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RESEARCH ARTICLE

Is the largest river valley west of the Andes a driver of diversification in Neotropical lowland birds?

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ABSTRACT

Physical and environmental barriers drive evolutionary diversification by limiting gene flow among populations. Rivers are barriers to gene flow in birds and other vertebrates, but differences in ecological conditions among sites also can affect the genetic structure of populations. The Magdalena River Valley (MRV) of Colombia, the largest South American river valley west of the Andes, is an appropriate location in which to test for the joint role of physical and ecological barriers because the river separates populations to the east and west, and a marked precipitation gradient leads to the occurrence of dry forests in the south and wet forests in the north. We conducted phylogeographic and population genetic analyses using mitochondrial DNA sequences of 4 avian species (*Xiphorhynchus susurrans, Mionectes oleagineus, Leptopogon amaurocephalus,* and *Eucometis penicillata*) sampled across 15 localities along the MRV. We found no spatial genetic structure in any of the species in the MRV region, and thus failed to find evidence for the role of the river or of the precipitation gradient as a driver of population differentiation. Gene flow across the river may be facilitated by its narrow headwaters and may occur as a consequence of river dynamics reducing its effectiveness as a barrier. Also, the dispersal abilities of the species may allow them to overcome the potential barrier that the river represents. As for the ecological barrier, we did not find that it caused any effect on the populations that we studied, probably because climatic differences were not strong enough to have led to population isolation along the MRV; alternatively, it is possible that the time elapsed since the origin of the precipititation gradient has been insufficient to lead to detectable effects on population structure. Our results contrast with work in other regions, which has shown marked genetic structure of vertebrate populations across major Neotropical rivers.

Keywords: Colombia, ecological barriers, *Eucometis*, *Leptopogon*, Magdalena Valley, *Mionectes*, riverine barriers, *Xiphorhynchus*

¿Es el valle más grande al occidente de los Andes un promotor de la diversificación en aves neotropicales de tierras bajas?

RESUMEN

Las barreras físicas y ambientales promueven la diversificación evolutiva limitando el flujo genético entre poblaciones. Los ríos son barreras al flujo genético en aves y otros vertebrados, pero las diferencias en las condiciones ecológicas entre sitios pueden igualmente tener un impacto sobre la estructura genética de las poblaciones. El valle del río Magdalena en Colombia (VRM), el valle más grande en Suramérica al occidente de los Andes, es un escenario apropiado donde se puede poner a prueba el papel conjunto de barreras físicas y ecológicas debido a que el río separa poblaciones al oriente y occidente, y un gradiente marcado en precipitación lleva a la presencia de bosques secos en el sur y bosques húmedos en el norte. Hicimos un análisis de filogeografía y genética de poblaciones usando secuencias de ADN mitocondrial de cuatro especies de aves (*Xiphorhynchus susurrans, Mionectes oleagineus, Leptopogon amaurocephalus* y *Eucometis penicillata*) muestreadas en 15 localidades a lo largo del VRM. Encontramos que no hay estructura genética espacial en ninguna de las especies en la región del VRM, por lo que no obtuvimos evidencia de que el río o el gradiente de precipitación jueguen un papel como promotores de diferenciación poblacional. El flujo genético a través del río podría ser facilitado por su cabecera angosta y podría ocurrir como consecuencia de su dinámica, que reduciría su efectividad como barrera. Igualmente, las habilidades de dispersión de las especies podríam permitirles sobreponerse a la barrera potencial que representa el río. En cuanto a la barrera ecológica, no encontramos que esté causando efecto alguno en las poblaciones estudiadas probablemente porque las diferencias climáticas no son lo suficientemente fuertes para conducir al aislamiento entre las poblaciones a lo largo del VRM; alternativamente, es posible que el tiempo desde el origen del gradiente de precipitación haya sido insuficiente para generar efectos detectables en la estructura de las poblaciones.

Nuestros resultados contrastan con estudios en otras regiones que muestran estructura genética marcada en poblaciones de vertebrados a través de ríos neotropicales de gran tamaño.

Palabras clave: barreras ecológicas, barreras fluviales, Colombia, *Eucometis*, *Leptopogon*, *Mionectes*, Valle del Magdalena, *Xiphorhynchus*

INTRODUCTION

Physical and ecological barriers to dispersal are known to restrict gene flow among populations, ultimately leading to evolutionary divergence and speciation (Haffer 1997, Bohonak 1999, Barraclough and Vogler 2000, Aleixo 2004, Smith et al. 2014). Although geographic isolation by physical barriers likely accounts for a large proportion of speciation events in tropical forest organisms (Moritz et al. 2000, Smith et al. 2014), natural selection in contrasting environments in the absence of physical isolation also might drive speciation and contribute to the buildup of tropical biodiversity (Moritz et al. 2000). However, studies examining the relative roles of physical and geographical barriers as drivers of population differentiation and speciation are scarce (Smith et al. 1997, 2014, Milá et al. 2009, Oliveira et al. 2015, Weir et al. 2015).

Several geographic features of the South American physical landscape contribute to the astounding avian diversity of the Neotropics. Inter-Andean valleys, rivers of the Amazon Basin, and the Andean mountain range are all important barriers to gene flow responsible for isolation and population differentiation in allopatry in Neotropical birds (e.g., Aleixo 2004, Cheviron et al. 2005, Ribas et al. 2012, Gutiérrez-Pinto et al. 2012, Maldonado-Coelho et al. 2013, Smith et al. 2014, Fernandes et al. 2014). A less-studied mechanism of population differentiation in Neotropical birds is environmental variation, which might play a role in restricting gene flow among populations (Rundle and Nosil 2005, Peterson and Nyári 2008, Nosil and Harmon 2009, Rodriguez et al. 2015). For example, spatial variation in food resources (Ryan et al. 2007), vegetation structure (Smith et al. 1997, Nicholls et al. 2006, Kirschel et al. 2011), or biotic and abiotic factors that vary with elevation (Freedman et al. 2010) may promote divergence in continuously distributed populations. The hypothesis of isolation by environment predicts that genetic distances between populations should increase with environmental differences, independent of geographic distance or of the presence of geographic barriers (Wang and Bradburd 2014).

An ideal setting in which to test the relative roles of physical isolation and isolation by environment as drivers of population differentiation would be a landscape in which species occurring on both sides of a geographical barrier are also distributed along an environmental gradient running parallel to the barrier. The Magdalena River Valley (hereafter, MRV) is the largest inter-Andean valley in northern South America, running south–north between the Eastern and Central Cordilleras of the Colombian Andes. The Magdalena River is the largest South American river west of the Andes and may reach >3 km in width near its mouth, thus potentially representing a major barrier for organisms with limited dispersal abilities (Moore et al. 2008). Additionally, the MRV exhibits a steep gradient in annual precipitation, ranging from ~700 mm in the south to ~4,000 mm in the north (Figure 1). This gradient results in a marked shift in vegetation, from tropical dry forest in the south to tropical wet forest in the north of the MRV; the transition between habitat types is rather sharp, occurring over ~60 km (Olson et al. 2001; Figure 1). Thus, the Magdalena River may constitute a physical barrier restricting east–west gene flow between populations located on opposite banks of the river, while varying climatic conditions may promote north–south differentiation of populations of species continuously distributed along its valley. Although the MRV is among the most important geographic and ecological barriers west of the Andes, its role in promoting divergence through vicariant and ecological mechanisms has seldom been proposed or tested (Haffer 1967, Link et al. 2015, Muñoz-Ortiz et al. 2015). This contrasts with ample research on the topic in lowland rivers east of the Andes, several of which are of comparable size to the Magdalena River (e.g., Patton et al. 1994, Bates et al. 2004, Maldonado-Coelho et al. 2013).

In this study, we examined the influence of the MRV on the diversification of lowland organisms by studying geographic variation in 4 species of widely distributed forest birds with presumably varied dispersal abilities. Specifically, we first evaluated whether the Magdalena River acts as a physical barrier for avian populations; this hypothesis predicts restricted gene flow across the river resulting in (1) populations from one bank of the river being more closely related to each other than to any population from the opposite bank (i.e. reciprocal monophyly of populations from separate banks), or (2) greater genetic distances between populations across the river from each other than between populations separated by similar distances on the same river bank. Additionally, we evaluated the extent to which differences in precipitation along the MRV might act as an ecological barrier to gene flow among populations; this hypothesis predicts (1) that populations from one section of the valley (either dry or wet) will be more closely related to each other than to populations from the other section of the valley, or (2) greater genetic distances between populations from different sections of the valley relative to populations separated by similar distances

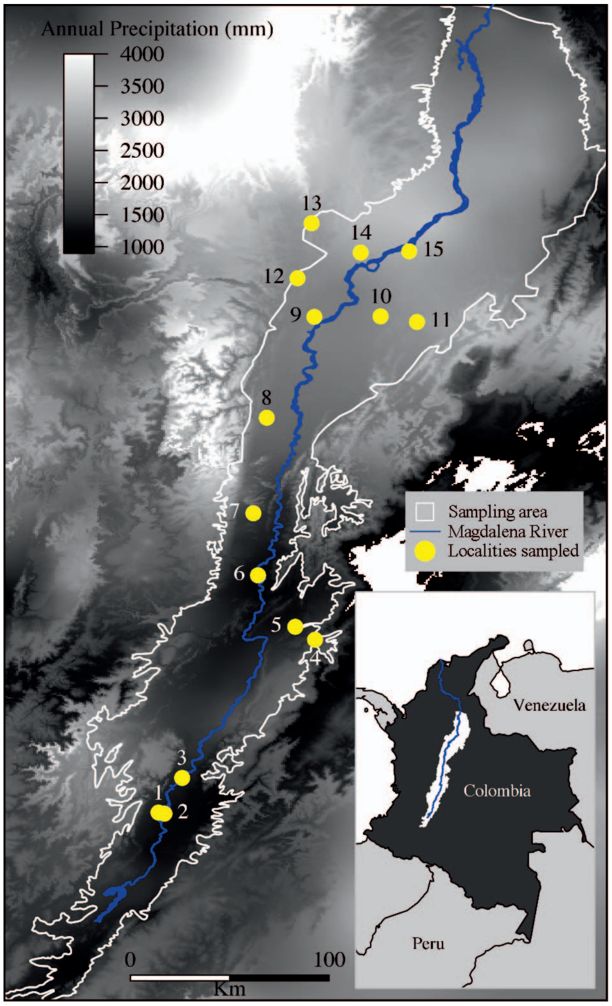

FIGURE 1. Map of central Colombia showing spatial patterns of variation in precipitation (data obtained from WorldClim; Hijmans et al. 2005) along the Magdalena River Valley (dry in the south, wet in the north) and the location of sampling sites selected for this study. Each numbered circle corresponds to a sampling locality: 1 = Arenosa, 2 = Bateas, 3 = Potosí, 4 = Boqueron, 5 = Mana Dulce, 6 = Venadillo, 7 = Jabiru, 8 = Rio Manso, 9 = La Suiza, 10 = El Cucuy, 11 = Cimitarra, 12 = Maceo, 13 = Remedios, 14 = Barbacoas, and 15 = San Juan.

within a section. Because the above hypotheses are not mutually exclusive, we also examined the extent to which physical isolation and ecology may have jointly structured populations of bird species in the region.

METHODS

Taxon and Geographic Sampling

We selected 15 localities (Figure 1) on both sides of the Magdalena River (8 on the western bank, 7 on the eastern bank) and distributed along the precipitation gradient of the valley (6 in dry forest, 9 in wet forest), in which we collected specimens of 4 locally abundant avian species (Appendix Table 3): Cocoa Woodcreeper (*Xiphorhynchus susurrans*, Furnariidae [$n = 33$]), Ochre-bellied Flycatcher (*Mionectes oleagineus*, Tyrannidae [$n = 19$]), Sepia-capped Flycatcher (*Leptopogon amaurocephalus*, Tyrannidae [$n = 18$]), and Gray-headed Tanager (*Eucometis penicillata*, Thraupidae [$n = 37$]). We selected these species because of their different habits, which likely influence their dispersal ability and hence the genetic structure of populations (Burney and Brumfield 2009, Smith et al. 2014, Paz et al. 2015). *X. susurrans* forages on tree trunks from the understory to the forest canopy for midsized insects and occasionally small vertebrates, and often follows army ant swarms (del Hoyo et al. 2003). *M. oleagineus* is largely frugivorous but also eats insects and other arthropods, and usually forages in the understory but may forage up to 10 m above the ground (del Hoyo et al. 2004). *L. amaurocephalus* forages on small arthropods in shady leafy settings, usually within 8 m of the ground, and occasionally eats small fruits (del Hoyo et al. 2004). Finally, *E. penicillata* feeds mainly on fruits and insects and forages close to the ground (Isler and Isler 1999). Birds were captured using mist nets, prepared as study skins, and deposited in the Museo de Historia Natural ANDES at Universidad de Los Andes in Bogotá, Colombia (Appendix Table 3).

Extraction, Amplification, and Sequencing of DNA

We extracted DNA from pectoral muscle tissue using a phenol chloroform protocol (see Gutiérrez-Pinto et al. 2012 for details). We then amplified the mitochondrial cytochrome *b* gene (cyt *b*; 1,056 bp [base pairs] for Cocoa Woodcreeper, 1,027 bp for Ochre-Bellied Flycatcher, 999 bp for Sepia-Capped Flycatcher, and 1,046 bp for Gray-Headed Tanager) using the primer pair L14996/H16064 (Sorenson et al. 1999, Sambrook and Russell 2001). The amplification reactions consisted of 2 μL of template extract (~50 ng of DNA), 1 μL of 10 mM dNTPs (deoxynucleotide triphosphates), 1.2 μLof each primer (10 mM), 2.5 μL of 10× buffer with 1.5 μL of $MgCl_2$, 0.125 μL Taq DNA polymerase, and 16.5 μL of sterile ddH_2O (double-distilled water). We ran PCRs in a PTC-200 Thermal Cycler (MJ Research, Waltham, Massachusetts, USA), beginning with an initial denaturation at 94°C for 2 min, followed by 34 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min, with a final extension phase at 72°C for 7 min. PCR products were purified with Exosap-IT (USB Corporation, Cleveland, Ohio, USA), and sequenced in both directions by Macrogen (Seoul, Korea). Sequences were deposited in GenBank (Appendix Table 3).

Phylogenetic and Population Genetic Analyses

For phylogenetic analyses, we enriched our dataset with cyt *b* sequences available in GenBank (Appendix Table 3) corresponding to individuals from outside the MRV. We determined the models of evolution that best fitted the data using jModelTest 2.1.7 (Posada 2008). We then employed the selected models in Bayesian phylogenetic analyses in the program MrBayes (Ronquist and Huelsenbeck 2003); 4 Monte Carlo Markov Chains were allowed to run for 6 million generations for each species. Chains converged in all cases, as indicated by effective parameter sample sizes >200, mean standard deviation of split frequencies <0.01, and potential scale reduction factors of ~ 1. Based on the trees retained, we constructed a 50% majority-rule consensus tree for each species. Outgroups were chosen based on existing phylogenetic information (Jetz et al. 2012): Buff-throated Woodcreeper (*Xiphorhynchus guttatus*) for Cocoa Woodcreeper, McConnell's Flycatcher (*Mionectes macconnelli*) for Ochre-bellied Flycatcher and for Sepia-capped Flycatcher, and Black-goggled Tanager (*Trichothraupis melanops*) for Gray-headed Tanager (Appendix Table 3). In addition, using only individuals captured in the MRV, we constructed median-joining haplotype networks separately for each species using the pegas package (Paradis 2010) for R 3.0.2 (R Core Team 2013).

We used analyses of molecular variance (AMOVA) to determine the extent to which populations were structured by the river barrier or the precipitation gradient. We assigned populations to a side of the river (east or west) and to the type of forest (wet or dry) using the boundaries of the dry and wet forest ecoregions of the MRV (Olson et al. 2001). Based on these divisions, we evaluated the structure among populations from different river banks and/or type of forest, among populations from the same bank and/or type of forest, and within populations (Maldonado-Coelho et al. 2013, Link et al. 2015).

We also tested for isolation by environment by examining the correlation between climatic distance and genetic distance among populations (Wang and Bradburd 2014). For this analysis, we correlated Euclidean distances in annual precipitation among localities based on WorldClim data (Hijmans et al. 2005) with mean Nei's (1972) genetic distance calculated using the R package adegenet (Jombart 2008). Additionally, we examined whether the geographic distance between populations affected the genetic structure of populations within the MRV (i.e. isolation by distance) using a Mantel test that related geographic and genetic distance matrices for each species. Geographic distances were estimated using ArcGIS 10.1 (ESRI, Redlands, California, USA). To test for an interaction between climatic distance and the effect of the river as a barrier, we performed partial Mantel tests using a third matrix in which the distance between populations on different sides of the river was scored as 1 and that between populations on the same bank was scored as 0 (Maldonado-Coelho et al. 2013). Mantel and partial Mantel tests were performed using Pearson's product moment correlation in the Vegan package for R (Oksanen et al. 2012), and significance was determined based on 1,000 permutations of the precipitation distance matrix.

RESULTS

Across our study sites, we observed 6 cyt *b* haplotypes in *X. susurrans* ($n = 31$) and in *L. amaurocephalus* ($n = 18$), 3 haplotypes in *M. oleagineus* ($n = 17$), and 7 haplotypes in *E. penicillata* ($n = 37$). Phylogenetic analyses that included a few samples from outside the MRV revealed somewhat different phylogeographic patterns among our study species. In *X. susurrans* and *L. amaurocephalus* we found little geographic structure and shallow divergence, whereas populations of *M. oleagineus* and *E. penicillata* showed greater structure and deeper divergence (Figure 2). In the latter 2 species, most samples from the MRV formed a clade separate from other populations, but in no case did we observe the existence of subclades separating populations from the MRV based on river bank or forest type.

Haplotype networks constructed for all species showed no structure among populations, a pattern inconsistent with the hypotheses that the river or the precipitation gradient cause population isolation. In all cases, the most frequently observed haplotypes occurred on both sides of the river and in both types of forest (Figure 3). However, a few rare haplotypes were present exclusively on one side of the river or in one type of forest (Figure 3). Despite this, AMOVAs showed that most of the variation for all species lay within populations (Table 1), and F_{ST} values were close to zero, indicating no genetic structure among the populations sampled. Geographic distance, the river barrier, and precipitation distance alone had no effect on the structure of populations within the MRV; only for the Gray-headed Tanager did we find a slight, but significant, positive relationship between precipitation and genetic distances ($r = 0.36$, $P = 0.007$), and geographic and genetic distances ($r = 0.36$, $P = 0.006$; Table 2, Figure 4). None of the partial tests including river bank as an explanatory variable for genetic distance were significant (Table 2).

DISCUSSION

We found no evidence to support the hypotheses that the Magdalena River or the precipitation gradient along its valley act as barriers to dispersal of individuals among populations of 4 lowland bird species. Our mitochondrial DNA sequence data revealed low overall genetic diversity and lack of population structure, even though some populations were separated by an $\sim$1-km-wide river and by nearly 700 km and occurred in environments that differed by up to $\sim$3,000 mm in annual rainfall (Hijmans et al. 2005).

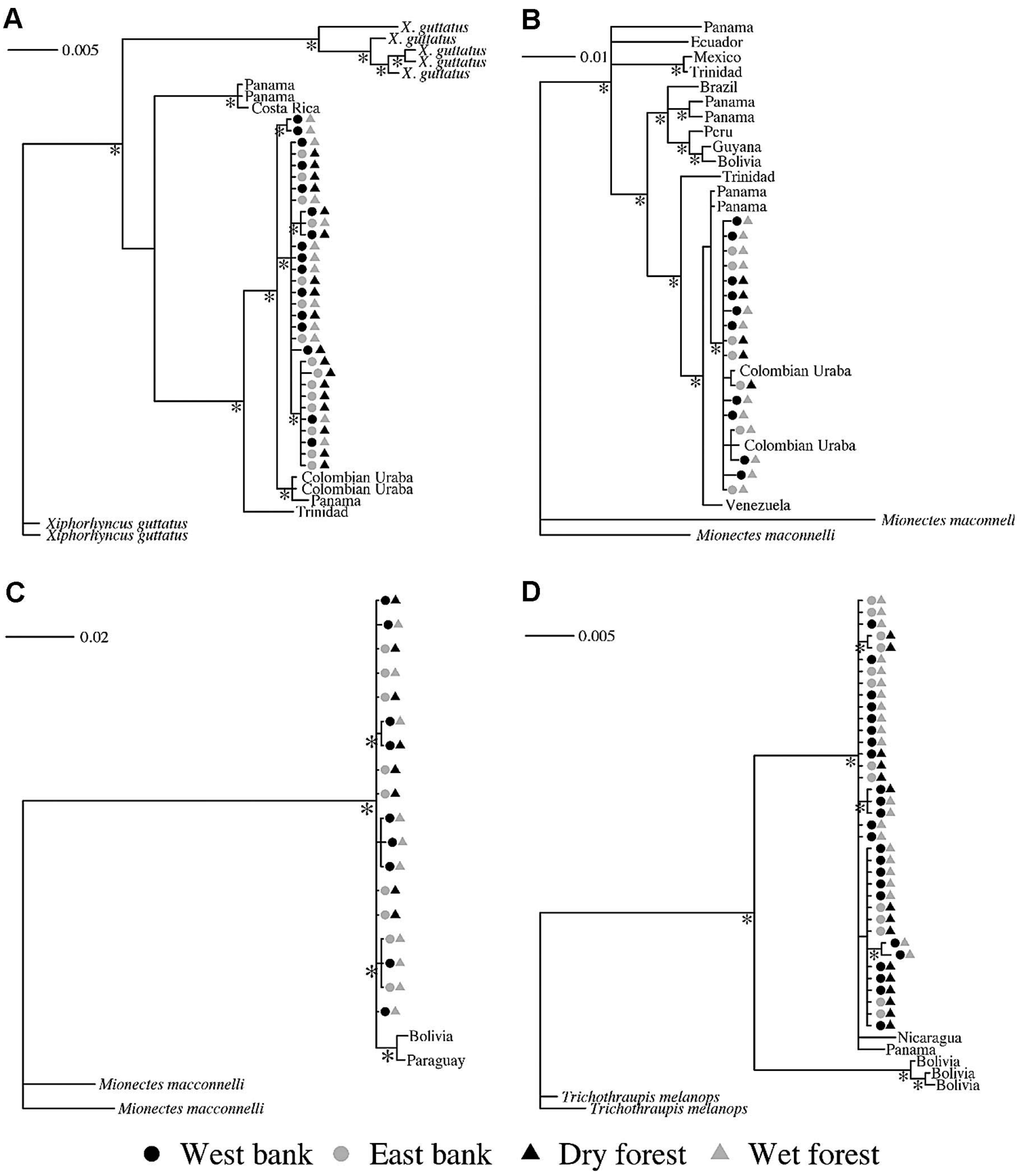

FIGURE 2. Fifty percent majority-rule consensus trees obtained based on Bayesian analyses of mtDNA (cytochrome *b*) sequences showing phylogenetic relationships among haplotypes of (**A**) *Xiphorhynchus susurrans*, (**B**) *Mionectes oleagineus*, (**C**) *Leptopogon amaurocephalus*, and (**D**) *Eucometis penicillata*. Samples from the Magdalena River Valley (MRV), Colombia, are labeled by the bank of the river (black circles for the west bank, gray circles for east bank) and by the type of forest (black triangles for dry forest, gray triangles for wet forest) from which they were collected, and samples from outside the MRV are labled with their locality of origin. *Xiphorhynchus guttatus*, *Mionectes macconnelli*, and *Trichotraupis melanops* were used as outgroups. The models of evolution that best fitted the data for each species were TrN (Tamura–Nei) for *X. susurrans*, TVM + I + G (transversion + invariable sites + rate variation among sites) for *M. oleagineus*, TPM1uf (Kimura 1981 + unequal base frequencies) for *L. amaurocephalus*, and HKY + I (Hasegawa–Kishino–Yano + invariable sites) for *E. penicillata*. Asterisks (*) represent posterior probability values of 0.95 or higher. Scale bars represent the number of expected mutations per site.

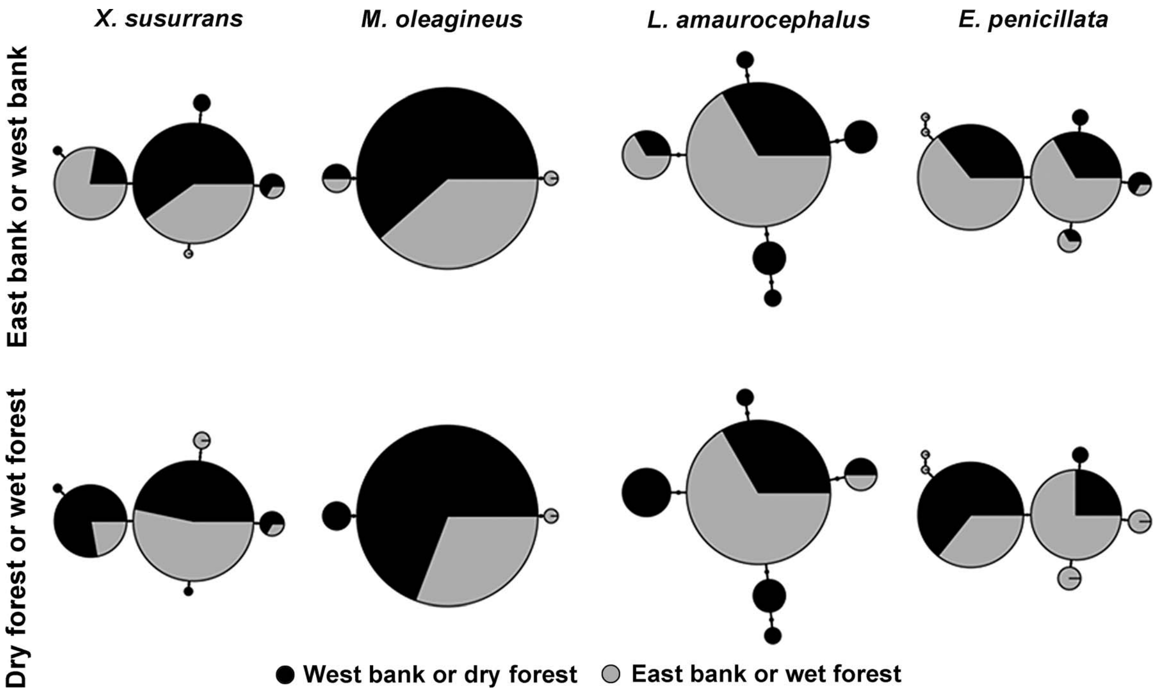

FIGURE 3. Haplotype networks obtained for each of 4 locally abundant species from the cytochrome *b* marker, using the pegas package for R (Paradis 2010), showing lack of structure with respect to river bank or type of forest. Portions in black indicate the frequency of individuals from the west bank of the river or dry forest, and portions in gray indicate the frequency of individuals from the east bank of the river or wet forest in the Magdalena River Valley, Colombia.

Considering that several rivers in the Neotropical region—some of which are not particularly large—are known to act as geographic barriers for birds (Ribas et al. 2012, Naka et al. 2012, Maldonado-Coelho et al. 2013, Fernandes et al. 2014, Smith et al. 2014) and other vertebrates (Gascon et al. 1996, Fouquet et al. 2012), why is it that it that the Magdalena River does not act as a barrier to gene flow among populations, despite it being the largest river west of the Andes in South America? Our results are not unique to birds, because similar findings (i.e. lack of genetic differentiation across the river and little to no genetic structure along the valley) were also obtained in a

TABLE 1. Results of analyses of molecular variance (AMOVA) showing that forest type (wet vs. dry) and the Magdalena River, Colombia, did not influence population structure in any of the 4 locally abundant species studied.

			Percent of variation	
Source of variation	Species	df	River	Forest
Among populations across the river and in different types of forest	*X. susurrans*	1	−3.7**	−8.0**
	M. oleagineus	1	9.6	3.4
	L. amaurocephalus	1	−5.7	−1.4
	E. penicillata	1	−6.6	11.8
Among populations from the same river side and type of forest	*X. susurrans*	6	34.9*	38.1**
	M. oleagineus	7	−33.0	−30.0
	L. amaurocephalus	6	9.6	6.9
	E. penicillata	9	22.8	11.4
Within populations	*X. susurrans*	23	68.8	69.9
	M. oleagineus	8	123.4	126.6
	L. amaurocephalus	10	96.1	94.5
	E. penicillata	26	83.8	76.8
Total	*X. susurrans*	30		
	M. oleagineus	16		
	L. amaurocephalus	17		
	E. penicillata	36		

* $P < 0.01$; ** $P < 0.001$.

TABLE 2. Results of Mantel tests examining the relationship between genetic distance and geographic and precipitation distances of 4 locally abundant species in the Magdalena River Valley, Colombia. An additional categorical matrix (river) was used in the Mantel tests to evaluate the effect of the Magdalena River on the genetic distances among individuals. If 2 individuals belonged to populations from different banks of the river, the matrix value was entered as 1, and if they were collected from populations on the same side, the matrix value was 0. Relationships were not significant except for *Eucometis penicillata*, for which genetic distance was weakly correlated with both geographic and environmental distances (significant results are highlighted in bold font). Upper 97.5% Quantile = the 97.5th percentile of the distribution of the correlation coeficient with randomized data.

	r	*P*	Upper 97.5% Quantile
Xiphorhynchus susurrans			
Geography	−0.02	0.37	0.49
Precipitation	0.15	0.10	0.31
Geography + River	−0.06	0.49	0.47
Precipitation + River	−0.18	0.84	0.84
Mionectes oleagineus			
Geography	0.20	0.18	0.62
Precipitation	0.10	0.28	0.60
Geography + River	0.13	0.19	0.40
Precipitation + River	0.02	0.30	0.47
Leptopogon amaurocephalus			
Geography	0.28	0.15	0.47
Precipitation	0.16	0.06	0.22
Geography + River	−0.17	0.86	0.54
Precipitation + River	−0.24	0.94	0.37
Eucometis penicillata			
Geography	**0.36**	**<0.01**	**0.26**
Precipitation	**0.36**	**<0.01**	**0.27**
Geography + River	0.14	0.13	0.37
Precipitation + River	0.06	0.19	0.40

recent study on brown spider monkeys (*Ateles hybridus*; Link et al. 2015). Gene flow across large rivers that may represent insurmountable barriers in their lower reaches may occur readily near their headwaters (Capparella 1988, 1991, Ayres and Clutton-Brock 1992, Peres et al. 1996, Gascon et al. 1998, Gehring et al. 2012, Naka et al. 2012); indeed, a recent study showed that distinct populations of Amazonian birds meet and hybridize in the headwater regions of the Tapajós, a major river known to promote population divergence (Weir et al. 2015). Thus, the lack of population differentiation across the Magdalena River may be explained by dispersal across its narrow headwaters. In addition, the effect of rivers such as the Magdalena as barriers may be transient because they form meanders and change course through time, allowing populations that were isolated in the past to reconnect after reconfigurations of basins. In addition, rivers such as the Magdalena form islands that may serve as stepping-stones, allowing for dispersal (Aleixo 2004, Jackson and Austin 2013, Link et al. 2015). Finally, during dry periods, rivers such as the Magdalena may become greatly reduced in size, allowing for greater connectivity of populations across the river than during wet periods. Nevertheless, some of the Amazonian rivers known to act as barriers to dispersal by birds also form islands or meanders and may fluctuate considerably in water discharge and width (Nanson and Knighton 1996, Mendes et al. 2010, Latrubesse et al. 2013), which suggests that properties of the Magdalena River may not fully account for our results.

Another potential explanation for the lack of genetic structure of populations across the Magdalena River is that our study species may have good dispersal abilities relative to the size of the barrier. A recent study has suggested that the dispersal ability of bird species is one of the most important factors accounting for speciation across geographical barriers, including rivers (Smith et al. 2014; see also Burney and Brumfield 2009, Claramunt et al. 2012). In fact, 2 of the 4 species evaluated in our study (*X. susurrans* and *M. oleagineus*) have been shown to be able to potentially overcome water barriers >300 m wide (Moore et al. 2008), and previous work has shown that Amazonian rivers with similar widths to the Magdalena do not represent barriers for other species in the genus *Xiphorhynchus* (Aleixo 2004). In contrast, examples of differentiation across other Neotropical rivers often involve species with limited dispersal abilities (e.g., trumpeters: Ribas et al. 2012; antbirds: Maldonado-Coelho et al. 2013). Work on species that occur in the MRV and that have lower dispersal abilities (e.g., some members of the Thamnophilidae or Troglodytidae) is necessary to determine whether the Magdalena River may indeed act as a geographic barrier for some birds (Hayes and Sewlal 2004, Moore et al. 2008).

We also failed to find evidence for the precipitation gradient as a factor causing genetic structure of populations along the MRV. Although this may suggest that habitat variation driven by precipitation has no effect on population structure in the region, our results may simply reflect the region's geological and climatic history. Until the Last Glacial Maximum, the MRV is thought to have been covered mostly by dry forest (Haffer 1967), which formed a continuous habitat north to the Caribbean lowlands in northern Colombia and Venezuela. Thus, the precipitation gradient is likely to have a relatively recent origin, with rainforests of the mid-MRV (and their avifaunas) dating to ~12,000 yr before present (Haffer 1967). Hence, if spatial changes in precipitation indeed represent an ecological barrier to dispersal by our study species, then it is possible that we failed to detect divergence in the genes that we studied because there has not been enough time for changes to accumulate and for populations to achieve significant structure, let alone reciprocal monophyly (Edwards and Beerli 2000).

Alternatively, the precipitation gradient may not represent a barrier to gene flow in neutral mtDNA genes, but

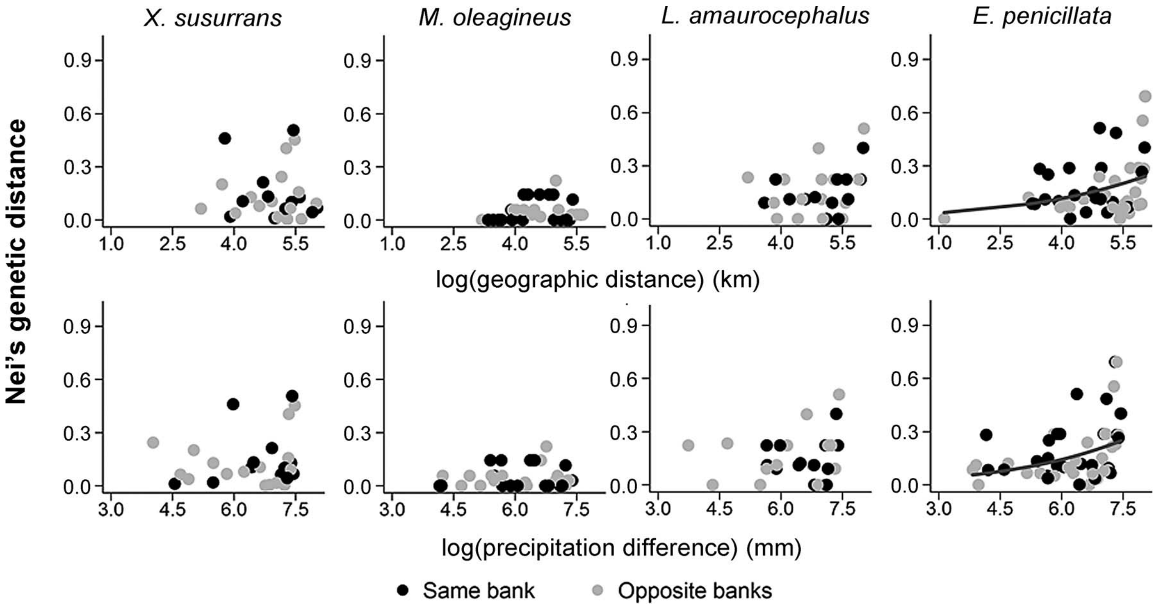

FIGURE 4. Isolation by environment tested through correlations between the genetic distances (Nei 1972) among populations and the geographic distances among localities for each study species (upper 4 graphs) and the precipitation distances among localities (lower 4 graphs). Black and gray circles indicate whether the comparison was among populations from the same bank of the Magdalena River, Colombia (black), or from opposite banks (gray). A slight positive but significant correlation between the genetic distance and the geographic ($r = 0.36$, $P = 0.006$) and environmental ($r = 0.36$, $P = 0.007$) distance was found only for *E. penicillata*.

may lead to divergence in functionally important traits owing to differential selection in contrasting environments (Smith et al. 1997, Doebeli and Dieckmann 2003, Milá et al. 2007, 2009, McCormack et al. 2008, Oliveira et al. 2015). Studies examining variation in morphological traits and in multiple loci across the genome would shed further light on the role that environmental variation along the MRV may have in driving phenotypic and genetic divergence among populations. In fact, as a follow-up to the present study, we are currently using RADseq (restriction site-associated DNA sequencing) data to explore genome-wide genetic structure in our focal species. Our goals are to evaluate the consistency of the patterns of low genetic structure among populations along the MRV found in this study and to explore the possibility that some functionally important loci might be under divergent selection by extreme differences in the environment despite gene flow in neutral markers.

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APPENDIX TABLE 3. Detailed information on sampling localities, species sampled, UNIANDES-O catalog numbers (Catalog), geographic coordinates (latitude and longitude), side of the Magdalena River (Side; E = east bank, W = west bank), mean annual precipitation in millimeters at each sampling locality (Prec), and GenBank accession numbers (GenBank) for each specimen used in our analyses of diversification of lowland birds in the Magdalena River Valley, Colombia. GenBank accession numbers of sequences generated during this study are highlighted in bold font. NA denotes individuals collected outside the Magdalena River Valley. These samples were used for phylogenetic analyses but were excluded from the construction of haplotype networks and from population genetics analyses. Species are listed in taxonomic order and then by GenBank accession number. Species abbreviations: XISU = *Xiphorhynchus susurrans*, XIGU = *Xiphorhynchus guttatus*, PSSI = *Pseudotriccus simplex*, MIOL = *Mionectes oleagineus*, MIMA = *Mionectes macconnelli*, LEAM = *Leptopogon amaurocephalus*, TRME = *Trichothraupis melanops*, and EUPE = *Eucometis penicillata*.

Locality	Species	Catalog	Latitude	Longitude	Side	Prec	GenBank
Uraba	XISU	985	7.52370	−76.58383	NA	NA	**KX836033**
Mana Dulce	XISU	942	4.35120	−74.65141	E	1,375	**KX836034**
Jabiru	XISU	948	5.06656	−74.84135	W	1,618	**KX836035**
Mana Dulce	XISU	984	4.35120	−74.65141	E	1,375	**KX836036**
Uraba	XISU	NA	7.52370	−76.58383	NA	NA	**KX836037**
Rio Manso	XISU	936	5.66584	−74.78178	W	2,240	**KX836038**
Mana Dulce	XISU	941	4.35120	−74.65141	E	1,375	**KX836039**
San Juan	XISU	934	6.71725	−74.13454	E	2,997	**KX836040**
Rio Manso	XISU	938	5.66584	−74.78178	W	2,240	**KX836041**
Barbacoas	XISU	930	6.71085	−74.35172	W	2,888	**KX836042**
Jabiru	XISU	950	5.06656	−74.84135	W	1,618	**KX836043**
San Juan	XISU	932	6.71725	−74.13454	E	2,997	**KX836044**
Jabiru	XISU	946	5.06656	−74.84135	W	1,618	**KX836045**
Mana Dulce	XISU	898	4.35120	−74.65141	E	1,375	**KX836046**
Mana Dulce	XISU	986	4.35120	−74.65141	E	1,375	**KX836047**
Barbacoas	XISU	929	6.71085	−74.35172	W	2,888	**KX836048**
Barbacoas	XISU	927	6.71085	−74.35172	W	2,888	**KX836049**
Barbacoas	XISU	926	6.71085	−74.35172	W	2,888	**KX836050**
Jabiru	XISU	944	5.06656	−74.84135	W	1,618	**KX836051**
San Juan	XISU	935	6.71725	−74.13454	E	2,997	**KX836052**
San Juan	XISU	945	6.27975	−74.10755	E	2,997	**KX836053**
Jabiru	XISU	949	5.06656	−74.84135	W	1,618	**KX836054**
Barbacoas	XISU	928	6.71085	−74.35172	W	2,888	**KX836055**
Bateas	XISU	955	3.17081	−75.25124	E	1,280	**KX836056**
Barbacoas	XISU	925	6.71085	−74.35172	W	2,888	**KX836057**
Mana Dulce	XISU	940	4.35120	−74.65141	E	1,375	**KX836058**
Jabiru	XISU	952	5.06656	−74.84135	W	1,618	**KX836059**
Jabiru	XISU	951	5.06656	−74.84135	W	1,618	**KX836060**
Mana Dulce	XISU	987	4.35120	−74.65141	E	1,375	**KX836061**
Bateas	XISU	953	3.17081	−75.25124	E	1,280	**KX836062**
Venadillo	XISU	947	4.67560	−74.82046	E	1,224	**KX836063**
Mana Dulce	XISU	943	4.35120	−74.65141	E	1,375	**KX836064**
Rio Manso	XISU	937	5.66584	−74.78178	W	2,240	**KX836065**
Rio Manso	MIOL	843	5.66584	−74.78178	W	2,240	**KX836067**
San Juan	MIOL	755	6.71725	−74.13454	E	2,997	**KX836066**
Rio Manso	MIOL	977	5.66584	−74.78178	W	2,240	**KX836068**
Rio Manso	MIOL	753	5.66584	−74.78178	W	2,240	**KX836069**
Remedios	MIOL	818	6.90883	−74.57204	W	2,824	**KX836070**
Mana Dulce	MIOL	835	4.35120	−74.65141	E	1,375	**KX836071**
Mana Dulce	MIOL	833	4.35120	−74.65141	E	1,375	**KX836072**
Mana Dulce	MIOL	995	4.35120	−74.65141	E	1,375	**KX836073**
Maceo	MIOL	829	6.55162	−74.64276	W	2,554	**KX836074**
La Suiza	MIOL	841	6.30360	−74.65141	W	2,461	**KX836075**
Uraba	MIOL	999	7.52370	−76.58383	NA	NA	**KX836076**
Uraba	MIOL	NA	7.52370	−76.58383	NA	NA	**KX836077**
San Juan	MIOL	967	6.27975	−74.10755	E	2,997	**KX836082**
Jabiru	MIOL	826	5.06656	−74.84135	W	1,618	**KX836078**
Jabiru	MIOL	976	5.06656	−74.84135	W	1,618	**KX836079**
San Juan	MIOL	975	6.27975	−74.10755	E	2,997	**KX836080**
San Juan	MIOL	974	6.27975	−74.10755	E	2,997	**KX836081**
Barbacoas	MIOL	821	6.71085	−74.35172	W	2,888	**KX836083**
Barbacoas	MIOL	830	6.71085	−74.35172	W	2,888	**KX836084**

APPENDIX TABLE 3. Continued.

Locality	Species	Catalog	Latitude	Longitude	Side	Prec	GenBank
Maceo	LEAM	870	6.55140	−74.64052	NA	2,554	**KX836085**
San Juan	LEAM	871	6.27975	−74.10755	E	2,997	**KX836086**
Mana Dulce	LEAM	990	4.35120	−74.65141	E	1,375	**KX836087**
Mana Dulce	LEAM	851	4.35120	−74.65141	E	1,375	**KX836088**
Barbacoas	LEAM	855	6.71085	−74.35172	W	2,888	**KX836089**
Rio Manso	LEAM	861	5.66584	−74.78178	W	2,240	**KX836090**
Barbacoas	LEAM	866	6.71085	−74.35172	W	2,888	**KX836091**
Mana Dulce	LEAM	991	4.35120	−74.65141	E	1,375	**KX836092**
Barbacoas	LEAM	849	6.71085	−74.35172	W	2,888	**KX836093**
Arenosa	LEAM	852	3.18115	−75.27187	W	1,333	**KX836094**
Barbacoas	LEAM	846	6.71085	−74.35172	W	2,888	**KX836095**
Mana Dulce	LEAM	993	4.35120	−74.65141	E	1,375	**KX836096**
El Cucui	LEAM	853	6.30707	−74.26435	W	2,604	**KX836097**
Mana Dulce	LEAM	992	4.35120	−74.65141	E	1,375	**KX836098**
Rio Manso	LEAM	873	5.66584	−74.78178	W	2,240	**KX836099**
San Juan	LEAM	863	6.71725	−74.13454	E	2,997	**KX836100**
Barbacoas	LEAM	865	6.71085	−74.35172	W	2,888	**KX836101**
Jabiru	LEAM	869	5.06656	−74.84135	W	1,618	**KX836102**
Arenosa	EUPE	774	3.18115	−75.27187	W	1,333	**KX835996**
Barbacoas	EUPE	777	6.71085	−74.35172	W	2,888	**KX835997**
Barbacoas	EUPE	794	6.71085	−74.35172	W	2,888	**KX835998**
Barbacoas	EUPE	766	6.71085	−74.35172	W	2,888	**KX835999**
Bateas	EUPE	786	3.17081	−75.25124	E	1,280	**KX836000**
Boqueron	EUPE	792	4.27002	−74.56168	E	1,668	**KX836001**
Boqueron	EUPE	769	4.27002	−74.56168	E	1,668	**KX836002**
Bateas	EUPE	795	3.17081	−75.25124	E	1,280	**KX836003**
Jabiru	EUPE	783	5.06656	−74.84135	W	1,618	**KX836004**
Jabiru	EUPE	773	5.06656	−74.84135	W	1,618	**KX836005**
Jabiru	EUPE	806	5.06656	−74.84135	W	1,618	**KX836006**
Jabiru	EUPE	808	5.06656	−74.84135	W	1,618	**KX836007**
La Suiza	EUPE	770	6.30360	−74.65141	W	2,461	**KX836008**
La Suiza	EUPE	802	6.30360	−74.65141	W	2,461	**KX836009**
La Suiza	EUPE	796	6.30360	−74.65141	W	2,461	**KX836010**
Maceo	EUPE	804	6.55140	−74.64052	W	2,554	**KX836011**
Barbacoas	EUPE	803	6.71085	−74.35172	W	2,888	**KX836012**
Maceo	EUPE	765	6.55140	−74.64052	W	2,554	**KX836013**
Remedios	EUPE	772	6.90883	−74.57204	W	2,824	**KX836014**
San Juan	EUPE	767	6.71725	−74.13454	E	2,997	**KX836015**
San Juan	EUPE	791	6.71725	−74.13454	E	2,997	**KX836016**
Maceo	EUPE	785	6.55162	−74.64276	W	2,554	**KX836017**
Potosí	EUPE	781	3.40542	−75.15874	E	1,379	**KX836018**
Potosí	EUPE	778	3.40542	−75.15874	E	1,379	**KX836019**
Potosí	EUPE	779	3.40542	−75.15874	E	1,379	**KX836020**
Potosí	EUPE	789	3.40542	−75.15874	E	1,379	**KX836021**
Potosí	EUPE	805	3.40542	−75.15874	E	1,379	**KX836022**
Rio Manso	EUPE	801	5.66584	−74.78178	W	2,240	**KX836023**
Rio Manso	EUPE	800	5.66584	−74.78178	W	2,240	**KX836024**
Rio Manso	EUPE	799	5.66584	−74.78178	W	2,240	**KX836025**
Rio Manso	EUPE	798	5.66584	−74.78178	W	2,240	**KX836026**
Rio Manso	EUPE	807	5.66584	−74.78178	W	2,240	**KX836027**
Rio Manso	EUPE	771	5.66584	−74.78178	W	2,240	**KX836028**
Rio Manso	EUPE	780	5.66584	−74.78178	W	2,240	**KX836029**
San Juan	EUPE	790	6.71725	−74.13454	E	2,997	**KX836030**
San Juan	EUPE	797	6.71725	−74.13454	E	2,997	**KX836031**
San Juan	EUPE	768	6.71725	−74.13454	E	2,997	**KX836032**
Panama	XISU						AY089800
Panama	XISU						EF202816
Panama	XISU						GU215206
NA	XIGU						KP775741
NA	PSSI						DQ294522

APPENDIX TABLE 3. Continued.

Locality	Species	Catalog	Latitude	Longitude	Side	Prec	GenBank
Bolivia	MIOL						DQ294509
Ecuador	MIOL						EF110848
Mexico	MIOL						EF110849
Panama	MIOL						EF110850
Panama	MIOL						EF110851
Peru	MIOL						EF110852
Guyana	MIOL						EF110853
Panama	MIOL						EF110854
Panama	MIOL						EF110855
Panama	MIOL						EF110856
Venezuela	MIOL						EF110857
Trinidad	MIOL						EF110858
Brazil	MIOL						EF110859
NA	MIMA						EF110846
Uruguay	LEAM						AF453808
Bolivia	LEAM						DQ294503
Argentina	TRME						FJ799899
Panama	EUPE						EF529961
Bolivia	EUPE						FJ799875
Bolivia	EUPE						FJ799876
Bolivia	EUPE						GU215311
Nicaragua	EUPE						GU215312