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RESEARCH ARTICLE

Conservative plumage masks extraordinary phylogenetic diversity in the *Grallaria rufula* (Rufous Antpitta) complex of the humid Andes

R. Terry Chesser,[1,2,*] Morton L. Isler,[2] Andrés M. Cuervo,[3,4] C. Daniel Cadena,[5] Spencer C. Galen,[2,6,a] Laura M. Bergner,[6,b] Robert C. Fleischer,[6] Gustavo A. Bravo,[7] Daniel F. Lane,[4] and Peter A. Hosner[2,8,9,c]

[1] U.S. Geological Survey, Patuxent Wildlife Research Center, Laurel, Maryland, USA
[2] Division of Birds, Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA
[3] Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia
[4] Museum of Natural Science, Louisiana State University, Baton Rouge, Louisiana, USA
[5] Laboratorio de Biología Evolutiva de Vertebrados, Departamento de Ciencias Biológicas, Universidad de los Andes, Bogotá, Colombia
[6] Center for Conservation Genomics, Smithsonian Conservation Biology Institute, National Zoological Park, Washington, D.C., USA
[7] Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA
[8] University of Kansas Biodiversity Institute, Lawrence, Kansas, USA
[9] Department of Biology, University of Florida, Gainesville, Florida, USA
[a] Present address: Department of Ornithology, Academy of Natural Sciences of Drexel University, Philadelphia, Pennsylvania, USA
[b] Present address: Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK
[c] Present address: Natural History Museum of Denmark and Center for Macroecology, Evolution, and Climate, GLOBE Institute, University of Copenhagen, Copenhagen, Denmark
*Corresponding author: tchesser@usgs.gov, chessert@si.edu

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ABSTRACT

The *Grallaria rufula* complex is currently considered to consist of 2 species, *G. rufula* (Rufous Antpitta) and *G. blakei* (Chestnut Antpitta). However, it has been suggested that the complex, populations of which occur in humid montane forests from Venezuela to Bolivia, comprises a suite of vocally distinct yet morphologically cryptic species. We sequenced nuclear and mitochondrial DNA for 80 individuals from across the distribution of the complex to determine the extent of genetic variation between and within described taxa. Our results revealed 18 geographically coherent clades separated by substantial genetic divergence: 14 within *rufula*, 3 within *blakei*, and 1 corresponding to *G. rufocinerea* (Bicolored Antpitta), a species with distinctive plumage found to be nested within the complex. Neither *G. rufula* nor *G. blakei* as presently defined was monophyletic. Although 6 of the 7 recognized subspecies of *G. rufula* were monophyletic, several subspecies contained substantial genetic differentiation. Genetic variation was largely partitioned across recognized geographic barriers, especially across deep river valleys in Peru and Colombia. Coalescent modeling identified 17 of the 18 clades as significantly differentiated lineages, whereas analyses of vocalizations delineated 16 biological species within the complex. The *G. rufula* complex seems unusually diverse even among birds of the humid Andes, a prime location for cryptic speciation; however, the extent to which other dispersal-limited Andean species groups exhibit similar degrees of cryptic differentiation awaits further study.

Keywords: Andes, cryptic species, *Grallaria rufula*, Grallariidae, Rufous Antpitta, species limits

El plumaje conservado enmascara una extraordinaria diversidad filogenética del complejo de *Grallaria rufula* en bosques húmedos de los Andes

RESUMEN

Actualmente se considera que el complejo de *Grallaria rufula* incluye dos especies (*G. rufula* y *G. blakei*). Sin embargo, se ha sugerido que el complejo, el cual incluye poblaciones de bosques húmedos de montaña desde Venezuela a Bolivia, corresponde a un conjunto de especies vocalmente diferenciables pero morfológicamente crípticas. Secuenciamos ADN nuclear y mitocondrial de 80 individuos de buena parte de la distribución geográfica del complejo para evaluar el grado de diferenciación genética existente en y entre los taxones descritos. Encontramos que existen 18 clados geográficamente coherentes que exhiben distancias genéticas considerables entre sí: 14 dentro de *rufula*, 3 dentro de *blakei* y 1 correspondiente a *G. rufocinerea*, una especie con plumaje notoriamente distinto que resultó ser parte del complejo. Tal como están definidas, *G. rufula* y *G. blakei* no forman grupos monofiléticos. Aunque 6 de las 7 subespecies reconocidas de *G. rufula* son monofiléticas, existe amplia diferenciación genética dentro de varias de ellas. La variación

genética en buena parte está estructurada por barreras geográficas conocidas, especialmente valles profundos de ríos en Perú y Colombia. Con base en modelos coalescentes identificamos a 17 de los 18 clados como linajes significativamente diferenciados, mientras que análisis de las vocalizaciones delimitaron 16 especies biológicas en el grupo. El complejo de *G. rufula* parece ser inusualmente diverso incluso entre las aves de zonas húmedas de los Andes, un escenario primordial de especiación críptica. Sin embargo, hasta qué punto otros grupos andinos con habilidades de dispersión limitadas presentan grados similares de diferenciación críptica está por estudiar.

Palabras clave: Andes, delimitación de especies, especie críptica, *Grallaria rufula*, Grallariidae

INTRODUCTION

Cryptic species are 2 or more species erroneously classified as a single species due to superficial similarity (Duellman and Trueb 1988). Although studies that reveal the existence of cryptic species are not uncommon (Winker 2005), the increased consideration of characters not associated with external morphology has recently led an increase in the number of cryptic species recognized (Bickford et al. 2007). In better known organisms, such as many vertebrates, cryptic species are routinely identified using DNA sequences (e.g., Cooke et al. 2012, Giarla et al. 2014), but other characters, such as vocalizations (e.g., Johnson 1959, Isler et al. 1998), osteology (e.g., Duellman and Trueb 1988, Woodman and Timm 2017), and cytogenetics (e.g., Patton and Dingman 1968, Tymowska and Fischberg 1973), are also used. For organisms for which behavioral data are scarce or non-existent, and for which morphology is conservative, cryptic species are often identified using genetic information alone (Bickford et al. 2007). The combination of genetic and non-genetic characters can make for particularly powerful conclusions regarding species delineation under a wide range of species concepts and species recognition criteria.

The tropical Andes are a biodiversity hotspot, containing more vertebrate species and more endemic vertebrate species than any other region (Myers et al. 2000). Nevertheless, the complex topography and diversity of environmental conditions in the tropical Andes make them a prime region for cryptic speciation, and the diversity of vertebrates there is undoubtedly underestimated (e.g., Duellman and Trueb 1988, Sanin et al. 2009, Rheindt et al. 2013, Giarla et al. 2014). Species of Andean humid forest are distributed over relatively narrow elevational ranges that can extend for many hundreds of kilometers, providing ample opportunities for geographical isolation across elevational and habitat discontinuities (Terborgh 1977, Graves 1982, 1988, Remsen 1984). Such discontinuities are especially prevalent in Colombia, where the Andes split into 3 main cordilleras and where other isolated highlands, such as the Sierra Nevada de Santa Marta, are present, and in Peru, where large rivers, such as the Marañón, the Huallaga, and the Apurímac/Ene, form deep arid intermontane canyons that are physical, ecological, and climatic barriers to dispersal (Chapman 1917, Graves 1985, O'Neill 1992, Weir 2009, Graham et al. 2010).

Geographical distributions and gene flow of Neotropical birds are known to be limited by landscape features such as rivers in Amazonia (Wallace 1852, Haffer 1974, Capparella 1988, 1991, Smith et al. 2014) and the aforementioned dry valleys of the tropical Andes (Vuilleumier 1969, Parker et al. 1985, Winger and Bates 2015). Although differentiation of populations on either side of such prominent barriers is a well-known phenomenon, the prevalence of genetic differentiation in species showing little or no morphological divergence (cf. Winger and Bates 2015, Pulido-Santacruz et al. 2018) is an open question. Levels of differentiation across geographical barriers are affected by avian ecology and behavior: for example, birds with limited dispersal abilities, such as those of tropical lowland forest understory or upland forest, show higher levels of genetic divergence across barriers than more dispersive species that occupy the canopy, forest edges, or seasonally flooded forests (Burney and Brumfield 2009, Smith et al. 2014, Harvey et al. 2017). Among the most reclusive terrestrial inhabitants of the understory of both lowland and highland forest are the antpittas (Grallariidae). As expected, based on their terrestrial behavior and limited dispersal abilities, patterns of population genetic structure suggest that antpittas are strongly affected by barriers and that the true species-level diversity of the group has been underestimated (Winger et al. 2015, Carneiro et al. 2018, van Doren et al. 2018).

The *Grallaria rufula* complex is currently considered to consist of 2 species of forest-inhabiting birds of the central and northern humid Andes: the Rufous Antpitta *G. rufula* (Lafresnaye, 1843) and the Chestnut Antpitta *G. blakei* (Graves, 1987). *Grallaria rufula* occurs from the Sierra Nevada de Santa Marta of northern Colombia and the adjacent Serranía de Perijá of the Venezuela-Colombia border south to central Bolivia. Elevational ranges vary among populations. In general, members of the complex are found at elevations of ~1,850–3,900 m, but some occupy considerably narrower ranges. Its 7 recognized subspecies (Krabbe and Schulenberg 2003, Dickinson and Christidis 2014; Table 1) are found in isolated highlands or are separated by arid river valleys (Figure 1). The more recently described *G. blakei*, a monotypic species, inhabits the Andes of north-central Peru, typically occurring at lower elevations than *G. rufula* (Figure 2). We provisionally treat 2 additional populations from south of the Río

TABLE 1. Distributions of currently recognized taxa within the *Grallaria rufula* complex, arranged from north to south.

Taxon	Author, date	Geographical distribution (type locality in parenthesis)	Elevation (m)
G. rufula spatiator	Bangs, 1898	Sierra Nevada de Santa Marta, Colombia (Páramo de Macotama, La Guajira, Colombia)	2,200–2,900
G. rufula saltuensis	Wetmore, 1946	Serranía de Perijá, Venezuela and Colombia (above Eroca, Cesar, Colombia)	2,500–3,250
G. rufula rufula	Lafresnaye, 1843	Eastern, Central, and Western Andes, Colombia, south to E. Andean slope in Piura and Cajamarca, Peru (uncertain "Bogotá" trade skin, Colombia)	1,850–3,650
G. rufula cajamarcae	Chapman, 1927	Piura and Cajamarca, Peru, west of Río Marañón and Río Huancabamba (Chugur, Cajamarca, Peru)	2,900–3,400
G. rufula obscura	Berlepsch and Stolzmann, 1896	Cerro Huicsacunga, NW Amazonas, Peru, south to Junín, Peru, west of Río Ene/Apurimac (Maraynioc, Junín, Peru)	2,400–3,900
G. rufula occabambae	Chapman, 1923	Junín and Cusco, Peru (Occabamba Valley, Urubamba region, Cusco, Peru)	2,450–3,650
G. rufula cochabambae	Bond and Meyer de Schauensee, 1940	Puno, Peru, east to Cochabamba, Bolivia (Incachaca, Cochabamba, Bolivia)[a]	1,950–3,500
G. blakei	Graves, 1987	East Andean slope of Amazonas and San Martín, Peru, south to Ayacucho, Peru, west of Río Apurimac (Carpish mountains, ~2,400 m, Huánuco, Peru)	1,700–3,500

[a] Populations in Puno, Peru, have traditionally been treated as *occabambae*, but analyses of genetics and vocalizations clearly group them with *cochabambae*.

Huallaga as part of *G. blakei*, the Pasco/Junín population (designated *blakei 2*) based primarily on plumage and elevational range relative to *G. rufula* (Graves 1987, Hosner et al. 2015), and the Ayacucho population (designated *blakei 3*) based on vocal similarity to the Pasco/Junín population (Hosner et al. 2015). The Pasco/Junín population occurs at a lower elevation within the range of *G. rufula*, as is typical of *blakei*, but the Ayacucho population is the only representative of the complex in its region and ranges to higher elevations.

Descriptions of species and subspecies in the *G. rufula* complex were based principally on variation in plumage color, as was the case for many passerines, but over the past 30–40 yr vocalizations have been recognized as key indicators of species delimitation in antpittas and other suboscine birds (e.g., Isler et al. 1998), in which vocalizations are stereotypical and likely innate (Kroodsma 1984, Kroodsma and Konishi 1991, Touchton et al. 2014). Substantial variation within *G. rufula*, especially in vocalizations (e.g., Isler and Whitney 2002), has led to suggestions that multiple cryptic species may be included within what is now recognized as a single species. For example, Wetmore (1946), in his description of the subspecies *saltuensis*, noted that its distinctive plumage might indicate species rather than subspecies status, and Krabbe and Schulenberg (2003) made note of geographic variation in vocalizations, including variation within subspecies, and suggested that some subspecies might be better treated as species.

As part of a comprehensive study of the systematics and evolution of the *G. rufula* complex, focusing primarily on investigations of genetics and vocalizations, we obtained DNA sequence data for 80 individuals from across the range of the complex, to assess the extent of genetic differentiation among morphologically differentiated populations (recognized species and subspecies), the extent of cryptic genetic differentiation, and the extent to which vocal differentiation in this complex matches genetic differentiation. Specifically, we addressed the following questions: (1) Is the *G. rufula* complex monophyletic? (2) Do *G. rufula*, *G. blakei*, and the 7 subspecies of *G. rufula* appear to be monophyletic? (3) Do populations currently unrecognized as taxa display degrees of genetic differentiation similar to those between recognized taxa? (4) How do nuclear and mitochondrial DNA compare in resolution of phylogenetic relationships? (5) Is genetic variation in the *G. rufula* complex congruent with vocal variation, presumably a key isolating mechanism in these birds? (6) How does a coalescent species delimitation method compare with vocal analyses of biological species status?

METHODS

Sampling and Laboratory Protocols

Samples from 80 individuals of the *G. rufula* complex were gathered from throughout its range, including 2 or more samples of each recognized subspecies (Table 2). Sampling for taxa of *G. rufula* and *G. blakei* totaled 78 individuals: 2 *spatiator*, 2 *saltuensis*, 26 *rufula*, 5 *cajamarcae*, 19 *obscura*, 9 *occabambae*, 5 *cochabambae*, and 10 *blakei* (including the provisional populations). Based on a preliminary tree from a species-level phylogenetic analysis of suboscine birds (M. G. Harvey, G. A. Bravo, R. T. Brumfield, and E. P. Derryberry, personal observation), an additional species, *G. rufocinerea* (Bicolored Antpitta),

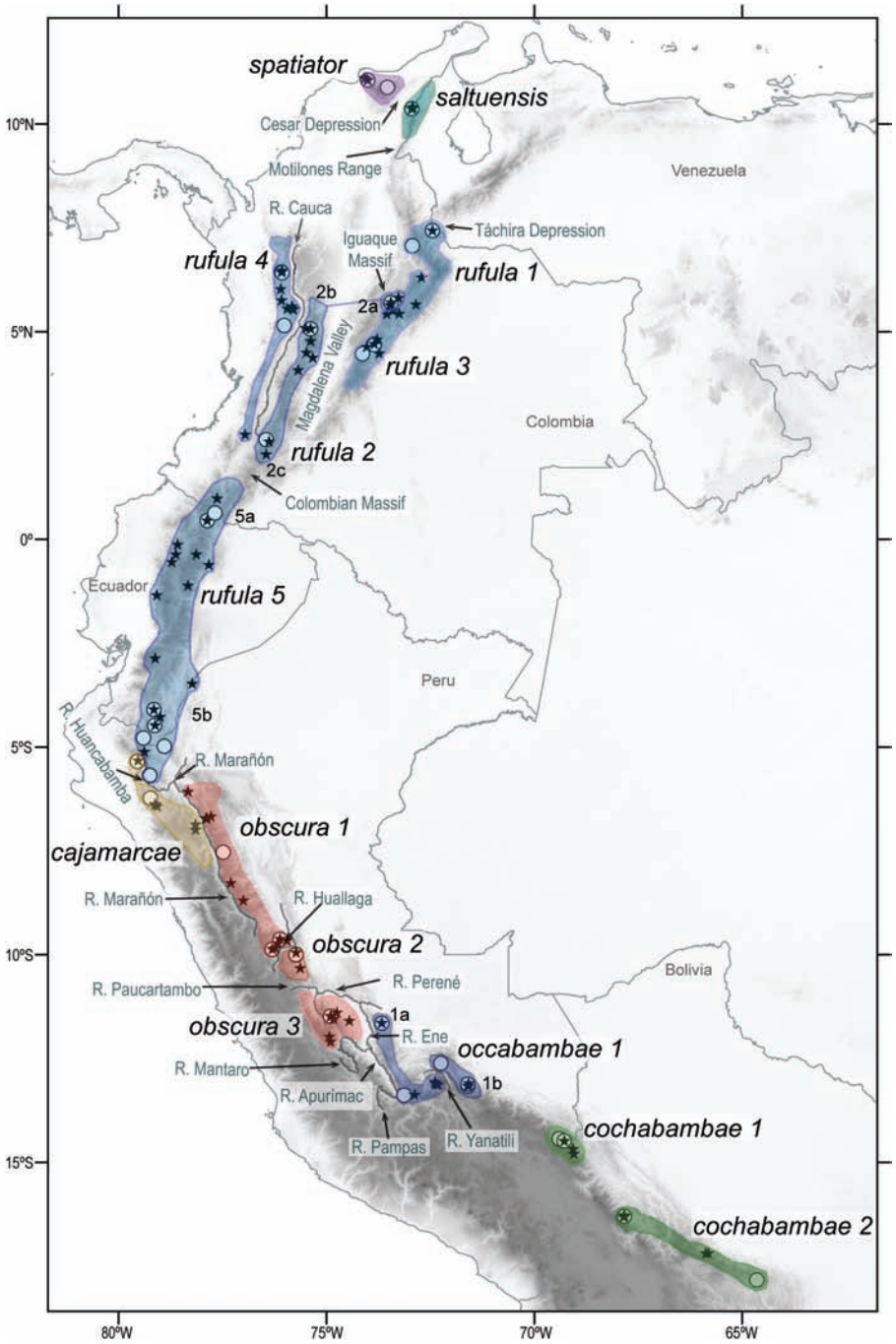

FIGURE 1. Distribution of *Grallaria rufula*, its seven currently recognized subspecies, and the genetic units identified in this study. Ranges are color-coded by subspecies as in Figures 3 and 4. Dots represent the locations of genetic samples used in this study, and stars represent the locations of recordings analyzed in the companion paper by Isler et al. (2020). Each symbol may represent more than one genetic or vocal sample. Geographic barriers that separate genetic units are indicated by arrows.

was determined to be part of the ingroup (see below), and 2 individuals of *rufocinerea* (one of these from the suboscine study) were added to our dataset, bringing the total of ingroup samples to 80. Representatives of 3 additional species were sampled as outgroups: *G. capitalis* (Bay Antpitta) and *G. ruficapilla* (Chestnut-crowned Antpitta), 2 species closely related to *G. rufula* (N. Rice and J. Bates personal communication), and the more distantly related congener *G. squamigera* (Undulated Antpitta). Most individuals were sampled using fresh tissues; however, both individuals of *spatiator* and *rufocinerea*, 2 individuals of *rufula*, and single individuals of *cajamarcae*

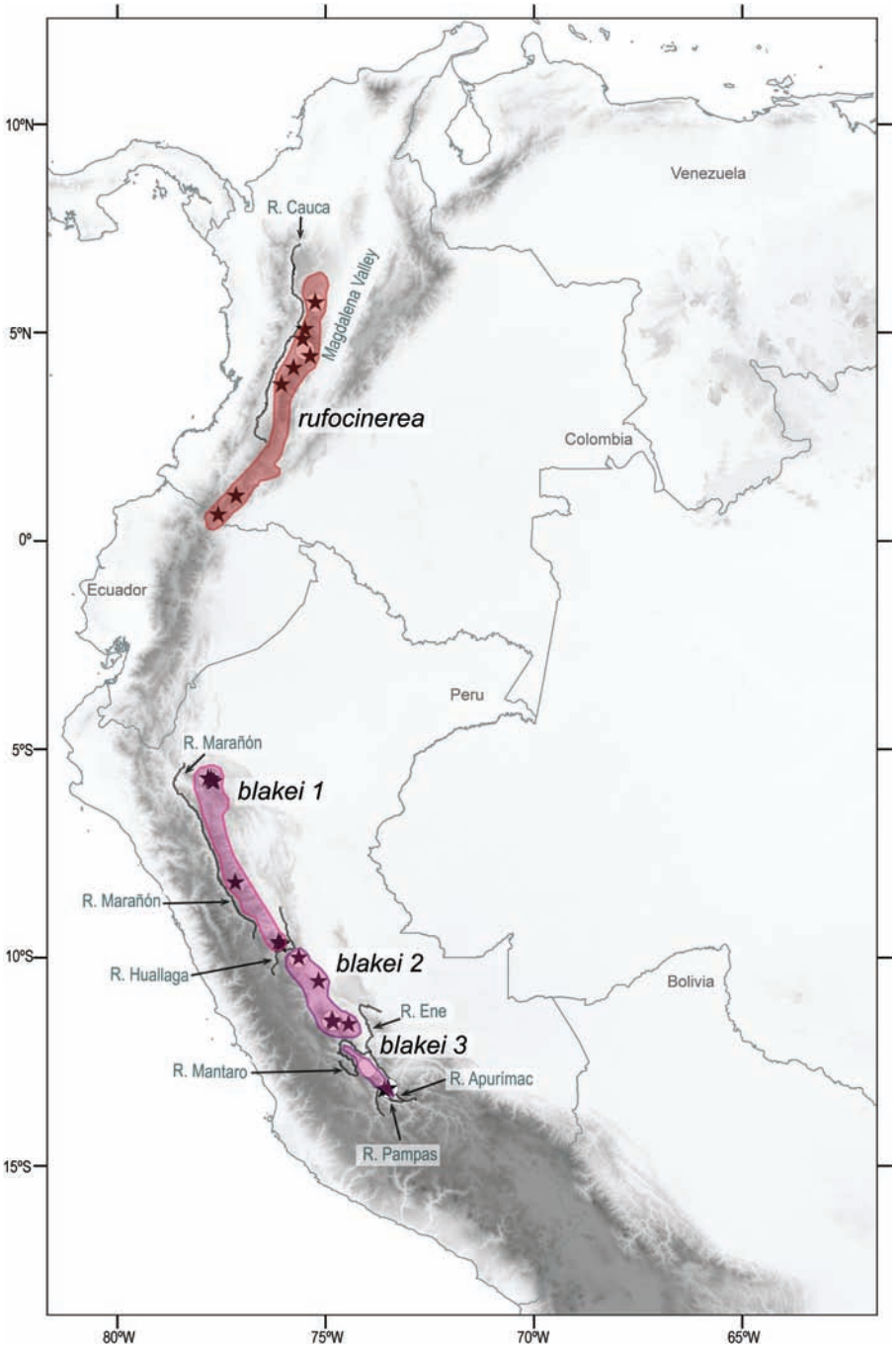

FIGURE 2. Distributions of *Grallaria blakei* and *G. rufocinerea* showing the genetic units identified in this study. Ranges are color-coded by species as in Figures 3 and 4. Dots represent the locations of genetic samples used in this study, and stars represent the locations of recordings analyzed in the companion paper by Isler et al. (2020). Each symbol may represent more than one genetic or vocal sample. Geographic barriers that separate genetic units are indicated by arrows.

and *cochabambae*, were sampled from the toepads of museum skins (Table 2).

DNA was extracted from tissue samples using Qiagen (Valencia, California, USA) DNeasy blood and tissue DNA extraction kits. For toepads, DNA was extracted in a physically isolated ancient DNA laboratory following strict protocols to minimize and detect contamination. All surfaces and equipment were regularly treated with a solution of 50% bleach and/or UV irradiation, and sterile, disposable blades were used for cutting toepad samples. Extraction blanks and negative controls were used to detect potential contamination. DNA extractions were conducted

via a phenol/chloroform procedure with subsequent centrifugal dialysis (Fleischer et al. 2000). DNA extractions and polymerase chain reaction (PCR) setup were carried out in the ancient DNA laboratory prior to moving to the separate contemporary DNA lab.

We sequenced 4 DNA fragments for fresh tissue samples: the mitochondrial gene nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2), intron 3 of the Z-linked muscle-specific kinase gene (MUSK), intron 15 of the Z-linked aconitase gene (ACO15), and intron 5 of the autosomal gene beta-fibrinogen (Fib5). For fresh tissue samples, ND2 was amplified in 2 fragments, using paired primers L5216 and H5766 (both Sorenson et al. 1999) for the first piece, and L5758 (Sorenson et al. 1999) and H6313 (Johnson and Sorenson 1998) for the second. Primers used for MUSK were MUSK-I3F and MUSK-I3R (Kimball et al. 2009), primers used for ACO15 were ACO Ai15fbb and ACO Ai15ra (Fernandes et al. 2013), and primers used for Fib5 were Fib5F and Fib6R (Kimball et al. 2009). For samples from museum specimens, ND2 and MUSK were amplified in smaller pieces using a variety of primers, many of which were designed specifically for this study (Table 3). ACO15 and Fib5 were not sequenced for museum specimens.

Amplification of mitochondrial genes and nuclear introns was performed on a Biorad DNA Engine Tetrad 2 thermocycler (Hercules, California, USA). PCRs were conducted in 25 μL reactions that typically consisted of 1X PCR Buffer, 2–4 mM $MgCl_2$, 200 μM deoxyribonucleotide triphosphates (dNTPs), 0.4 μM of each primer, 0.8 μg bovine serum albumin (BSA), and 1 U AmpliTaq DNA polymerase (Life Technologies, Carlsbad, California, USA). The thermocycling conditions were as follows: 95°C for 4 min; 40 cycles of 94°C for 45 s, 52°C for 45 s, and 72°C for 90 s; and 72°C for 10 min. The annealing temperature was increased to 54–60°C for amplification of ACO15 for several samples. PCR products were cleaned for cycle sequencing using ExoSAP-IT (Affymetrix, Santa Clara, California, USA). Sequencing reactions were performed using the Big Dye Terminator 3.1 cycle sequencing kit (Applied Biosystems, Foster City, California, USA). Samples were sequenced in both directions using an ABI 3130xl automated sequencer and assembled, edited, and aligned using Sequencher 5.1 (GeneCodes, Ann Arbor, Michigan, USA). All sequences have been submitted to GenBank.

For the sample of *G. rufocinerea* from the suboscine study, total genomic DNA was extracted from a toepad using the Qiagen DNeasy kit, following standard protocols with the addition of an extended lysis time in dithiothreitol. Because DNA from this sample was highly degraded, library preparation did not include DNA shearing and proceeded directly to ligating barcoded Illumina (San Diego, California, USA)-compatible adapters, PCR amplification, probe hybridization, and enrichment of hybridized fragments. This sample was multiplexed and sequenced on 3 independent 150-base pair (bp) paired-end Illumina HiSeq 2,000 lanes targeting 2,321 ultraconserved elements (Faircloth et al. 2012) and 96 exons based on a probe set that contained 4,715 probes (Harvey et al. 2016). This sample yielded data for 2,146 target loci at an average depth of 160X. Sequences of ND2 were recovered as a by-product of the target capture at an average coverage of 166X. Using the software Geneious 11.1.4 (Kearse et al. 2012), contigs of these sequences were assembled and mapped to an ND2 sequence from an individual of *G. guatimalensis* (Scaled Antpitta; GenBank Accession numbers: MF925490, MF925493, and MF925502, respectively; Van Doren et al. 2018).

Phylogenetic Analyses

Numbers of variable and phylogenetically informative characters were calculated using PAUP* 4.0 (Swofford 2003), and phylogenetic trees were estimated using maximum likelihood (ML) and Bayesian approaches as implemented in RAxML 7.7.1 (Stamatakis et al. 2008; http://embnet.vital-it.ch/raxml-bb/) and *BEAST 2.2.0 (Bouckaert et al. 2014), respectively. For the ML analyses, separate phylogenetic trees were inferred for mitochondrial sequences (partitioned by codon), nuclear sequences (partitioned by gene), and for the combined dataset. RAxML analyses were performed with the GTR + G model of sequence evolution and included 100 bootstrap replicates in addition to the search for the most likely tree.

To produce a time-calibrated, multispecies coalescent tree, we inferred the phylogeny using *BEAST 2.2.0 (Heled and Drummond 2009, Bouckaert et al. 2014). We used PartitionFinder (Lanfear et al. 2012) and the Bayesian information criterion to select a partitioning scheme and models of DNA sequence evolution for each locus, drawing from symmetric DNA sequence evolution models implemented in BEAST, and treating each codon position of ND2 as data subsets. PartitionFinder identified each ND2 codon position as its own partition, and the HKY + G, HKY + I, and GTR + G models for the first, second, and third codon positions, respectively. The HKY + I model was selected for each intron. Preliminary Markov Chain Monte Carlo (MCMC) runs suffered from poor mixing; thus, we substituted the simpler HKY + G model for the ND2 3rd codon position, which solved this problem. In preliminary runs using an uncorrelated lognormal relaxed clock, the standard deviation of the coefficient of rate variation included zero, justifying use of a strict molecular clock (Ho and Duchêne 2014). We chose a Yule tree prior and a linear with constant root population function. We treated each genetic group (Table 2) as an a priori population (tip). To time-calibrate the tree and to produce results directly comparable with another study of Andean antpittas (Winger et al. 2015), we used a 2.1% per million year substitution rate (Weir and Schluter 2009). We executed 4 independent 50,000,000 generation MCMC chains, sampling every

50,000 states, resulting in 4,000 samples from the posterior distribution of trees. We summarized both MCMC runs as a maximum clade credibility (MCC) tree after discarding the first 25% of each run as burn-in; we assessed convergence with Tracer 1.5 (Rambaut and Drummond 2007), ensuring each parameter estimate had an effective sample size greater than 200. To estimate lineage divergence in relation to biogeographical barriers (and for direct comparison with the results of Winger et al. 2015), we conducted a second analysis following their methodological choices. We produced a reduced alignment using a single ND2 sequence from each genetic group (Table 2), did not partition, used an HKY + G model of sequence evolution, and we implemented an uncorrelated lognormal relaxed molecular clock with an average rate of 2.1%. We executed 2 50,000,000 generation MCMC chains, sampling every 50,000 states, resulting in 2,000 posterior samples. We discarded the initial 25% of samples as burn-in, and summarized the remaining 1,500 samples as a maximum clade credibility tree.

Genetic Clustering Using the General Mixed Yule-Coalescent Model

To obtain an objective perspective on population differentiation using molecular data, we implemented a Bayesian version (bGMYC; Reid and Carstens 2012) of the General mixed Yule-coalescent model (GMYC; Pons et al. 2006) for species delimitation. Although the GMYC model has been used to test and justify species limits alone, and is often described as a method to infer species limits, we prefer to interpret lineages identified by the GMYC model as populations that exhibit substantial genetic differentiation unexpected under panmixia. Mitochondrial lineages identified by the GMYC models are genetic clusters treated as candidates for species status, because in some cases mitochondrial evolution may not accurately represent population and species history (Rubinoff and Holland 2005), and because such methods detect lineages, which do not necessarily equate to species (Sukumaran and Knowles 2017). Candidate lineages identified by the GMYC model are to be further examined for concordance with phenotypic (song and plumage) and other genotypic (nuclear markers) characters. To implement bGMYC, we randomly subsampled 100 trees from the mitochondrial ND2 treeset inferred with *BEAST 2.2.0. Analysis was limited to ND2 because the GMYC model is for single loci, and ND2 contains the most informative sites, and mitochondrial markers exhibit rapid coalescence times compared with nuclear markers. We ran bGMYC (Reid and Carstens 2012) in R 3.0.1 (R Core Team 2014). MCMC chain length was 50,000, with a 40,000 generation burn-in, 100 generation thinning, and considered 2 to 81 candidate species (t1 = 2 and t2 = 81). We considered lineages to be candidates for species status if they were identified to be different from all other samples with 0.95 posterior probability.

RESULTS

Sequence Characteristics

Complete or near-complete ND2 sequences (1,021–1,041 bp) were obtained for virtually all tissue, blood, and toepad samples of G. *rufula, G. blakei*, and *G. rufocinerea*, although for 3 tissues, 1 blood sample, and 4 toepads, we were only able to obtain partial ND2 sequence data (525–917 bp; Table 2). We obtained at least partial nuclear sequence data for all but 6 samples (2 tissue, 3 blood, and 1 toepad). Sequences of MUSK were obtained for the other 7 toepads, and sequences of the complete suite of nuclear genes for 57 of the other 67 tissue and blood samples (Table 2). Total ingroup individuals with sequence data for each gene were as follows: ND2 = 80, MUSK = 73, Fib5 = 61, and ACO15 = 60. All genes were successfully sequenced for all outgroups.

The total number of aligned nucleotides was 3,071. ND2 included 1,041 aligned nucleotides, MUSK included 580, ACO15 885, and Fib5 565 (figures for introns include small pieces of flanking sequence). A large insertion of 242 bp (in *cajamarcae*) added considerably to the length of the alignment for ACO15. As expected, the mitochondrial sequence contained a large percentage of both the phylogenetically informative and variable characters. Among individuals of *rufula-blakei*, these were distributed as follows: 345 phylogenetically informative characters/381 variable characters in ND2, 33/40 in MUSK, 47/60 in ACO15, and 32/41 in Fib5.

Phylogenetic Analyses

ML analyses of the combined data distinguished 18 geographically coherent ingroup clades separated by at least 3% mitochondrial sequence divergence: 14 within *G. rufula*, 3 within *G. blakei*, and 1 corresponding to *G. rufocinerea* (Figures 3 and 4). Six of the 7 recognized subspecies of *G. rufula* were reciprocally monophyletic. The exception was the nominate subspecies, which consisted of 3 groups, one of which also included *G. rufocinerea*. Two clades endemic to the Eastern Andes of Colombia (designated *rufula 1* and *rufula 3*; Figure 1), together with *G. rufocinerea* of the Central Andes, formed one monophyletic group. A clade endemic to the Western Andes (*rufula 4*) formed another, and the third group consisted of a clade (*rufula 2*) that contained 2 individuals from the Central Andes and 1 individual from the western slope of the Eastern Andes of Colombia (wedged against the ranges of *rufula 1* and *rufula 3*), and a clade (*rufula 5*) found from northern Ecuador (and presumably southern Colombia) south to extreme northern Peru. Although *rufula 4* and *rufula 2/5* were sisters in the best concatenated ML tree, bootstrap support for this relationship was not strong (58%) and they were not sisters in the *BEAST tree. More than 1 clade was detected within 2 other subspecies of *G. rufula*: 3 within *obscura* and 2 within *cochabambae.*

TABLE 2. Voucher/tissue number, identification, geographical locality data, and sequences obtained for samples of *G. rufula*, *G. blakei*, *G. rufocinerea*, and outgroup species. Most samples were taken from fresh tissue, mainly from collected birds, but eight samples (labeled "toepad") were taken from museum skins and four samples (labeled "blood sample") consisted of blood from live birds. Complete or near-complete sequences of ND2, MUSK, Fib5, and ACO15 were obtained for all samples except as noted (* indicates partial sequence). Fib5 and ACO15 were not sequenced for toepads. AMNH = American Museum of Natural History, ANDES = Museo de Historia Natural de la Universidad de los Andes, ANSP = Academy of Natural Sciences of Philadelphia, DMNH = Delaware Museum of Natural History, FMNH = Field Museum of Natural History, IAvH = Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, ICN = Instituto de Ciencias Naturales (Universidad Nacional de Colombia), JJ = Jill Jankowski, KU = University of Kansas Museum of Natural History, LSUMZ = LSU Museum of Natural Science, MSB = Museum of Southwestern Biology, MUA-AVP = Museo Universitario de la Universidad de Antioquia, and USNM = US National Museum of Natural History.

Voucher/ Tissue No.	Species	Subspecies	Genetic Group	Country	Departamento	Locality	Elev.	Latitude, Longitude	Sequences obtained
USNM 387369 (toepad)	*rufula*	*spatiator*	*spatiator*	Colombia	Cesar	Río Guatapurí, Sierra Nevada de Santa Marta	2440–2900 m	10.88°N, 73.53°W	ND2*, MUSK*
USNM 387370 (toepad)	*rufula*	*spatiator*	*spatiator*	Colombia	Magdalena	San Lorenzo, Sierra Nevada de Santa Marta	2440–2650 m	11.17°N, 74.12°W	ND2*, MUSK*
ICN 36771 (ANDES-BT 744)	*rufula*	*saltuensis*	*saltuensis*	Colombia	Cesar	Serranía de Perijá; Manaure, above El Cinco	2500 m	10.37°N, 72.95°W	all genes
ICN 36756 (ANDES-BT 743)	*rufula*	*saltuensis*	*saltuensis*	Colombia	Cesar	Serranía de Perijá; Manaure, above El Cinco	2500 m	10.37°N, 72.95°W	all genes
IAvH-A-14947 (IAvH-CT 8452)	*rufula*	*rufula*	*rufula 1*	Colombia	Norte de Santander	Herrán, PNN Tamá, sector Orocué, Alto El Pesebre	2800 m	07.43°N, 72.45°W	ND2, Fib5
USNM 411894 (toepad)	*rufula*	*rufula*	*rufula 1*	Colombia	Santander	Hacienda Las Vegas, 12 miles [19 km] up valley from Piedecuesta	1830 m	07.07°N, 72.93°W	ND2*, MUSK*
IAvH-A 12296 (IAvH-CT 2275)	*rufula*	*rufula*	*rufula 2*	Colombia	Boyacá	Villa de Leyva, Santuario de Iguaque, Sector Morro Negro	3245 m	05.70°N, 73.45°W	all genes
MUA-AVP 0556	*rufula*	*rufula*	*rufula 2*	Colombia	Caldas	Manizales, Vereda el Desquite, Finca Martinica	3400 m	05.07°N, 75.38°W	ND2*
FMNH 255649 (toepad)	*rufula*	*rufula*	*rufula 2*	Colombia	Cauca	San Rafael, Puracé	3375 m	02.40°N, 76.45°W	ND2, MUSK*
ANDES-O 004 (ANDES-BT 770)	*rufula*	*rufula*	*rufula 3*	Colombia	Cundinamarca	Bogotá, Usme, El Destino, margen derecha Río Curubital	3050 m	04.47°N, 74.13°W	ND2
IAvH-A 12688 (IAvH-CT 2573)	*rufula*	*rufula*	*rufula 3*	Colombia	Cundinamarca	Guasca, Vereda San Francisco, Sector de Palacio, Reserva Forestal de Río Blanco	3220 m	04.70°N, 73.85°W	all genes
IAvH-A-12727 (IAvH-CT 2603)	*rufula*	*rufula*	*rufula 3*	Colombia	Cundinamarca	La Calera, Mundo Nuevo, vereda El Manzano,Laguna Brava, Reserva Forestal de Río Blanco	3220 m	04.68°N, 73.85°W	all genes
IAvH-A 13358 (IAvH-CT 3997)	*rufula*	*rufula*	*rufula 4*	Colombia	Risaralda	Pueblo Rico, La Cumbre, PNN Tatamá	2620 m	05.15°N, 76.02°W	all genes
IAvH-CT 5225 (blood sample)	*rufula*	*rufula*	*rufula 4*	Colombia	Antioquia	Urrao, Páramo de Frontino	3150 m	06.43°N, 76.08°W	ND2
IAvH-CT 5246 (blood sample)	*rufula*	*rufula*	*rufula 4*	Colombia	Antioquia	Urrao, Páramo de Frontino	2600 m	06.42°N, 76.07°W	ND2

TABLE 2. Continued

Voucher/ Tissue No.	Species	Subspecies	Genetic Group	Country	Departamento	Locality	Elev.	Latitude, Longitude	Sequences obtained
IAvH-CT 5247 (blood sample)	*rufula*	*rufula*	*rufula 4*	Colombia	Antioquia	Urrao, Páramo de Frontino	2600 m	06.42°N, 76.07°W	ND2*
ANSP 3923	*rufula*	*rufula*	*rufula 5a*	Ecuador	Carchi	ca. 3 km SE Impueran; Cerro Mongus	3300 m	00.45°N, 77.87°W	ND2, MUSK
ANSP 3982	*rufula*	*rufula*	*rufula 5a*	Ecuador	Carchi	ca. 3 km SE Impueran; Cerro Mongus	3300 m	00.45°N, 77.87°W	ND2, MUSK, Fib5
USNM 614861	*rufula*	*rufula*	*rufula 5a*	Ecuador	Sucumbíos	2 km SW of Cocha Seca	3210 m	00.63°N, 77.68°W	ND2*
ANSP 4978	*rufula*	*rufula*	*rufula 5b*	Ecuador	Zamora Chinchipe	25 km SSE Jimbura; E slope Cordillera Las Lagunillas	2950 m	04.78°S, 79.40°W	ND2, MUSK
ANSP 8707	*rufula*	*rufula*	*rufula 5b*	Ecuador	Zamora Chinchipe	Cord. de Sabanillia; Quebrada Honda	2550 m	04.48°S, 79.13°W	all genes
ANSP 8718	*rufula*	*rufula*	*rufula 5b*	Ecuador	Zamora Chinchipe	Cord. de Sabanillia; Quebrada Honda	2550 m	04.48°S, 79.13°W	all genes
ANSP 3792	*rufula*	*rufula*	*rufula 5b*	Ecuador	Loja	Cajanuma, Parque Nacional Podocarpus	2800 m	04.10°S, 79.15°W	ND2, MUSK
LSUMZ B-34837	*rufula*	*rufula*	*rufula 5b*	Peru	Cajamarca	Cordillera del Condor; Picorana	2250 m	04.98°S, 78.90°W	all genes
LSUMZ B-32561	*rufula*	*rufula*	*rufula 5b*	Peru	Cajamarca	Quebrada Lanchal, ca 8 Km ESE Sallique	2850 m	05.68°S, 79.25°W	all genes
LSUMZ B-32391	*rufula*	*rufula*	*rufula 5b*	Peru	Cajamarca	Quebrada Lanchal, ca 8 Km ESE Sallique	2925 m	05.68°S, 79.25°W	all genes
LSUMZ B-32387	*rufula*	*rufula*	*rufula 5b*	Peru	Cajamarca	Quebrada Lanchal, ca 8 Km ESE Sallique	2925 m	05.68°S, 79.25°W	ND2, MUSK, ACO
LSUMZ B-32345	*rufula*	*rufula*	*rufula 5b*	Peru	Cajamarca	Quebrada Lanchal, ca 8 Km ESE Sallique	2925 m	05.68°S, 79.25°W	all genes
LSUMZ B-32293	*rufula*	*rufula*	*rufula 5b*	Peru	Cajamarca	Quebrada Lanchal, ca 8 Km ESE Sallique	2860 m	05.68°S, 79.25°W	all genes
LSUMZ B-32257	*rufula*	*rufula*	*rufula 5b*	Peru	Cajamarca	Quebrada Lanchal, ca 8 Km ESE Sallique	2925 m	05.68°S, 79.25°W	all genes
FMNH 222151 (toepad)	*rufula*	*cajamarcae*	*cajamarcae*	Peru	Piura	Tambo, Huancabamba	2880 m	05.35°S, 79.55°W	ND2, MUSK*
MSB:Bird 162567	*rufula*	*cajamarcae*	*cajamarcae*	Peru	Lambayeque	Tres Lagunas	3421 m	06.23°S, 79.23°W	all genes
MSB:Bird 162570	*rufula*	*cajamarcae*	*cajamarcae*	Peru	Lambayeque	Tres Lagunas	3382 m	06.23°S, 79.23°W	all genes
MSB:Bird 162593	*rufula*	*cajamarcae*	*cajamarcae*	Peru	Lambayeque	Tres Lagunas	3414 m	06.23°S, 79.23°W	all genes
MSB:Bird 162677	*rufula*	*cajamarcae*	*cajamarcae*	Peru	Lambayeque	Tres Lagunas	3276 m	06.23°S, 79.23°W	all genes
LSUMZ B-810	*rufula*	*obscura*	*obscura 1*	Peru	San Martín	Puerto Del Monte, ca 30 km NE Los Alicios	3250 m	07.53°S, 77.48°W	all genes
LSUMZ B-809	*rufula*	*obscura*	*obscura 1*	Peru	San Martín	Puerto Del Monte, ca 30 km NE Los Alicios	3250 m	07.53°S, 77.48°W	all genes
LSUMZ B-7713	*rufula*	*obscura*	*obscura 1*	Peru	Huánuco	Unchog Pass NNW Acomayo	3450 m	09.68°S, 76.12°W	all genes
LSUMZ B-7690	*rufula*	*obscura*	*obscura 1*	Peru	Huánuco	Unchog Pass NNW Acomayo	3450 m	09.68°S, 76.12°W	all genes

TABLE 2. Continued

Voucher/ Tissue No.	Species	Subspecies	Genetic Group	Country	Departamento	Locality	Elev.	Latitude, Longitude	Sequences obtained
LSUMZ B-3520	*rufula*	*obscura*	*obscura 2*	Peru	Huánuco	Bosque San Marcos, 16km W Panao	3350 m	09.98°S, 76.08°W	all genes
LSUMZ B-3510	*rufula*	*obscura*	*obscura 2*	Peru	Huánuco	Bosque Potrero, 14km W Panao	3000 m	09.98°S, 76.08°W	all genes
LSUMZ B-8336	*rufula*	*obscura*	*obscura 2*	Peru	Pasco	Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail	3450 m	09.90°S, 75.73°W	all genes
LSUMZ B-8324	*rufula*	*obscura*	*obscura 2*	Peru	Pasco	Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail	3450 m	10.00°S, 75.73°W	all genes
LSUMZ B-8286	*rufula*	*obscura*	*obscura 2*	Peru	Pasco	Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail	3450 m	10.00°S, 75.73°W	all genes
LSUMZ B-8260	*rufula*	*obscura*	*obscura 2*	Peru	Pasco	Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail	3450 m	10.00°S, 75.73°W	all genes
LSUMZ B-8256	*rufula*	*obscura*	*obscura 2*	Peru	Pasco	Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail	3450 m	10.00°S, 75.73°W	all genes
LSUMZ B-8253	*rufula*	*obscura*	*obscura 2*	Peru	Pasco	Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail	3450 m	10.00°S, 75.73°W	all genes
LSUMZ B-8246	*rufula*	*obscura*	*obscura 2*	Peru	Pasco	Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail	3450 m	10.00°S, 75.73°W	all genes
LSUMZ B-8241	*rufula*	*obscura*	*obscura 2*	Peru	Pasco	Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail	3450 m	10.00°S, 75.73°W	all genes
LSUMZ B-8217	*rufula*	*obscura*	*obscura 2*	Peru	Pasco	Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail	3700 m	10.00°S, 75.73°W	all genes
LSUMZ B-8197	*rufula*	*obscura*	*obscura 2*	Peru	Pasco	Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail	3450 m	10.00°S, 75.73°W	all genes
LSUMZ B-8350	*rufula*	*obscura*	*obscura 2*	Peru	Pasco	Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail	3450 m	10.00°S, 75.73°W	all genes
KU 17662	*rufula*	*obscura*	*obscura 3*	Peru	Junín	Toldopampa	3225–3625 m	11.48°S, 74.90°W	all genes
KU 17666	*rufula*	*obscura*	*obscura 3*	Peru	Junín	Toldopampa	3225–3625 m	11.48°S, 74.90°W	all genes
FMNH 390659	*rufula*	*occabambae*	*occabambae 1a*	Peru	Junín	Cordillera Vilcabamba, headwaters Río Pomureni	3350 m	11.65°S, 73.67°W	all genes
FMNH 390660	*rufula*	*occabambae*	*occabambae 1a*	Peru	Junín	Cordillera Vilcabamba, headwaters Río Pomureni	3350 m	11.65°S, 73.67°W	all genes
FMNH 390661	*rufula*	*occabambae*	*occabambae 1a*	Peru	Junín	Cordillera Vilcabamba, headwaters Río Pomureni	3350 m	11.65°S, 73.67°W	ND2, MUSK, ACO
FMNH 390662	*rufula*	*occabambae*	*occabambae 1a*	Peru	Junín	Cordillera Vilcabamba, headwaters Río Pomureni	3350 m	11.65°S, 73.67°W	all genes
KU 29422	*rufula*	*occabambae*	*occabambae 1a*	Peru	Cusco	Paccaypata	3000 m	13.38°S, 73.13°W	all genes
FMNH 429996	*rufula*	*occabambae*	*occabambae 1b*	Peru	Cusco	Pillahuata	2460 m	13.16°, 71.60°W	all genes
FMNH 433522	*rufula*	*occabambae*	*occabambae 1b*	Peru	Cusco	La Esperanza, 39 km (road) NE Paucartambo	2850 m	13.17°, 71.60°W	all genes
JJ 5247 (blood sample)	*rufula*	*occabambae*	*occabambae 1b*	Peru	Cusco	Trocha Union, Manu National Park	3250 m	13.12°S, 71.60°W	all genes

TABLE 2. Continued

Voucher/ Tissue No.	Species	Subspecies	Genetic Group	Country	Departamento	Locality	Elev.	Latitude, Longitude	Sequences obtained
MSB:Bird 168436	*rufula*	*occabambae*	*occabambae 1b*	Peru	Cusco	Quebrada Honda	2858 m	12.62°S, 72.25°W	ND2, MUSK, ACO
LSUMZ B-529	*rufula*	*cochabambae*	*cochabambae 1*	Peru	Puno	Valcon, 5 km NNW Quiaca	3000 m	14.43°S, 69.40°W	all genes
KU 21173	*rufula*	*cochabambae*	*cochabambae 1*	Peru	Puno	Sina	3125 m	14.48°S, 69.28°W	all genes
KU 21234	*rufula*	*cochabambae*	*cochabambae 1*	Peru	Puno	Sina	3125 m	14.48°S, 69.28°W	all genes
LSUMZ B-1218	*rufula*	*cochabambae*	*cochabambae 2*	Bolivia	La Paz	ca. 1km S Chuspipata	3050 m	16.30°S, 67.83°W	all genes
DMNH 67057 (toepad)	*rufula*	*cochabambae*	*cochabambae 2*	Bolivia	Cochabamba	Epizana [Ipajama], 101 km SE (by road); Siberia cloud forest, Cordillera Oriental	2990 m	17.83°S, 64.65°W	ND2, MUSK*
LSUMZ B-43720	*blakei*	"north"	*blakei 1*	Peru	San Martín	ca. 22 km ENE Florida	ca. 2400 m	05.72°S, 77.75°W	ND2, MUSK, Fib5
LSUMZ B-43817	*blakei*	"north"	*blakei 1*	Peru	San Martín	ca. 22 km ENE Florida	ca. 2400 m	05.72°S, 77.75°W	all genes
LSUMZ B-43818	*blakei*	"north"	*blakei 1*	Peru	San Martín	ca. 22 km ENE Florida	ca. 2400 m	05.72°S, 77.75°W	ND2, MUSK, Fib5
LSUMZ B-5620	*blakei*	"north"	*blakei 1*	Peru	Amazonas	30 km by road E Florida on road to Rioja	2200 m	05.70°S, 77.82°W	all genes
LSUMZ B-8104	*blakei*	"south"	*blakei 2*	Peru	Pasco	Playa Pampa, ca. 8 km NW Cushi on trail Chaglla	1750–2400 m	09.95°S, 75.70°W	all genes
KU 14763	*blakei*	"south"	*blakei 2*	Peru	Junín	Río Satipo	2500 m	11.52°S, 74.85°W	all genes
KU 14767	*blakei*	"south"	*blakei 2*	Peru	Junín	Río Satipo	2500 m	11.52°S, 74.85°W	all genes
KU 16918	*blakei*	"south"	*blakei 3*	Peru	Ayacucho	Ccano	2700 m	12.78°S, 74.60°W	all genes
KU 29298	*blakei*	"south"	*blakei 3*	Peru	Ayacucho	Chupón	3400 m	13.24°S, 73.49°W	all genes
KU 29375	*blakei*	"south"	*blakei 3*	Peru	Ayacucho	Chupón	3400 m	13.24°S, 73.49°W	all genes
USNM 436486 (toepad)	*rufocinerea*	*rufocinerea*	*rufocinerea*	Colombia	Antioquia	Páramo de Sonsón	2680–2740 m	05.72°N, 75.25°W	ND2
AMNH 111976 (toepad)	*rufocinerea*	*rufocinerea*	*rufocinerea*	Colombia	Quindío	Laguneta	3140 m	04.58°N, 75.50°W	ND2*, MUSK*
outgroup species									
KU 16717	*G. capitalis*			Peru	Ayacucho	Rumichaca	3000 m	13.28°S, 73.98°W	all genes
MSB 35251	*G. ruficapilla*			Peru	Cajamarca	5.4 km SE Contumaza	2625 m	07.40°S, 78.78°W	all genes
MSB 33988	*G. squamigera*			Peru	Apurimac	Ccocha	3680 m	13.48°S, 72.98°W	all genes

TABLE 3. Primers used for amplifying ND2 and MUSK from samples from museum specimens of the *Grallaria rufula* complex.

Primer name	Sequence 5′ – 3′	Source
ND2 Primers		
L5219	CCCATACCCCGAAAATGAGWSG	Zwiers et al. (2008)
H5419	AARTAYTTGRTTGCRGCYTCGAT	this study
L5419	GAAGCTGCAACAAAATACTT	Fleischer et al. (2006)
L5565g	TYGCRATRAARCTCGGRCTWG	this study
H5578	CCTTGAAGCACTTCTGGGAATCAGA	Fleischer et al. (2006)
H5578g	CCTTGAAGGACTTCTGGAAATCAAA	this study
L5758g	GGAGGATGAGCCGGACTNAAYCARAC	modified from Sorenson et al. (1999)
H5766	RGAKGAGAARGCYAGGATYTTKCG	Sorenson et al. (1999)
L5969g	AACTATCCACAATAAYAACAGCATG	this study
H5977g	GTCCGGCTAAAGAGAGAAGTGTTA	modified from Zwiers et al. (2008)
L6077g	ACCAAACAAGAAATAACCCCCACAGCA	this study
H6113	CAGTATGCAAGTCGGAGGTAGAAG	Zwiers et al. (2008)
H6313	ACTCTTRTTTAAGGCTTTGAAGGC	Johnson and Sorenson (1998)
MUSK Primers		
MUSK-I3F	CTTCCATGCACTACAATGGGAAA	Kimball et al. (2009)
MUSK-151F	TGATTTCTAGTTGCTAGGAAGC	this study
MUSK-166R	ATTTGTGASRTGAGCCCTSG	this study
MUSK-197F	TCACAAGTGWCTKCATCTGC	this study
MUSK-284R	TAYACTCACAAATGCATTCATCAG	this study
MUSK-303F	TGCTGATGAATGCATTTGTGAG	this study
MUSK-332R	TGCAGAGCTGTGAATTATAGC	this study
MUSK-434F	TCCTTCAGTTGTAAGACAACAC	this study
MUSK-447R	CAAAGATTCAGCTTACATGC	this study
MUSK-I3R	CTCTGAACATTGTGGATCCTCAA	Kimball et al. (2009)

The 18 clades received 100% ML bootstrap support, except for *rufula 2* (98%) and *obscura 1* (93%). Two of the 18 clades contained geographical subgroups, designated *rufula 5a* and *5b* and *occabambae 1a* and *1b*, respectively, separated by 1–2% ND2 sequence divergence. Although the 2 subgroups within *occabambae* were geographically partitioned, only *occabambae 1a* formed a clade in the combined analyses; *occabambae 1b* instead formed a grade. Samples of *G. blakei* formed 3 clades; only the 2 southern forms provisionally treated as *blakei* were sister taxa, and all were nested within *G. rufula*. Thus, neither *G. rufula* nor *G. blakei* was monophyletic, although monophyly of *rufula* + *blakei* + *rufocinerea* was strongly supported. One clade of *blakei* (*blakei 1*) extended from northern to central Peru, whereas the 2 sister clades (*blakei 2* and *blakei 3*) were found in central and south-central Peru, respectively; *blakei 2* was separated from *blakei 1* to the north by the Huallaga Valley (Figure 2). All clades of *blakei* received 100% bootstrap support.

Distributions of taxa in major clades were characterized by distinct geographical patterns in both the ML and *BEAST analyses. The northernmost subspecies *spatiator* and *saltuensis* were sister taxa, and these were sister to *rufula 1* + *rufula 3* + *rufocinerea* of the Eastern and Central Andes of Colombia; these 5 northern taxa formed a moderately to well-supported clade (77% ML bootstrap/1.0 posterior probability) sister to all other ingroup taxa, which formed a well-supported clade (98%/1.0). The remaining taxa were divided into 2 groups, a reasonably well-supported (84%/0.99) southern group consisting of *blakei 2*, *blakei 3*, *occabambae*, and both clades of *cochabambae*, and a north-central group consisting of *cajamarcae*, *rufula 4*, *rufula 2*, *rufula 5*, *blakei 1*, and the 3 clades of *obscura*. This latter group received only weak to moderate support (51%/0.94), due in part to the difficulty of placing *rufula 4*. Relationships within the northern and southern clades were generally strongly supported (82–100%/0.97–1.0, except for the node uniting *rufula 1* and *rufula 3*, which was 54%/0.91), but relationships within the northern-central clade were poorly supported. Branching patterns differed slightly between the ML concatenated tree and the *BEAST maximum clade credibility tree. For example, *rufula 4* was sister to the remainder of the northern-central clade identified above in the *BEAST tree, rather than sister to *rufula 2* + *rufula 5*, as in the ML tree. However, these differences did not indicate strong conflict between phylogenetic hypotheses produced by the 2 methods: collapsing the few nodes in each tree that were not strongly supported (i.e. bootstrap values <70%, posterior probabilities <0.95) resulted in topologies that were entirely congruent.

Taxa in the northernmost group were highly differentiated from taxa in the north-central and southern groups, and, generally, from each other as well.

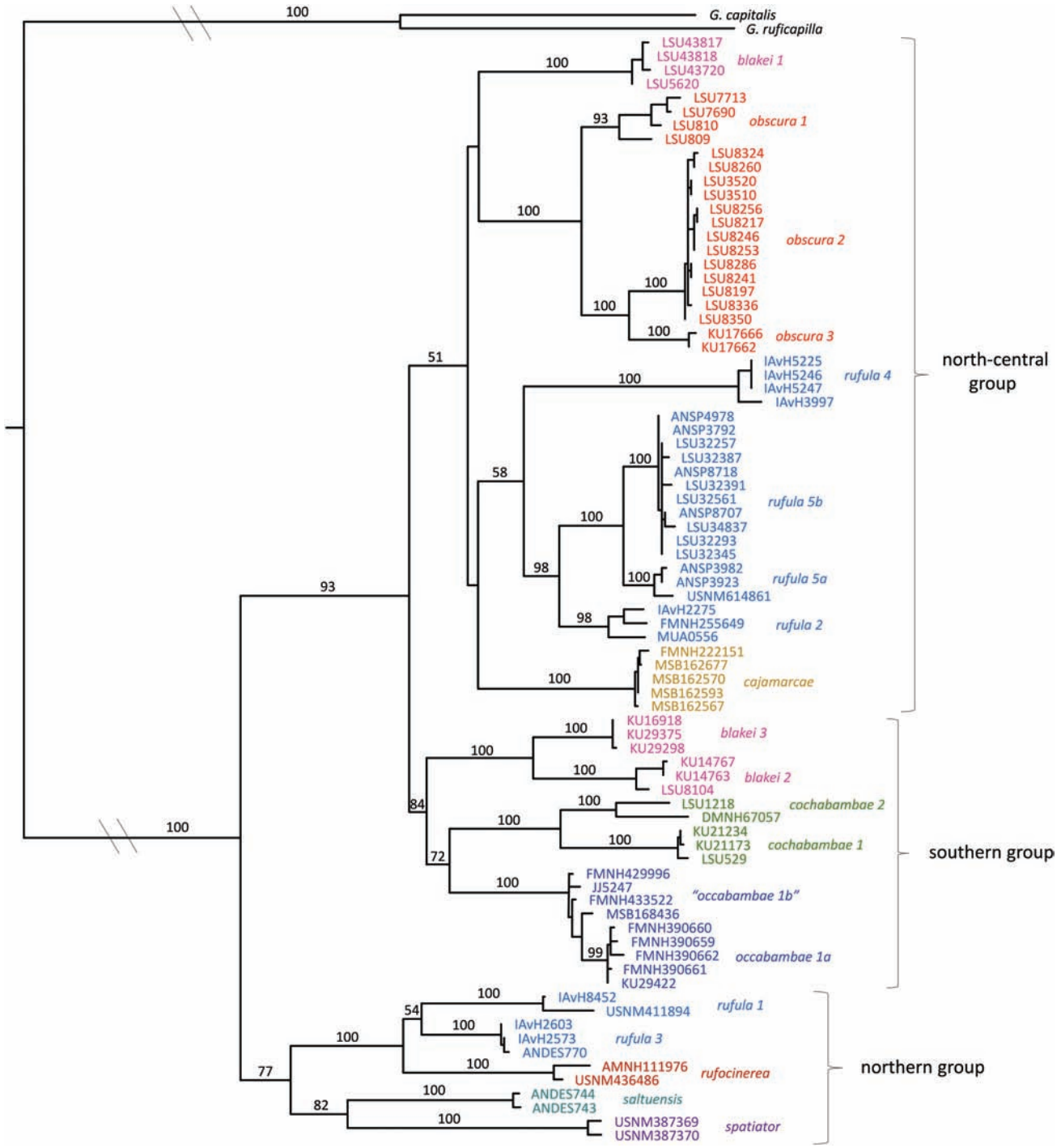

FIGURE 3. Most likely tree for the *Grallaria rufula* complex, based on the combined mitochondrial and nuclear data, produced using the program RAxML. Numbers above nodes represent bootstrap support values based on 100 bootstrap replicates. This tree differs from the ML bootstrap tree largely in its placement of *rufula 4*; hence, the low support values in this part of the tree.

For example, *saltuensis* and *spatiator* were 7.5% divergent in ND2 (uncorrected pairwise distance) from each other and at least 8.5% divergent from all other taxa. Maximum ND2 divergence between resolved sister taxa (7.5% uncorrected pairwise distance) was between the northernmost subspecies, *spatiator* and *saltuensis*. By contrast, divergence between sister lineages in the north-central and southern groups ranged from 3% (between *obscura 2* and *obscura 3*) to 5.5% (between *cochabambae 1* and *cochabambae 2*).

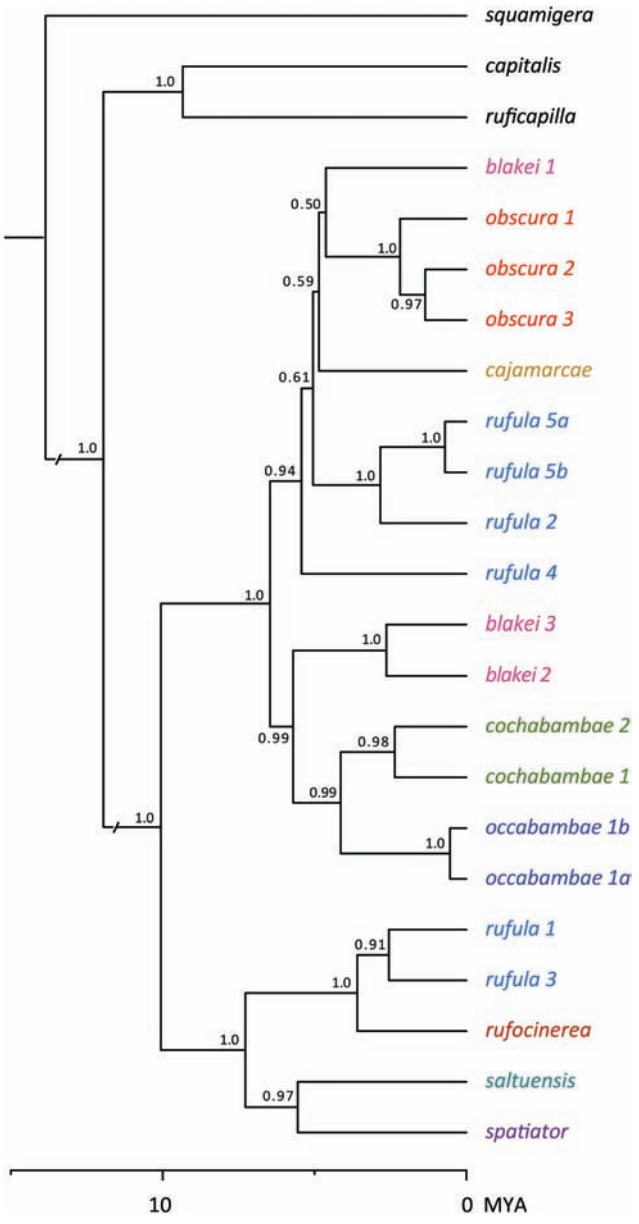

FIGURE 4. Maximum clade credibility tree for the *Grallaria rufula* complex, based on the combined mitochondrial and nuclear data, produced for single individuals per taxon using the program *BEAST. Numbers beside nodes represent posterior probabilities; timescale is in millions of years ago (mya). See Table 5 for mean divergence times and 95% confidence intervals for nodes in this tree.

The mitochondrial ML tree topology was generally consistent with that of the concatenated ML tree, identifying the same clades within *rufula* and *blakei* and for the most part identifying the same higher level clades (Appendix Figure 6). The main topological difference was that *rufula 4* and *cajamarcae* were sisters to the entire north-central group in the mtDNA tree, rather than sisters to the *rufula 2/5* clade within this group, as in the combined tree. Other differences between the mitochondrial and concatenated trees were (1) reciprocal monophyly of both *occabambae 1a* and *occabambae 1b* in the mitochondrial tree, (2) generally stronger bootstrap support for relationships in the north-central clade in the mitochondrial tree, and (3) slightly lower bootstrap support at deeper nodes in the mitochondrial tree.

Resolution of individual clades in trees using only nuclear sequence data was also high: 12 of the 18 clades identified in the concatenated analysis formed lineages in ML analyses of the nuclear data alone (Table 4, Appendix Figure 7). Lineages not recovered in the nuclear-only analyses were (1) *rufula 2* and *rufula 5*, which were not reciprocally monophyletic but instead formed a single clade consisting of *rufula 2/5*; (2) *rufula 1* and *rufula 3*, which were also not reciprocally monophyletic but instead formed a single clade consisting of *rufula 1/3*; (3) *cochabambae 2*, which was paraphyletic with respect to *cochabambae 1* (however, the 2 samples of *cochabambae 2* did form a clade when analyses were restricted to MUSK, the only nuclear gene for which data were available for both samples); and (4) *obscura 2*, which was paraphyletic with respect to both *obscura 1* and *obscura 3*. Nuclear sequence data were available for only one individual of *rufocinerea*, but this individual formed a lineage distinct from its close relatives *rufula 1* and *rufula 3*.

The higher level topology of the nuclear tree differed somewhat from those of the combined and mitochondrial trees (Appendix Figure 7). The northern clade present in the combined and mitochondrial trees was only partially resolved in the nuclear tree: *saltuensis* and *rufula 1/3* formed a clade, but this clade and *rufocinerea* formed 2 branches of a 3-way polytomy at the base of the tree, the third branch consisting of all other individuals of the complex. Taxon *spatiator* did not cluster with the rest of the northern clade, but instead grouped with *rufula 4* and *rufula 2/5*, which were sisters as in the combined tree but not in the mitochondrial tree. The central lineages *obscura, cajamarcae*, and *blakei 1*, rather than forming part of a north-central group, instead clustered with the southern lineages *cochabambae, occabambae*, and *blakei 2* and *3*.

The bGMYC analysis identified 17 distinct lineages as candidates for species status (Table 4, Figure 5). These were identical to the 18 clades revealed by the concatenated and mtDNA phylogenetic analyses, except that *obscura 2* and *obscura 3* did not individually meet the 0.95 posterior probability threshold under the bGMYC analysis, but instead were considered a single lineage. At the more liberal 0.5 posterior probability threshold, *obscura 2* and *obscura 3* were identified as independent lineages.

Geographic Barriers and Dates of Divergence

Geographic barriers separating lineages within the *G. rufula* complex include river valleys and other lowlands below the elevations at which *rufula*, *blakei*, and *rufocinerea* occur (Figures 1 and 2; Table 5).

TABLE 4. Comparison of results of analyses of nuclear and mitochondrial DNA, molecular species delimitation using bGMYC (based on the ND2 sequence data), and analysis of vocalizations (Isler et al. 2020). Only one nuclear sequence was available for *G. rufocinerea*, so this is treated as a distinct nuclear lineage rather than a clade. Major discrepancies occur only in the treatment of *obscura*, and minor discrepancies in the treatment of *rufula 1* and *rufula 3*, *rufula 2* and *rufula 5*, and *cochabambae 2*.

Taxon	nucDNA	mtDNA	Molecular species delimitation [a]	Vocalizations
saltuensis	clade	clade	species	species
spatiator	clade	clade	species	species
rufula 1	clade	clade	species	species
rufula 3		clade	species	
rufula 4	clade	clade	species	species
rufula 2	clade	clade	species	species
rufula 5a		clade [a]	species	
rufula 5b				
cajamarcae	clade	clade	species	species
blakei 1	clade	clade	species	species
obscura 1	clade [b]	clade	species	species
obscura 2		clade		species
obscura 3		clade		species
blakei 2	clade	clade	species	species
blakei 3	clade	clade	species	species
occabambae 1a	clade	clade [c]	species	species [d]
occabambae 1b				
cochabambae 1	clade	clade	species	species
cochabambae 2	clade [e]	clade	species	species
G. rufocinerea	distinct lineage	clade	species	species

[a] With *rufula 5a* and *5b* as geographical subgroups.

[b] With *obscura 1* and *3* also clades.

[c] With *occabambae 1a* and *1b* as geographical subgroups.

[d] With *occabambae 1a* and *1b* as subspecies.

[e] *cochabambae 1* and *cochabambae 2* were reciprocally monophyletic when analyses were restricted to MUSK, the only nuclear gene sequenced for all individuals of these taxa, although relationships of the individual for which only MUSK was sequenced (toepad DMNH 6757 of *cochabambae 2*) were unresolved when all nuclear genes were analyzed.

Prominent among river valleys and low-elevation gaps are those separating the cordilleras of the Andes in Colombia (ríos Magdalena and Cauca), *spatiator* and *saltuensis* in northern Colombia (Cesar Depression), *saltuensis* and *rufula 1* along the Colombia/Venezuela border (Motilones Range), and numerous Peruvian river valleys, including those of the Marañón, Huancabamba, Huallaga, and Apurímac. Gaps less obviously associated with specific geographical barriers separate *rufula 2* and *rufula 5* in southern Colombia, *occabambae* and *cochabambae 1* in southern Peru, and *cochabambae 1* and *cochabambae 2* in northern Bolivia. Some gaps include high elevation ridges with unsuitable habitat, such as those that appear to separate *rufula 1/3* and *rufula 2* in the Eastern Andes.

The *BEAST maximum-clade credibility tree indicated that the *G. rufula* complex began diversifying in the mid-to-late Miocene, ~10 mya (Table 5, Figure 4). The earliest divergences within the complex were all dated to the Late Miocene, and most diversification appears to have occurred within the Pliocene. Only 3 divergences among the 18 clades were dated to the Pleistocene. Divergence

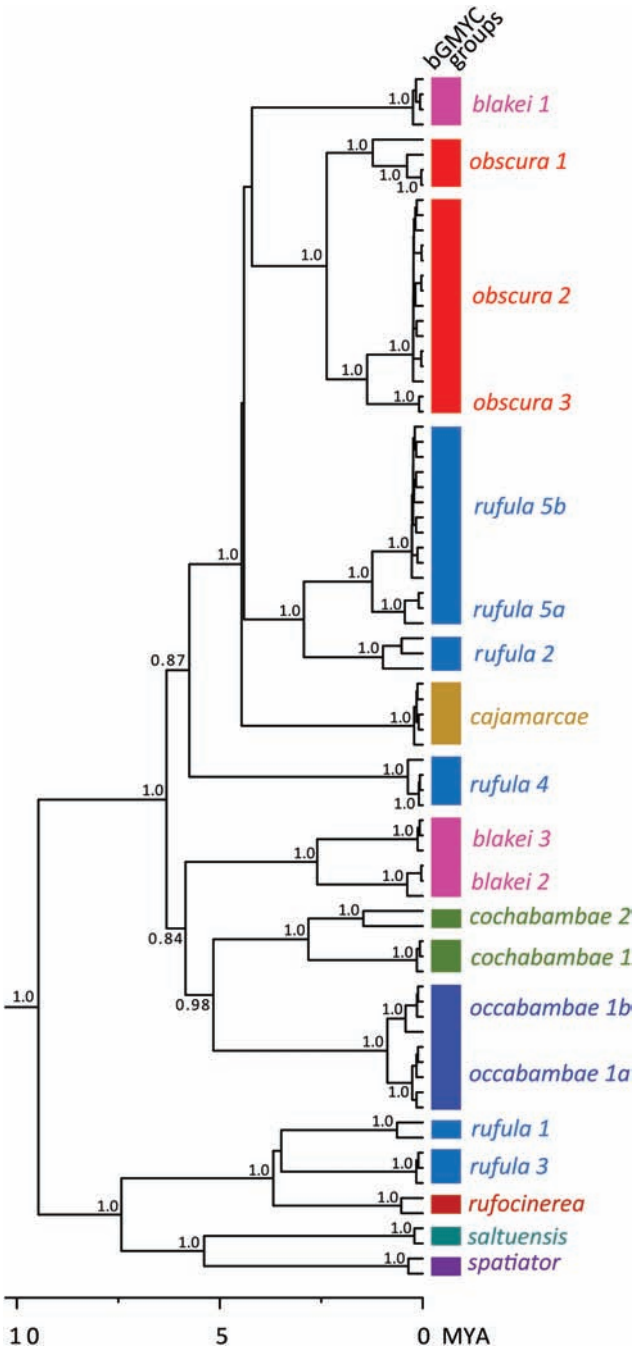

FIGURE 5. Candidates for species status in the *Grallaria rufula* complex, based on the mitochondrial (ND2) data, as estimated using the program bGMYC.

estimates from *BEAST tree and mitochondrial gene-tree analyses were in general similar to those from the analyses of the combined nuclear and mitochondrial data (Table 5).

DISCUSSION

We found remarkable genetic diversity within the *G. rufula* complex, contrasting with the relatively conservative morphology reflected in the currently recognized taxa in this complex. Neither *G. rufula* nor the group of populations provisionally considered to constitute *G. blakei* proved to be monophyletic, and *G. rufocinerea*, a species not traditionally considered to be closely related to *G. rufula*, was nested within the complex. Six of the 7 described subspecies of *rufula* were monophyletic, indicating some congruence of genetics with plumage variation, but 9 currently unrecognized populations of the *rufula* complex were monophyletic and genetically divergent to a similar degree. Populations in the Colombian Andes and in north-central Peru contained the most cryptic diversity: nominate *rufula* of Colombia and Ecuador consisted of 5 divergent lineages, *obscura* of Peru consisted of 3 lineages (although the bGMYC analysis recognized only 2), and the Peruvian populations considered to constitute *G. blakei* consisted of 3 lineages. An additional unrecognized population of similar divergence was present within the southernmost subspecies *cochabambae*, and additional populations of lesser divergence within *occabambae* and one population of *rufula* (*rufula 5*). However, sampling within *rufula 5* consisted only of individuals from the ends of the range (Figure 1); sampling of additional individuals may affect the pattern of divergence within this group.

Analyses of mitochondrial and nuclear DNA sequences were largely congruent. Fourteen lineages were identified in analyses of nuclear DNA: 11 lineages were identical to those recovered in analyses of the mtDNA and the other 3 lineages each formed clades consisting of 2 mtDNA sister lineages (Table 4). In each case, the mtDNA sister lineages either could not be resolved as reciprocally monophyletic in the nuclear analyses (*rufula 1* and *rufula 3*, and *rufula 2* and *rufula 5*) or were paraphyletic with respect to their mitochondrial sister clades in the nuclear analyses (*obscura 2*). Thus, the lack of congruence in these clades seems to be the result of lack of resolution, as would be expected from the more slowly evolving and coalescing nuclear genome, rather than conflict.

Species Delimitation

Vocal analyses in a companion study, which identified 16 biological species within the *G. rufula* complex (Isler et al. 2020), are remarkably coincident with our genetic results (Table 4). With one exception (*obscura 2*, which could not be resolved as a separate lineage in the nuclear tree), these 16 biological species are identical to the lineages identified in the analysis of the nuclear sequence data, including the lumping together of distinct mitochondrial lineages *rufula 1 + 3* and *rufula 2 + 5* (Table 4). None of the less divergent clades that appeared only in the mitochondrial trees (*rufula 5a* and *5b*, and *occabambae 1a* and *1b*) were distinctive enough to merit biological species status using the vocal analyses, although *occabambae 1a* and *1b* did show moderate vocal differences and are being described as subspecies (Isler et al. 2020). Of special interest is the

TABLE 5. Geographical barriers between adjacent *rufula*/*blakei* populations. Taxon pairs listed from north to south. Asterisks indicate sister species. Divergence times (with 95% confidence intervals) are from *BEAST maximum clade credibility trees.

Taxa	Geographic barrier	Divergence time (mya)	
		all data	ND2 data
spatiator and *saltuensis**	Cesar Depression, lowlands between Santa Marta and Perijá mountain systems, Colombia	5.6 (4.1–7.1)	5.8 (4.5–7.4)
saltuensis and *rufula 1**	Motilones Range, low-elevation, narrow ridge connecting the Perijá and the Eastern Andes in Norte de Santander, Colombia	7.3 (5.6–8.9)	8.0 (6.5–9.6)
rufula 1 northeastern limit	Táchira Depression	n/a	n/a
rufula 1 and *rufula 3**	North-South divide along the Eastern Andes [populations not clearly defined by existing data]	2.9 (0.8–3.7)	3.6 (2.5–4.2)
rufula 1/3 and *rufula 2*	Magdalena Valley, with respect to the Central Andes population. The population of *rufula 2a* was restricted to the Iguaque Massif of western Boyacá and southern Santander, a spur of the Eastern Andes	10.1 (8.4–11.6)	10.5 (9.0–11.9)
rufula 3 and *rufocinerea*	Río Magdalena Valley	3.4 (2.7–4.6)	3.8 (3.0–4.7)
rufula 2 and *rufula 4*	Río Cauca Valley	5.3 (4.5–6.4)	6.2 (5.3–7.1)
rufula 2 and *rufula 5a*	Colombian Massif in southern Colombia, with multiple upper drainages on both slopes of the Andes	2.8 (1.8–3.8)	3.1 (2.2–3.9)
rufula 5b and *cajamarcae*	Río Huancabamba	5.0 (4.3–5.8)	4.8 (4.1–5.6)
obscura 1/*blakei 1* and *rufula 5b*/*cajamarcae*	Río Marañón Valley	4.6–5.0 (4.1–5.6)	4.8 (3.8–5.3)
obscura 1 and *blakei 1**	Elevationally parapatric. *obscura, 1* 2,400–3,500 m; *blakei 2,* 1,800–2,900 m [elevation at parapatry may vary regionally; may be sympatric in some locations, e.g., Carpish Tunnel Trail in Huánuco]	4.4 (3.5–5.2)	4.5 (3.7–5.1)
obscura 1 and *obscura 2*	Río Huallaga	2.2 (1.4–2.9)	2.4 (1.8–3.0)
obscura 2 and *blakei 2*	Elevationally parapatric. *obscura 2,* 2,750–3,500 m; *blakei 2,* 2,400–2,700 m	6.4 (5.6–7.4)	6.9 (6.1–7.9)
obscura 2 and *obscura 3*	Río Perené and Río Paucartambo	1.4 (0.8–1.9)	1.4 (0.9–1.8)
obscura 3 and *blakei 2*	Elevationally parapatric *obscura 3,* 3,000–3,600 m; *blakei 2,* 2,400–2,700 m	6.4 (5.6–7.4)	6.9 (6.1–7.9)
blakei 2 and *blakei 3**	Río Mantaro	2.6 (1.8–3.4)	2.9 (2.0–3.8)
blakei 3 and *occabambae 1a*	Río Apurímac	5.7 (4.7–6.6)	6.4 (5.4–7.2)
blakei 3 southern limit	Río Pampas	n/a	n/a
occabambae 1a and *occabambae 1b*	Río Yanatili Valley	0.5 (0.2–1.0)	0.7 (0.3–1.1)
occabambae 1b and *cochabambae 1*	No known physical barrier; considerable distance separates known localities	4.1 (2.8–5.4)	5.6 (4.7–6.4)
cochabambae 1 and *cochabambae 2**	No known physical barrier; considerable distance separates known localities	2.4 (1.3–3.2)	3.1 (2.4–3.8)
cochabambae 2 southern limit	Low elevations of humid Andes on the east side of Amboro National Park, Bolivia	n/a	n/a

finding that the songs of the Eastern Andes and Central Andes populations of *rufula 2* appear to be indistinguishable (Isler et al. 2020), supporting the unusual genetic result mentioned above, in which clade *rufula 2* contained 2 individuals from the Central Andes of Colombia and 1 individual from the Eastern Andes. Phylogeographic analyses have revealed genetic differentiation between hummingbird populations from the western slope of the Eastern Andes of Colombia and other populations from this cordillera (Chaves and Smith 2011); however, the pattern we observed, in which a population from the western slope of the Eastern Andes is more closely allied, and similar genetically, to populations from the Central Andes, has not been previously documented. Such a pattern is surprising considering the role of the Magdalena Valley as a barrier to dispersal between the Eastern and Central cordilleras in other birds (e.g., Cadena et al. 2007, Gutiérrez-Pinto et al. 2012, Valderrama et al. 2014).

Results of the bGMYC analysis, which delineated 17 significantly differentiated lineages based solely on the mitochondrial data, were identical to the results of species identification using vocalizations, with 3 exceptions: (1) *rufula 1* and *rufula 3* were considered 2 distinct model-based lineages but only a single biological species, (2) *rufula*

2 and *rufula 5* were likewise considered 2 distinct model-based lineages but only a single biological species, and (3) *obscura 2* and *obscura 3* were considered a single model-based lineage but 2 biological species (Table 4). Whether those speces identified using mtDNA but not found to be distinct in analyses of vocal characters or in analyses of nuclear DNA (i.e. *rufula 1*, *2*, *3*, and *5*) would be considered species under alternate species concepts, such as the evolutionary and phylogenetic species concepts, would depend on the degree to which mtDNA is considered representative of true lineages or sufficient for diagnosability.

Biogeography

Geographical barriers play a key role in structuring populations of Andean birds (Chapman 1917, Graves 1985, O'Neill 1992, Weir 2009, Hazzi et al. 2018). Our study of genetic differentiation in the extraordinarily diverse *rufula* complex provides the opportunity to investigate the role of Andean geographic barriers at a fine-grained scale. Our results support the importance of river valleys and other well-known topographic features in separating lineages of *rufula*, although in some lineages, range delimitation is not clearly associated with geographical barriers, and in one case a distribution extends across a recognized geographical barrier.

The distribution of the *rufula* complex in Colombia encompasses the Eastern, Central, and Western Andes, as well as the Santa Marta and Perijá mountains to the north (Table 5, Figure 1). The Santa Marta and Perijá taxa (*spatiator* and *saltuensis*, respectively) are isolated from the Andes by the Cesar Depression and the low elevation Motilones Range. A single lineage (*rufula 4*) is endemic to the Western Andes and separated from other lineages to the east by the Cauca Valley. Our genetic samples and recordings (Isler et al. 2020) for *rufula 4* are from Antioquia and Risaralda in the northern Western Andes, but specimens with similar plumage from the "Coast Range west of Popayán" (AMNH 109634 and 109635) and from Cerro Munchique (FMNH 249750 and LACM 57383) indicate that its distribution may extend south to Cauca. The Magdalena Valley, a major barrier that separates the Central and Eastern Andes, forms the western boundary of the distributions of *rufula 1* and *rufula 3*, 2 lineages restricted to the Eastern Andes. Range limits between *rufula 1* and *rufula 3* are not clearly delineated by current data, but the Táchira Depression forms the northern limit to *rufula 1*, and the low passes of the much narrower southern portion of the Eastern Andes (Las Cruces or Andalucía) may limit the range of *rufula 3* south to the Sumapaz Massif. A single lineage (*rufula 2*) occurs in the Central Andes, but its distribution also extends across the Magdalena Valley to the Iguaque Massif of western Boyacá and southern Santander, a spur of the Eastern Andes. The Colombian Massif in southern Colombia, with a number of nascent valleys and complex topography, appears to separate sister clades *rufula 2* and *rufula 5*, but considerable geographical distance separates their known distributions and it is not possible to single out a particular geographic feature.

River valleys predominate as geographical barriers in northern and central Peru. The deep canyons of the Marañón, its tributary the Huancabamba, and other large Amazonian tributaries such as the Huallaga and the Apurímac-Ene are recognized dispersal barriers to Andean birds (Weir 2009, Hazzi et al. 2018) that also form barriers to gene flow in the *rufula* complex (Table 5; Figures 1 and 2). Less prominent Peruvian river valleys, such as the Perené, the Pampas, the Mantaro, and the Yanatili, also appear to separate or delimit lineages of the *rufula* complex, although their effects are less clear. Genetic and vocal sampling in the regions of these rivers is too sparse to be certain of their role in delineating distributional limits.

The distributions of several Peruvian lineages do not appear to be associated with geographical barriers, instead exhibiting elevational parapatry or geographical separation in the absence of a physical barrier (Table 5; Figures 1 and 2). The lineage described as *blakei* (Graves 1987), here identified as *blakei 1*, has long been known to be elevationally parapatric or even sympatric with the *rufula* lineage identified here as *obscura 1*. Another lineage considered a form of *blakei*, identified here as *blakei 2*, is elevationally parapatric with the *obscura 2* and *obscura 3* lineages of *rufula*. This parapatry, together with plumage similarities, led to initial treatment of *blakei 2* as a newly discovered population of *blakei* (Hosner et al. 2015). This lineage, *blakei* 2, appears to be separated by the Río Mantaro from a third population also considered a form of *blakei* (Hosner et al. 2015), here identified as *blakei 3*, whose southern distributional limit appears to be the Río Pampas.

In southern Peru, considerable distance separates known localities of lineages *occabambae* and *cochabambae 1*, and no obvious geographical barrier occurs in this area. Separation of lineages in southern Peru in the absence of a barrier has been noted in other taxa, including *Arremon assimilis* (Gray-browed Brushfinch) and *Arremon torquatus* (White-browed Brushfinch; Cadena et al. 2007, Cadena and Cuervo 2010), *Adelomyia melanogenys* (Speckled Hummingbird) subspecies *A. m. chlorospila*/*A. m. inornata* (Chaves and Smith 2011), *Basileuterus tristriatus* (Three-striped Warbler) subspecies *B. t. tristriatus* and *B. t. punctipectus* (Gutiérrez-Pinto et al. 2012), and *G. erythroleuca* (Red-and-white Antpitta) and *G. erythrotis* (Rufous-faced Antpitta; Winger et al. 2015). Fjeldså et al. (1999) suggested that fine-scale differences in ecological and climatic stability in this area may have promoted persistence and divergence of lineages despite the lack of a physical barrier. At the southern end of the distribution, in Bolivia, *cochabambae 1* and *cochabambae 2* may be separated by the Río Consata Valley near Sorata, La Paz, but considerable distance separates known localities in this seldom visited region,

and further fieldwork is required. Finally, low elevations in western Santa Cruz form the southern boundary of the *rufula* complex (Table 5, Figure 1).

As described above, populations traditionally referred to *G. blakei* and *G. rufula* replace each other with elevation in the Peruvian Andes. The discovery that *G. rufocinerea* is part of the *G. rufula* complex provides another case of elevational replacement in the complex. Both *G. rufocinerea* and *G. rufula* occur in the central Andes of Colombia south into extreme northern Ecuador, but they do not seem to be syntopic in most areas. As far as is known in regions where forms of both *G. rufocinerea* and *G. rufula* exist, *G. rufocinerea* occurs at lower elevations in upper montane forests (~2,400–3,000 m), being replaced above ~3,000 m by *G. rufula* (Hilty and Brown 1986, Kattan and Beltrán 2002; F. Ayerbe personal communication). Because populations in the *G. rufula* complex replacing each other with elevation are not sister to each other in either case, our results are consistent with the emerging pattern that elevational replacements in Neotropical mountains reflect secondary contact of previously allopatric lineages as opposed to parapatric divergence along elevational gradients (Patton and Smith 1992, Caro et al. 2013, Cadena and Céspedes 2020).

The time-calibrated *BEAST analysis indicated that the *G. rufula* complex originated in the mid-to-late Miocene and diversified over the past 10 myr. Most diversification appears to have occurred in the Pliocene and Pleistocene, with the majority occurring in the Pliocene. In contrast to some Andean groups, in which most diversification can be traced to the Pleistocene (e.g., Chesser 2000, Benham et al. 2015), only 5 of the 18 clades of the complex (*obscura 1*, *2*, and *3*, and *cochabambae 1* and *2*) were dated to the Pleistocene in our maximum clade credibility tree.

The timing of diversification across specific geographical barriers is similar to that identified in some previous studies. For example, our figures from ND2 for ages of sister taxa separated by the ríos Marañón (4.8 mya, CI: 3.7–5.1 mya) and Huallaga (2.4 mya, CI: 1.8–3.0 mya) are close to those estimated from ND2 for the *G. hypoleuca* complex, which were 4.4 mya for sisters separated by the Marañón and 2.2 mya for those separated by the Huallaga (Winger et al. 2015). However, our estimate for the Apurímac (6.4 mya, CI: 5.3–7.2 mya) is not close to theirs (4.2 mya). Winger et al. (2015) presented lines of evidence that argued for a vicariant origin for taxa on either side of these and other geographical barriers, and our data are consistent with this for the Marañón and Huallaga valleys.

Plumage

In contrast to substantial differentiation in genetics and vocalizations (Isler et al. 2020), plumage in the *G. rufula* complex has remained remarkably unchanged during its diversification, contributing heavily to the traditional classification of all but 2 taxa of the complex into only a single species. The only characters that show appreciable variation are (1) color of the back, head, and breast, which ranges from olive-gray brown in *saltuensis* and dull rufous in *cochabambae* to bright chestnut or dark chestnut in *blakei 1* and *rufula 4*, respectively; (2) color or patterning of the belly, including the extent of white coloration, which ranges from substantial in *spatiator* to virtually non-existent in some individuals of *cochabambae*; contrasting light feather tips, especially notable in the southern forms *cochabambae* and *occabambae*; and the presence of indistinct barring on the lower belly in *blakei 1* and at least some individuals of *rufula 4*; and (3) presence or absence of a dull whitish eye-ring. That plumage coloration is not constrained in this group is indicated by the surprising placement of the distinctive *G. rufocinerea* within the *G. rufula* complex. Although Sclater and Salvin (1879), in their description of *rufocinerea*, indicated that it should directly precede *G. rufula* in the linear sequence, the 2 species have not in general been considered closely related. Coloration of the back, head, and throat of *rufocinerea* is similar to that of other members of the *rufula* complex, its dark chestnut back color being especially similar to that of *rufula 4*, but coloration of the rest of the underparts is leaden gray, a unique character state and color in this complex. The pattern of soft whistle vocalizations, however, also supports the placement of *rufocinerea* in the *rufula* complex (Isler et al. 2020), confirming the surprising genetic result. This differentiation in plumage did not occur on one of the deeper branches of the phylogenetic tree; instead, according to the *BEAST MCC tree (Figure 4), *rufocinerea* is sister to *rufula 1* + *rufula 3*, having evolved some 3.4 mya in the late Pliocene.

Other notable plumage differences in the *rufula* complex, as mentioned above, include the distinctive olive-gray brown plumage of *saltuensis* and the chestnut or dark reddish-brown plumage characteristic of some taxa. The latter was described as a key feature when *blakei* was recognized as distinct from *rufula*, although similarities of plumage with specimens of *rufula* from the Western Andes and a single specimen of *rufula* from Pasco, Peru, were also noted (Graves 1987). Typical *blakei* is identified here as *blakei 1*, and the similar forms mentioned by Graves correspond to *rufula 4* (Western Andes) and *blakei 2* (Pasco), respectively. None of these are sister taxa, although *blakei 2* is sister to the more recently diagnosed *blakei 3*. Thus, the 2 low-elevation chestnut-plumaged forms described as *blakei* or provisionally ascribed to it (i.e. *blakei 2*) appear to have been independently derived from higher elevation populations of *rufula*, one lineage north of the Huallaga Valley, and one lineage to the south.

Cryptic Diversity in the Andes

The Andes are already considered an area of extraordinary biodiversity (Myers et al. 2000). How unusual is the high degree of cryptic diversity found in the *G. rufula* complex? Among Andean birds, only the *Henicorhina*

leucophrys (Gray-breasted Wood-Wren) complex seems to be similar in terms of mitochondrial genetic diversity. The *H. leucophrys* complex currently encompasses 3 species: *H. leucophrys*, a widespread species found from central Mexico south to Bolivia and consisting of 19 subspecies, and 2 monotypic Colombian endemics, *H. anachoreta* (Hermit Wood-Wren) of the Sierra Nevada de Santa Marta and *H. negreti* (Munchique Wood-Wren) of western Colombia. Cadena et al. (2019) found extraordinary mitochondrial diversity within this complex, identifying 38 presumptive species using the 0.95 threshold in bGMYC. Although the geographical distribution of the *H. leucophrys* complex greatly exceeds that of the *G. rufula* complex, some 24 of its presumptive species occur within the geographical range occupied by the *G. rufula* complex, making the *H. leucophrys* complex somewhat more diverse as measured by mitochondrial DNA over the same geographical area. However, Cadena et al. (2019) did not sequence nuclear DNA for their study, nor were the complex vocalizations studied. Hence, the extent to which mitochondrial diversity in *Henicorhina* is reflected in nuclear DNA and biological species limits remains to be determined, and the species diversity of the 2 complexes cannot be directly compared at this time.

In terms of cryptic biological species diversity, the *Scytalopus magellanicus* (Magellanic Tapaculo) complex, another group of short-winged species of forest understory, in many respects mirrors the *G. rufula* complex. *Scytalopus magellanicus* was traditionally considered a single widespread species with as many as 13 subspecies (Zimmer 1939, Peters 1951, Meyer de Schauensee 1970, Sibley and Monroe 1990, but see Fjeldså and Krabbe 1990, Whitney 1994, Krabbe and Schulenberg 1997). Krabbe and Schulenberg (1997) elevated 10 of the subspecies to biological species status based on vocal characters. New revisions including vocal characters and genetic markers have described and recognized 5 additional species (Krabbe and Cadena 2010, Cadena et al. 2020, Krabbe et al. 2020). As in the *G. rufula* complex, vocal and genetic differences across the *S. magellanicus* complex are remarkably coincident, although a few geographically partitioned mitochondrial lineages seemingly lack corresponding vocal differences. Recent studies of the *S. magellanicus* complex have also identified 2 independent instances of evolution of elevational parapatry.

Whether the high levels of cryptic diversity found in these species complexes are characteristic of avian species of the Andes remains to be determined. The examples of *G. rufula*, *H. leucophrys*, and *S. magellanicus* suggest that widely distributed, drab-plumaged species of limited dispersal ability are prime candidates for cryptic diversity. Studies of other Andean birds regularly reveal cryptic differentiation, but generally not approaching the scale shown in these species groups (e.g., Valderrama et al. 2014). However, many species of limited dispersal capability have yet to be studied.

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Author contributions: R.T.C. and M.L.I. conceived this study and its companion study and worked together throughout. A.M.C. and C.D.C. collected genetic data for samples from Colombia, S.C.G. and L.M.B. for most samples from Ecuador, Peru, and Bolivia, and P.A.H. for other samples from Peru. R.C.F. provided extractions for most toepad samples. G.A.B. determined that *rufocinerea* is part of the *rufula* complex and provided mitochondrial sequence for this species. R.T.C. edited and aligned the sequences and P.A.H. and R.T.C. conducted phylogenetic and other data analyses and created the relevant figures. M.L.I., R.T.C., A.M.C., C.D.C., P.A.H., and D.F.L. supplied data on distribution and biogeography. A.M.C. created the maps. R.T.C. wrote the initial manuscript, which was improved by comments from all other authors.

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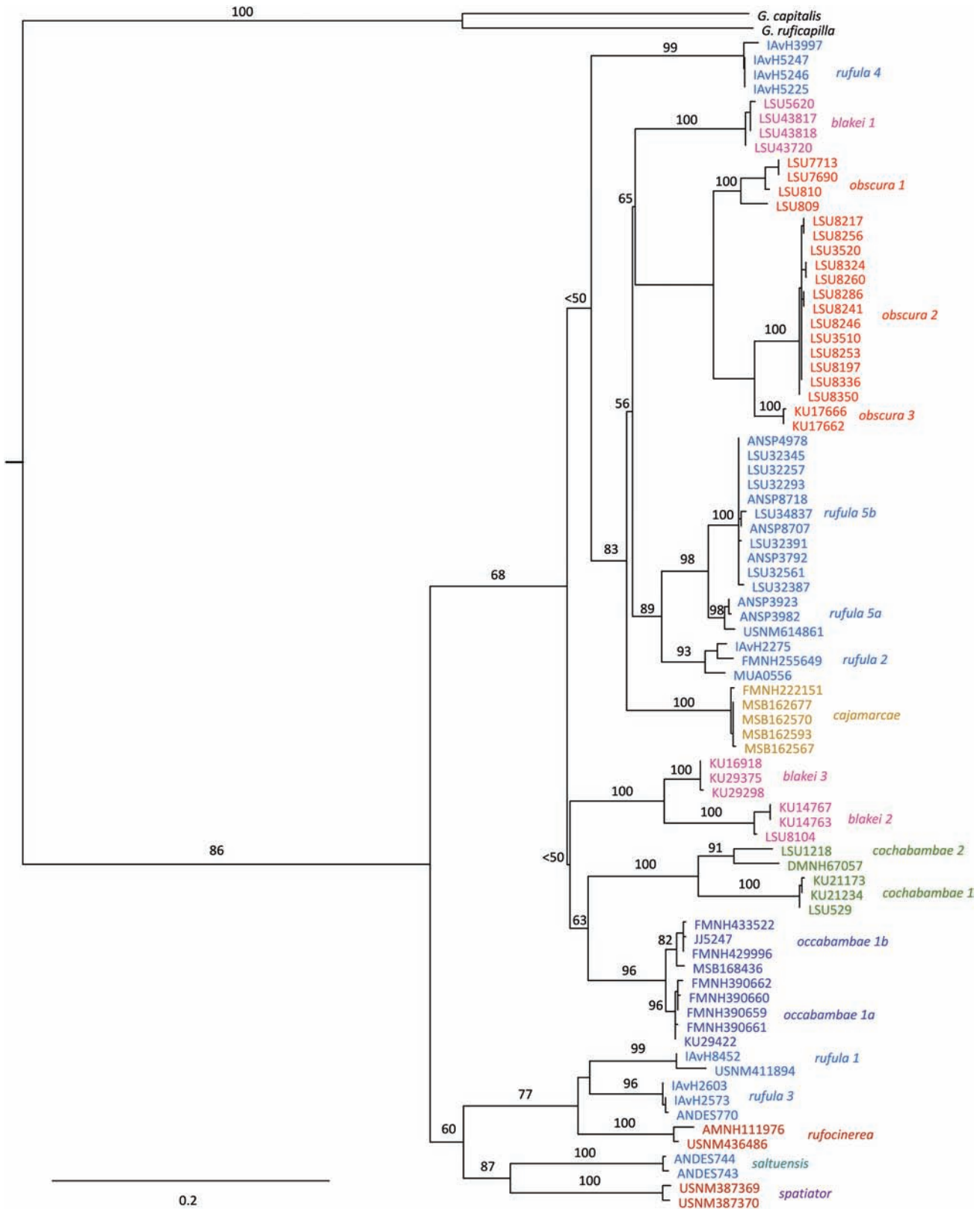
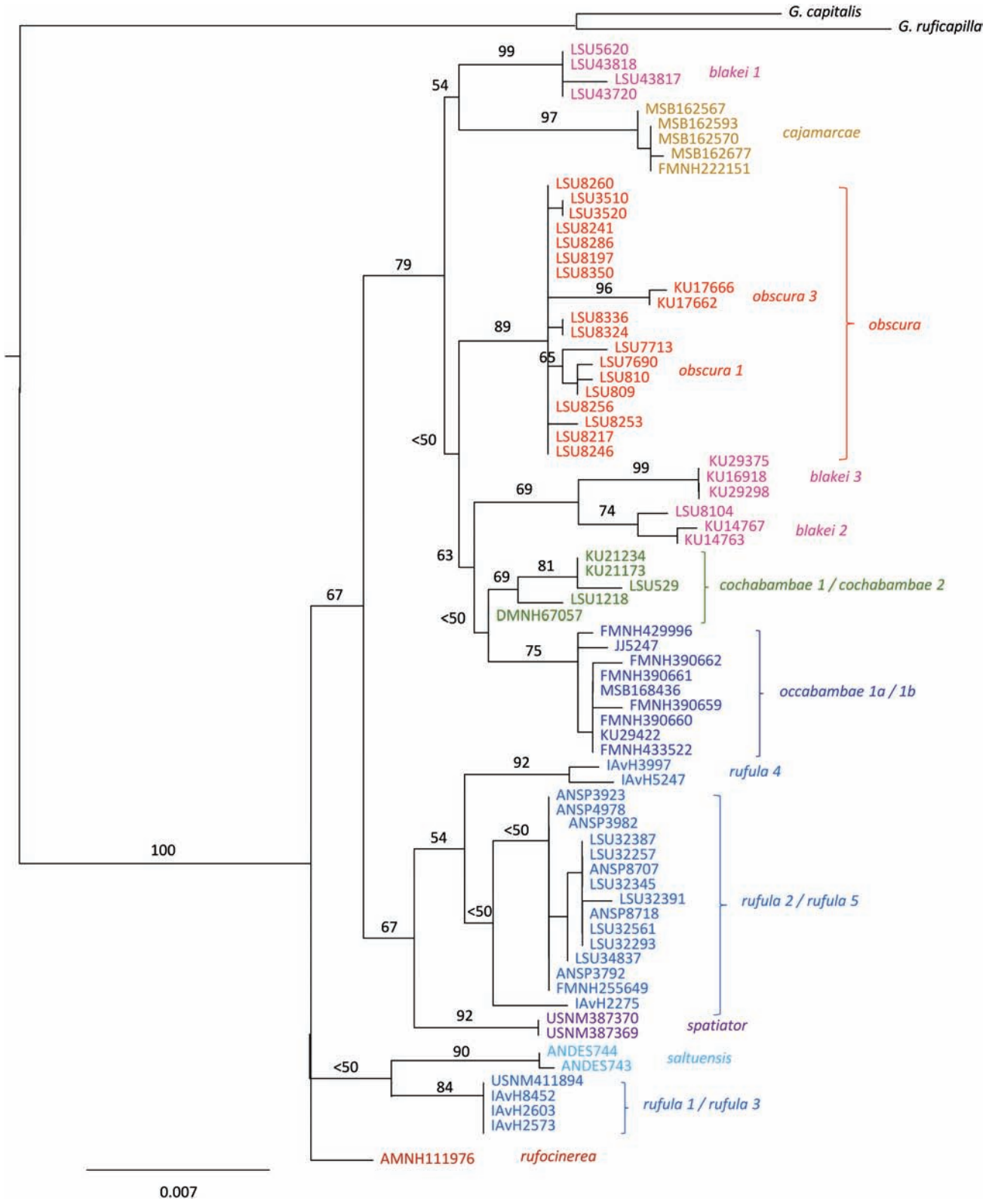


APPENDIX FIGURE 6. Most likely tree for the *Grallaria rufula* complex, based on the mitochondrial data, produced using the program RAxML. Numbers above nodes represent bootstrap support values based on 100 bootstrap replicates.



APPENDIX FIGURE 7. Most likely tree for the *Grallaria rufula* complex, based on the combined nuclear data, produced using the program RAxML. Numbers above nodes represent bootstrap support values based on 100 bootstrap replicates.