

Genetic diversity in an Andean forest bird: the Rufous Antpitta (*Grallaria rufula*)

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Introduction

The Rufous Antpitta (*Grallaria rufula*) is a subsocial passerine bird found in high elevation Andean forest from Colombia to Bolivia. Seven subspecies of *G. rufula* are currently recognized. Many subspecies differ qualitatively in vocalizations and some subspecies appear to consist of multiple vocal types. Thus, it has been suggested that this complex may consist of multiple biological species (Graves 1987, Krabbe and Schulenberg 2003). Graves (1987) separated *Grallaria blakei* as a cryptic species that occurs sympatrically with *G. rufula* in Peru. However, as he noted, it is more difficult to determine the species status of allopatric populations.

Genetic and vocal analyses are powerful tools for assessing evolutionary divergence. As part of a study of genetic and quantitative vocal variation in the *G. rufula* complex, we studied genetic variation in this species from Ecuador to Bolivia. This part of the distribution of *G. rufula* encompasses the entire range of subspecies *G. r. obscura*, *G. r. occabambae*, *G. r. cochabambae*, and part of the range of the nominate subspecies *G. r. rufula*, as well as the entire range of *G. blakei* (Fig. 1).



Methods

Forty tissue samples and one blood sample of *G. rufula* and three tissue samples of *G. blakei* were obtained from six tissue collections (see Acknowledgments). DNA was extracted from these samples using Qiagen extraction kits, and the mitochondrial gene NADH dehydrogenase 2 (ND2) was amplified using standard polymerase chain reaction (PCR). The PCR products were sequenced and the sequences were edited using Sequencher. Maximum parsimony (MP) and maximum likelihood (ML) analyses of the sequence data were performed using PAUP*. To assess nodal support, 1,000 MP and 100 ML bootstrap replicates were conducted. The congeneric species *Grallaria squamigera* was used as an outgroup; ND2 sequence for this species was obtained from GenBank.

Results

Complete ND2 sequence was obtained for 32 individuals, including 30 individuals of *G. rufula* and two individuals of *G. blakei*. Both phylogenetic analyses revealed eight genetically distinct groups, which were 2.7–10.2% divergent (Figs. 1,2).

Samples of *G. r. cochabambae*, *G. r. rufula*, and *G. blakei* all formed single, genetically distinct groups. Samples of *G. r. obscura* formed three genetic groups, which comprised a clade. Samples of *G. r. occabambae* formed two groups that were not sister taxa; instead the single individual of *G. r. cochabambae* was sister to the southern samples of *G. r. occabambae*. Samples of *G. blakei* fell within the clade formed by *G. r. rufula* and *G. r. obscura* in both analyses (Fig. 2).

MP and ML analyses differed only in the placement of a group of subspecies *G. r. occabambae* from Cuzco, Peru (clade 3). MP analysis placed this group as sister to the clade consisting of *G. r. rufula*, *G. r. obscura*, and *G. blakei*. ML analysis placed this group as sister to the clade consisting of *G. r. cochabambae* and the remaining *G. r. occabambae* samples (Fig. 2). With this placement, the deepest division of the ML phylogeny separates 'southern' groups consisting of birds from southern Peru and Bolivia from 'northern' groups consisting of specimens from central Peru, northern Peru, and Ecuador.

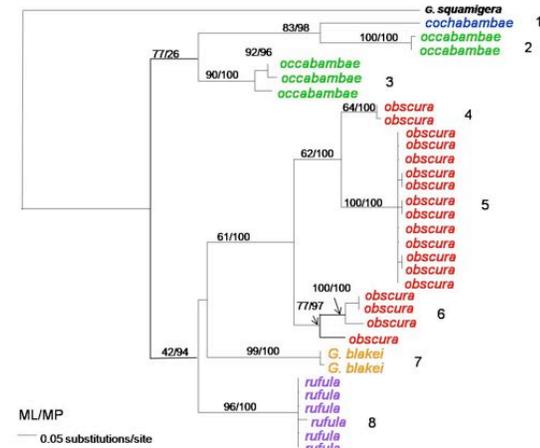


Figure 2. Maximum likelihood tree for ND2 sequences of *G. rufula* and *G. blakei*. Bootstrap support for likelihood and maximum parsimony analyses are listed above the branches.

Discussion

Whereas species limits in North American birds are relatively well known, there is still much to be learned about the South American avifauna (e.g., Sanin et al. 2009). Analysis of sequences of the mitochondrial gene ND2 revealed significant genetic variation within the *G. rufula* complex. Notably, all genetic groups identified by the ND2 data differ substantially in vocalizations (Isler et al. unpub. data) and are likely valid biological species in addition to being valid evolutionary and phylogenetic species.

When data for populations in Colombia are considered (Cadena unpub. data), there may be a dozen or more biological species within the *G. rufula* complex. Further analyses of intraspecific variation in South American birds will undoubtedly uncover many other cryptic species and enhance our understanding of the patterns of biodiversity in the Neotropics.

Acknowledgements

We thank the Natural History Research Experience internship program of the National Museum of Natural History, museum director Cristian Samper, and the Department of Vertebrate Zoology for their generosity and support of this research. We thank Robert Fleischer, Jesus Maldonado, and Nancy Rozeal for access to the genetics lab at the Center for Conservation and Evolutionary Genetics, as well as all lab members who helped along the way. We thank the following institutions for contributing tissue samples to this project: Louisiana State University Museum of Natural Science, Baton Rouge; Field Museum of Natural History, Chicago; University of Kansas Natural History Museum; Jill Jankowski, University of Florida; Academy of Natural Sciences, Philadelphia.

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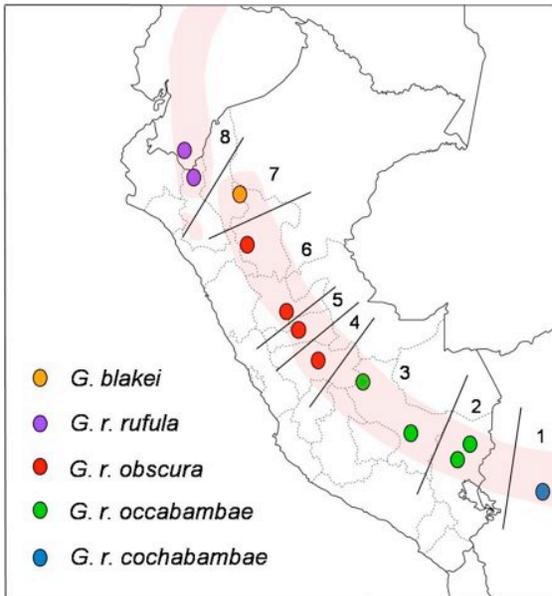


Figure 1. Geographic range of *G. rufula* (shaded red) with specimen locations for each subspecies. Numbers correspond to genetic groupings in Figure 2.