaná rivers as well: *Thamnophilus aethiops* (23-30g; Thom & Aleixo 2015) and *Malacoptila rufa* (36-44g; Ferreira 2013). As discussed by Smith *et al.* (2014) differences in life history attributes, effective population sizes, lineage ages, and dispersal rates can together account for highly disparate responses of avian lineages across important physical barriers in the Neotropics such as the Andes and some large Amazonian rivers, including the Madeira River. Further tests, controlling for phylogeny, habitat, wing shape and loading, and behavioral responses to open spaces, will be necessary to determine the extent to which body mass is a useful predictor of genetic population structure in suboscine passerines.

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APPENDIX

Information on the specimens analyzed.

Population/Locality	Taxon: Voucher/Genbank accession numbers
0/ AM: right bank of lower Juruá, RESEX Baixo Juruá, comunidade Socó. 3°36'S; 66°4'W	*G. spirurus*: INPA A 808/HM164938
1/ AM:"Campo do Lago Preto", left bank of Madeira river, 39 km W Novo Aripuanã. 5°09'S; 60°44'W	*W. poecilinotus*: INPA A 420/HM164985,421/HM164980, 422/HM164994, 424/HM164975. *S. turdina*: INPA A 395/HM165029, 413/HM165028
2/ AM: right bank Purús river, Ussuá stream, tributary of Mucuim river (right bank). 7°13'S; 64°10'W	*W. poecilinotus*: INPA A 101/HM164978
3/ RO: left bank of the Madeira river, near Jacy Paraná, ca. 45 km southwest Porto Velho. 9°10'S; 64°23'W	*G. spirurus*: INPA A 349/HM164942, 359/HM164941. *W. poecilinotus*: INPA A 345/HM164983, 347/HM164999, *S. turdina* INPA A 348/HM165032
4/ RO: left bank of the Madeira river, ca. 20 km N Abunã. 9°31'S; 65°21'W	*G. spirurus*: INPA A 173/HM164943, 191/HM164960. *W. poecilinotus*: 182/HM164984
5/ AM: left bank of Aripuanã river, Arauazinho stream, 130 km S Novo Aripuanã. 6°18'S; 60°24'W	*G. spirurus*: INPA A 461/HM164951, 466/HM164950, 510/HM164952, 553/HM164963, 562/HM164962. *W. poecilinotus*: INPA A 465/HM164974, 472/HM164967, 475/HM165009, 504/HM164971. *S. turdina*: INPA A 533/HM165014
6/ AM: left bank of lower Roosevelt river, confluence with Aripuanã river. 7°35'S; 60°43'W	*G. spirurus*: INPA A 906/HM164945. *W. poecilinotus*: INPA A 902/HM164976, 904/HM164992. *S. turdina*: INPA A 903/HM165017.

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7/ AM: right bank of lower Roosevelt river, confluence with Aripuaná river. 7°38'S; 60°40'W	*G. spirurus*: INPA A 895/HM164948. *W. poecilinotus*: INPA A 894/HM164997, 896/HM165007, 900/HM165003, 898/HM165001. *S. turdina*: INPA A 893/HM165015
8/ RO: right bank lower Jiparaná river, comunidade Demarcação, ca. 20 km southeast Calama. 8°09'S; 62°47'W	*G. spirurus*: INPA A 875/HM164946, 877/HM164958. *W. poecilinotus*: INPA A 876/HM164989, 884/HM164990, 907/HM165008. *S. turdina*: INPA A 885/HM165016
9/ RO: left bank of lower Jiparaná river, opp. Comunidade Demarcação, ca. 20 km southeast Calama. 8°14'S; 62°46'W	*G. spirurus*: INPA A 870/HM164964, 886/HM164940. *W. poecilinotus*: INPA A 871/HM164982, 872/HM164996, 873/HM165000, 878/HM164988, 881/HM165010, 882/HM164977. *S. turdina*: INPA A 874/HM165034, 880/HM165021, 883/HM165018
10/ RO: right bank of Madeira river, 9.5 km southeast Porto Velho. 8°52'S; 64°0'W	*G. spirurus*: INPA A 329/HM164959. *W. poecilinotus*: INPA A 307/HM164966, 308/HM164970, 326/HM164968, 327/HM164973, 334/HM164972
11/ RO: right bank of Madeira river, near Jacy Paraná, ca. 45 km southwest Porto Velho	*W. poecilinotus*: INPA A 367/HM165002, 368/HM164969. *S. turdina*: INPA A 371/HM165030, 372/HM165025, 374/HM165026
12/ RO: right bank of Madeira river, ca. 20 km N Abunã. 9°35'S; 65°21'W	*G. spirurus*: INPA A 208/HM164949. *W. poecilinotus*: INPA A 248/HM165005, 264/HM165006, 265/HM164993. *S. turdina*: INPA A 249/HM165031, 266/HM165012
13/ AM: right bank of Aripuaná, Extremo stream, 135 km S Novo Aripuaná. 6°18'S; 60°20'W	*G. spirurus*: INPA A 536/HM164944, 559/HM164947, 561/HM164955. *W. poecilinotus*: INPA A 478/HM164987, 479/HM164979. *S. turdina*: INPA A 525/HM165027, 527/HM165023, 538/HM165024
14/ AM: right bank of middle Aripuaná, confluence with Roosevelt river. 7°37'S; 60°40'W	*G. spirurus*: INPA A 890/HM164954. *W. poecilinotus*: INPA A 887/HM164995, 888/HM164981, 891/HM165004. *S. turdina*: INPA A 892/HM165033, 889/HM165019
15/ AM: Floresta Estadual do Sucunduri, right bank of upper Sucunduri river. 8°34.5'S; 59°08.5'W	*G. spirurus*: INPA A 845/HM164957. *W. poecilinotus*: INPA A 849/HM164998. *S. turdina*: INPA A 846/HM165013, 848/HM165022, 850/HM165020
16/ AM: Parque Estadual do Sucunduri; right bank of Bararati river. 8°21'S; 58°37'W	*G. spirurus*: INPA A 852/HM164953, 855/HM164956. *W. poecilinotus*: INPA A 856/HM164986, 857/HM164991
Outgroup/ AM: right bank of upper Negro river, 3 km SW São Gabriel da Cachoeira. 0°8'S; 67°5'W	*G. spirurus*: INPA A 1153/HM164939
Outgroup/ AM: left bank upper Negro river, 10 km east São Gabriel da Cachoeira. 0°10'S; 66°59'W	*G. spirurus*: INPA A 1118/HM164961
Outgroup/ AM: left bank middle Solimões river; RDS Amanã, Comunidade Nova Canaã, Centro Grande stream. 2°36'S; 64°52'W	*W. poecilinotus*: INPA A 398/HM164965
Outgroup/ AM: ca. 60 km N Manaus; highway BR-174, km 43; Campina reserve/INPA	*S. turdina*: INPA A 777/HM165011