

# Patterns of genetic differentiation in *Myrmotherula brachyura* (Pygmy Antwren)

Adam Martin<sup>1,2</sup> and R. Terry Chesser<sup>2,3</sup>

<sup>1</sup>Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR; <sup>2</sup>Division of Birds, Department of Vertebrate Zoology, National Museum of Natural History, Washington DC, USA; <sup>3</sup>USGS Patuxent Wildlife Research Center, National Museum of Natural History, Washington DC, USA

## Introduction

- Distributions of many Amazonian species and subspecies are delimited by the Amazon and its major tributaries, including the rios Negro, Madeira, and Tapajós (Wallace 1852, Sneath 1913).
- The monotypic species *Myrmotherula brachyura* is a subsocial passerine bird found in canopy and sub-canopy of lowland forest throughout the Amazon Basin.
- Canopy species are thought to respond to riverine barriers to a lesser extent than understory species (Burney and Brumfield 2009), suggesting that *M. brachyura* may show little genetic structure across Amazonia.
- However, vocalizations of *M. brachyura* are known to be geographically variable (M. Isler, unpubl. data) – differences in vocalizations suggest that distinct evolutionary units may exist within *M. brachyura*.
- We studied genetic variation in *M. brachyura* across its geographic range to address the following questions:
  - Does *M. brachyura* show genetic variation across Amazonia?
  - If so, is this variation consistent with major rivers as barriers to gene flow?
  - Is genetic divergence across rivers lower in *M. brachyura* than in co-distributed congeners that inhabit forest understory (e.g., *M. menetriesii* and *M. longipennis*)?
  - Does genetic variation mirror vocal variation in *M. brachyura*?
  - Do genetic and vocal data suggest that *M. brachyura* is comprised of more than one species?

## Methods

- Tissue samples of *M. brachyura* from localities in Brazil, Guyana, Ecuador, Peru, and Bolivia (Fig. 1) were obtained from five museum collections (see Acknowledgments).
- Two samples of the sister species *M. obscura* were used as the outgroup.
- Genomic DNA was extracted from all samples using QIAGEN extraction kits.
- The mitochondrial gene NADH Dehydrogenase 2 (ND2) was amplified using standard PCR protocols.
- Samples were sequenced using an ABI PRISM 3130 Genetic Analyzer, and Sequencher 4.10.1 was used to align and edit the sequences.
- Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses were conducted using PAUP\* 4.0b10 and RAxML, respectively.
- Support for phylogenies was assessed using 1000 parsimony bootstrap replicates and 100 likelihood bootstrap replicates.

## Results

- ND2 sequences were obtained for 48 individuals: 46 *M. brachyura* and 2 *M. obscura*.
- Individuals of *M. brachyura* grouped into four well-supported and geographically coherent clades (Fig. 2).
- Phylogenetic relationships among clades were poorly resolved.
- Geographic boundaries between clades coincide with the lower Amazon and three main tributaries: the Rio Madeira, Rio Negro, and Rio Tapajós (Fig. 1).
- Genetic divergence across these rivers was virtually uniform, ranging from 1.7% to 2.0% (Table 1).

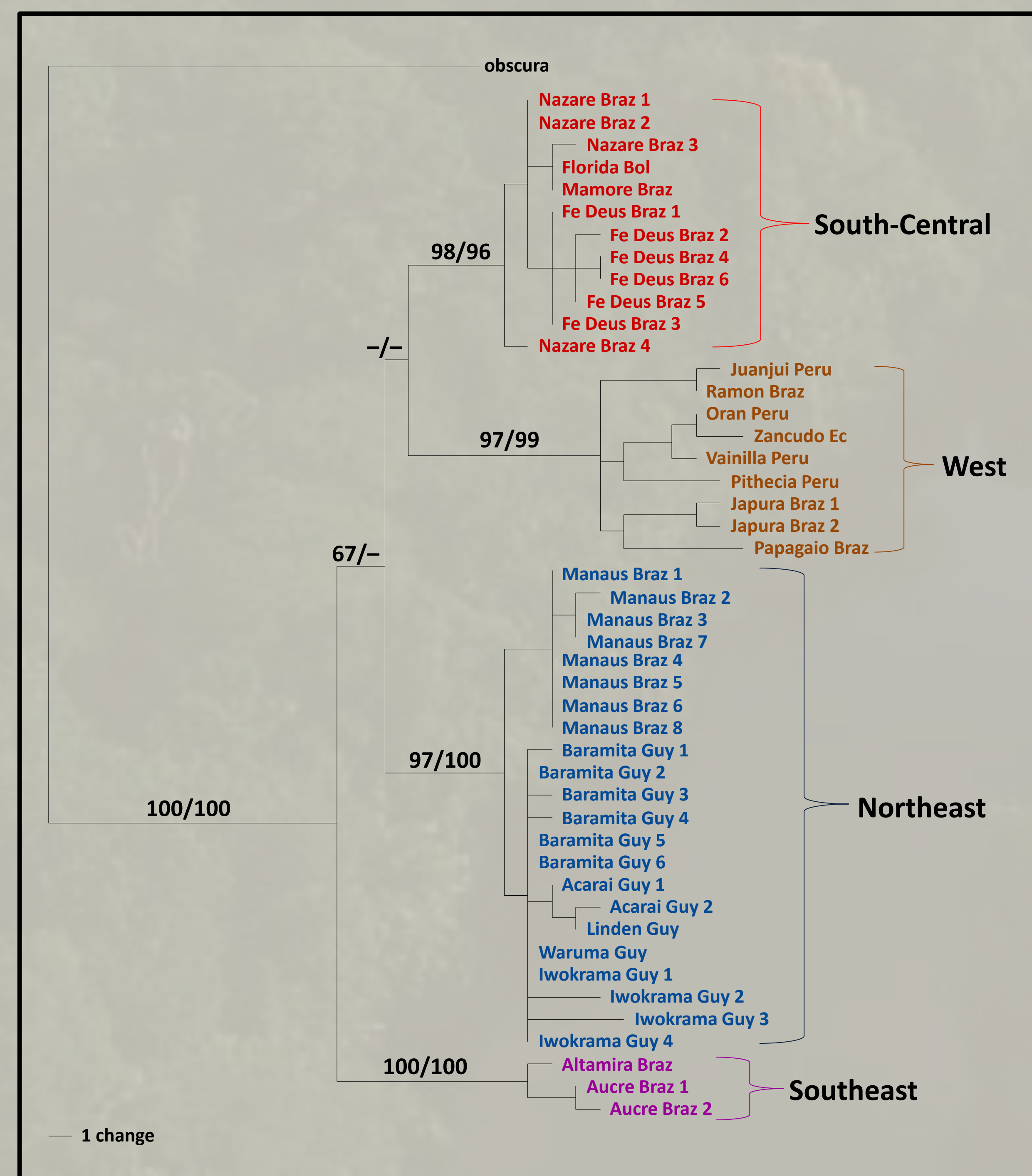


Figure 2. One of 750 most parsimonious trees. Numbers above branches indicate bootstrap support (MP/ML). Individuals of *M. brachyura* are identified by locality and color coded by geographic region.

Table 1. Comparison of genetic divergence across major Amazonian rivers for species of *Myrmotherula* antwren that inhabit different forest strata.

species	stratum	Amazon	Tapajós	Madeira	Negro
<i>M. brachyura</i>	canopy	2.0%	1.8%	1.7%	1.8%
<i>M. menetriesii</i> / <i>M. longipennis</i>	understory	4.5 - 6%	3 - 5%	2%	1%

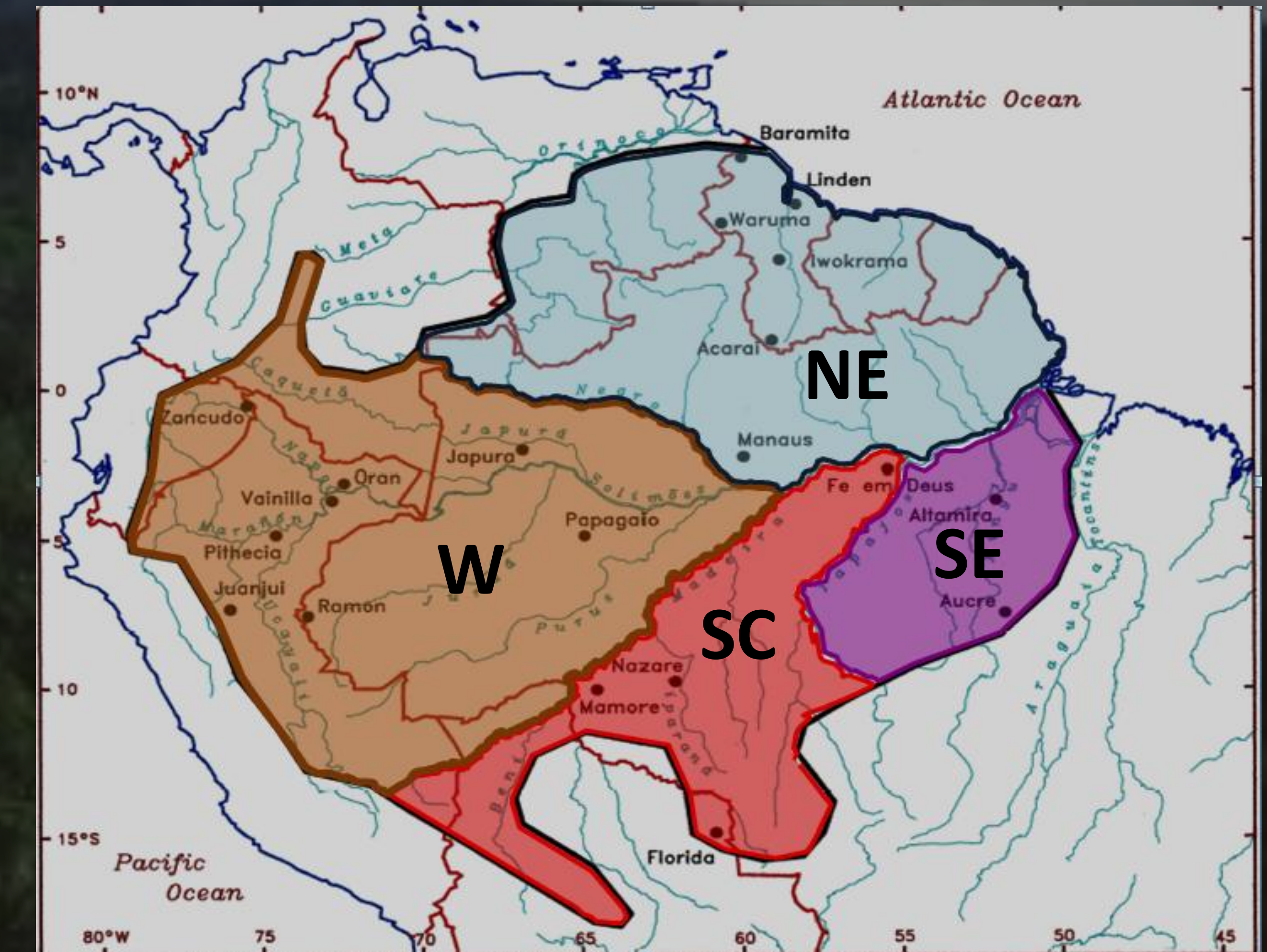


Figure 1. Map showing localities of *M. brachyura* samples. Ranges of genetically distinct clades are color coded to correspond to colors on the phylogenetic tree.

## Discussion

- Maximum genetic divergence within canopy species *M. brachyura* (2%) was much lower than that within understory species *M. menetriesii* (5%) and *M. longipennis* (6%) (Chesser, unpubl. data) (Table 1).
- Divergence across individual rivers in *M. brachyura* was lower than that of *M. menetriesii* and *M. longipennis*, except for the Rio Negro.
- The variability in divergence across rivers was much greater in *M. menetriesii* and *M. longipennis* than in *M. brachyura*.
- Preliminary analyses of vocalizations of *M. brachyura* (M. Isler, unpubl. data) identified three distinct groups corresponding to the NE, SE, and SC+W genetic clades.
- Vocal and genetic data suggest that *M. brachyura* is likely comprised of at least 3 biological species and of at least 4 phylogenetic species.

## Acknowledgments

- We thank:
- The Smithsonian Natural History Research Experiences Program, Cristián Samper, Elizabeth Cottrell, Eugene Hunt, and Virginia Power.
  - Robert Fleischer, Jesus Maldonado, and Nancy Rotzel of the Center for Conservation and Evolutionary Genetics.
  - The following institutions for contributing tissue samples to this project: Louisiana State University Museum of Natural Science, Baton Rouge; Museu Paraense Emilio Goeldi, Belém, Brazil; National Museum of Natural History, Washington, DC; Field Museum of Natural History, Chicago; Academy of Natural Sciences, Philadelphia.
  - Mort Isler for contributing the base map and unpublished data on vocalizations.
  - Yesha Shrestha and Tammy Wilbert for laboratory assistance.

## Literature cited

- Burney, C. W., and R. T. Brumfield. 2009. Ecology predicts levels of genetic differentiation in Neotropical birds. *Amer. Nat.* 174: 358-368.
- Sneath, E. 1913. Über die verbreitung der vogelarten in unteramazonien. *J. Ornithol.* 61: 469-539.
- Wallace, A.R. 1852. On the monkeys of the Amazon. *Proc. Zool. Soc. London* 20: 107-110.



Smithsonian Institution



Oregon State University



USGS science for a changing world