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Molecular systematics and biogeography of lowland antpittas (Aves, Grallariidae): The role of vicariance and dispersal in the diversification of a widespread Neotropical lineage

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ABSTRACT

We infer phylogenetic relationships, divergence times, and the diversification history of the avian Neotropical antpitta genera *Hylopezus* and *Myrmothera* (Grallariidae), based on sequence data (3,139 base pairs) from two mitochondrial (ND2 and ND3) and three nuclear nuclear introns (TGFB2, MUSK and FGB-I5) from 142 individuals of the 12 currently recognized species in *Hylopezus* and *Myrmothera* and 5 outgroup species. Phylogenetic analyses recovered 19 lineages clustered into two major clades, both distributed in Central and South America. *Hylopezus nattereri*, previously considered a subspecies of *H. ochroleucus*, was consistently recovered as the most divergent lineage within the *Grallaricula/Hylopezus/Myrmothera* clade. Ancestral range estimation suggested that modern lowland antpittas probably originated in the Amazonian Sedimentary basin during the middle Miocene, and that most lineages within the *Hylopezus/Myrmothera* clade appeared in the Plio-Pleistocene. However, the rate of diversification in the *Hylopezus/Myrmothera* clade appeared to have remained constant through time, with no major shifts over the 20 million years. Although the timing when most modern lineages of the *Hylopezus/Myrmothera* clade coincides with a period of intense landscape changes in the Neotropics (Plio-Pleistocene), the absence of any significant shifts in diversification rates over the last 20 million years challenges the view that there is a strict causal relationship between intensification of landscape changes and cladogenesis. The relative old age of the *Hylopezus/Myrmothera* clade coupled with an important role ascribed to dispersal for its diversification, favor an alternative scenario whereby long-term persistence and dispersal across an ever-changing landscape might explain constant rates of cladogenesis through time.

1. Introduction

In recent years, several features of land surface evolution have been invoked to explain Neotropical biogeographic patterns. For instance, changes in drainage and sedimentation patterns within large wetlands in western Amazonia during the Miocene (Figueiredo et al., 2009; Hoorn et al., 2013; Jaramillo et al., 2017; Wesselingh et al., 2002), and the Andean uplift unleashed climate changes across the continent (e.g., Insel et al., 2009) that ultimately affected current distributional patterns of a wide array of organisms (Antonelli and Sanmartín, 2011; Hoorn et al., 2013, 2010). Given this scenario, closely related taxa distributed across different Neotropical biomes offer good opportunities not only to infer their biogeographic history, but also to shed light on the processes underlying biological diversification of Neotropical biotas (Aleixo, 2004; D'Horta et al., 2012; Fernandes et al., 2014). Furthermore, the advent of event-based biogeography now allows the integration of all relevant processes (i.e. dispersal, extinction, vicariance, and founder-event speciation) through the use of explicit models (Matzke, 2014; Sanmartín et al., 2008). These event-based estimations specify both the ancestral distributions and associated causal events,

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thus making it easier to compare alternative evolutionary/biogeographical scenarios (Matzke, 2014; Ronquist and Sanmartín, 2011; Sanmartín et al., 2008).

Passerine understorey birds with low dispersal propensity are good models for testing biogeographical hypotheses because they are particularly susceptible to be delimited by geographical barriers, such as rivers and mountains (Aleixo, 2004; Burney and Brumfield, 2009; Winger et al., 2015). The Grallariidae (*sensu* Moyle et al., 2009a; Rice, 2005), commonly known as antpittas, is a Neotropical family of suboscine passerines that comprises ~50 species restricted primarily to the understorey of forested habitats in tropical South and Central America. They are known for their secretive behavior and their tendency to have small distribution ranges that, in combination with their rotund body, long legs, and short wings and tail, suggest they are poor dispersers (Krabbe and Schulenberg, 2003). In fact, recent biogeographic analyses support the notion that current distribution ranges of Andean antpittas are consistent with the idea of vicariant speciation, rather than dispersal events across pre-existing barriers (Winger et al., 2015). Recent DNA-based phylogenies support the monophyly of the family (Moyle et al., 2009a; Ohlson et al., 2013; Rice, 2005), which is also partially supported by morphological characters (Ames, 1971; Galvão and Gonzaga, 2011; Lowery and O'Neil, 1969). Antpittas are currently organized into the genera *Grallaria*, *Grallaricula*, *Myrmothera*, and *Hylopezus* (Remsen et al., 2017), and molecular data suggest that *Hylopezus* and *Myrmothera* are sister taxa, that *Grallaricula* is their closest relative, and that this entire clade is sister to *Grallaria* (Moyle et al., 2009a; Ohlson et al., 2013; Rice, 2005).

In this study, we inferred phylogenetic relationships, divergence times, and the biogeographic history of the lowland genera *Hylopezus* and *Myrmothera* using mitochondrial and nuclear loci. We inferred biogeographic patterns and estimated a comprehensive time-calibrated phylogeny for these genera. We also estimated ancestral distributional ranges and tested for temporal shifts in diversification rates. Our main goal was to verify whether major late Miocene and Plio-pleistocene landscape changes in the Neotropics (e.g., uplift of the Andes and the consolidation of the modern Amazonian trans-continental drainage) were correlated with diversification in this widespread lineage, as inferred for other Neotropical taxa (Brumfield and Edwards, 2007; Lima et al., 2017; Ribas and Miyaki, 2007; Smith et al., 2014). A central prediction derived from the purported correlation between landscape change events and diversification is that cladogensis is expected to accelerate as a direct response to increased opportunities for vicariance following the appearance of novel barriers (Chaves et al., 2011; Moore and Donoghue, 2007; Weir and Price, 2011).

2. Material and methods

2.1. Taxon sampling and molecular phylogeny

To infer phylogenetic relationships within *Hylopezus* and *Myrmothera*, we sequenced a total of 142 vouchered individuals (76 *Hylopezus*, 61 *Myrmothera,* 2 *Grallaricula,* 3 *Grallaria*) from localities throughout their distributions (Figs. A1 and A2, Table A1). Our sampling spanned the geographical distributions of all 12 currently recognized species within *Hylopezus* (10) and *Myrmothera* (2) (Carneiro et al., 2012; Krabbe and Schulenberg, 2003; Remsen et al., 2017). We included as outgroups five samples from the two other genera currently recognized in the Grallariidae (Krabbe and Schulenberg, 2003; Rice, 2005): *Grallaricula* (*G. flavirostris* and *G. nana*) and *Grallaria* (*G. rufula*, *G. ruficapilla* and *G. guatimalensis*). The choice of *Grallaria* and *Grallaricula* taxa included in the analyses was guided by a complete and yet unpublished phylogeny of the Grallariidae family (Bravo et al. in prep).

Total genomic DNA was extracted using DNeasy tissue extraction kits (Qiagen, Valencia, CA, USA). For most samples, we sequenced the mitochondrial genes – NADH dehydrogenase subunit 2 (ND2: 1,041 base pairs, bp), and NADH dehydrogenase subunit 3 (ND3: 351 bp). We also sequenced three nuclear introns representing the main lineages inferred from our complete mtDNA data, as follows: transforming growth factor beta 2 intron 5 (TGFB2: 625 bp); the 3rd intron of the Z-linked muscle-specific kinase (MUSK: 582 bp); and a fragment of the beta-fibrinogen intron 5 (FGB-I5: 549 bp). We used standard methods described elsewhere (Brumfield et al., 2007; Brumfield and Edwards, 2007; Kimball et al., 2009) to amplify and obtain sequences for these five markers. For primer details see Table A2.

Electropherograms were inspected, assembled in contigs and edited in Geneious 9.1.2 (http://www.geneious.com, Kearse et al., 2012). Sequences were aligned with MAFFT v. 6 (Katoh et al., 2002) using the default parameters, and further inspected visually. Heterozygous sites were coded according to IUPAC when double peaks were present in both strands of the same individual's electropherograms. The softwares used in our phylogenetic inferences treat by default IUPAC codes of DNA base combinations as missing data (Potts et al., 2014). Simulations and empirical analysis suggest that this magnitude of missing data (< 0.5% in our case) does not affect phylogenetic estimates at the scale carried out herein (Wiens and Moen, 2008).

2.2. Phylogeny estimation

To identify geographical lineages to be used in subsequent analyses, we generated a concatenated multilocus phylogeny including all individuals sampled (n = 142 including outgroups) using Bayesian inference (BI) on MrBayes 3.2.1 (Ronquist et al., 2012). In this analysis only three species of the genus *Grallaria* were chosen to root the tree.

Lineages were defined as population clusters separated by pairwise genetic distances equal to or greater than that separating the sister species pair of lowland antpittas *H. whittakeri* and *H. paraensis*, which are also distinguished by conspicuous vocal differences (Carneiro et al., 2012). We accepted values of Bayesian posterior probability ≥0.95 as statistically well-supported nodes (Bryson et al., 2014; Huelsenbeck and Rannala, 2004).

Models of molecular evolution and best-fit partitioning schemes were selected using the Bayesian Information Criterion (BIC) (Minin et al., 2003) as implemented in PartitionFinder V1.1.1 (Lanfear et al., 2012). We defined separate data blocks for the three codon positions of the protein-coding genes (ND2, ND3) and a single data block for each intron (TGFB2, MUSK, FGB-I5). The optimal partition scheme and the best-fit models used on the BI are described in details in the Appendix (Table A3). Two independent runs were conducted, each with three heated and one cold Markov chain sampling every 1000 generations for 20 million generations. Output parameters were visualized using Tracer 1.6 (Rambaut et al., 2014) to evaluate stationarity and convergence (Effective Sample Size – ESS values > 200). We further assessed convergence between runs using the 'RWTY' package, which implements functions of the AWTY in the R environment (https://github.com/danlwarren/RWTY; Nylander et al., 2008). The first 25% of generations were discarded as burn-in. MrBayes analyses were carried out in the CIPRES Science Gateway (Miller et al., 2010).

2.3. Species trees and divergence time estimate

We used ∗BEAST (Heled and Drummond, 2010), part of the BEAST 1.7.4 package (Drummond et al., 2012), to generate a time-calibrated species tree and a mtDNA chronogram, using all individuals from each currently recognized species or geographical lineages recovered by our MrBayes analysis. Following recommendations contained in the BEAST manual (Drummond et al., 2012), we did not root the tree in any specific outgroup. We considered the same partitions and models estimated in our PartitionFinder analysis.

We used a Yule speciation prior and a relaxed uncorrelated log-normal clock for each gene tree (Drummond et al., 2006). We applied two different calibration strategies to obtain absolute divergence times. First, we constrained the ages of relevant nodes based on normally