

Species limits in the Rusty-breasted Antpitta (*Grallaricula ferrugineipectus*) complex

Benjamin M. Van Doren,^{1,2*} Benjamin G. Freeman,^{1,3,4} Natalia Aristizabal,⁵ Mauricio Alvarez-R,^{6,7} Jorge Pérez-Emán,^{8,9} Andrés M. Cuervo,^{5,6} and Gustavo A. Bravo^{5,10}

ABSTRACT—The Rusty-breasted Antpitta (*Grallaricula ferrugineipectus*) is widely distributed within the tropical Andes of South America. We analyzed 73 study specimens, 25 vouchered tissue samples, and 123 audio recordings to assess geographic variation in genetics, vocalizations, and morphology and evaluate species limits. We found that *Grallaricula ferrugineipectus* as currently defined is polyphyletic because populations from Colombia and Venezuela form a clade closely related to Andean populations of *G. nana*, whereas populations from Peru and Bolivia are recovered as sister to *G. lineifrons*. Birds in Colombia and Venezuela (the northern group) last shared a common ancestor with birds from Peru and Bolivia (the southern group) more than 10 million years ago. Northern and southern groups additionally differ in song, suggesting they may have evolved substantial premating reproductive isolation. Discriminant function analysis reliably distinguished songs from northern and southern groups in multivariate acoustic space, but univariate analyses found non-overlapping acoustic variation between northern and southern groups in only one trait: mean note maximum frequency (and other correlated measures of song pitch). This finding suggests that the ‘three-trait’ threshold for using vocalizations to inform species limits, which was developed for another suboscine group, the antbirds (Thamnophilidae), may be conservative when applied to antpittas (Grallariidae). In addition, we document apparent clinal variation in song pace within the southern group, a rare example of a suboscine with geographic clinal variation in a vocal trait. Finally, we show that northern and southern groups differ markedly in morphology. In summary, northern and southern groups of Rusty-breasted Antpittas are divergent in genetics, vocalizations, and morphology, demonstrating that these taxa are best classified as 2 monophyletic, biological species with allopatric distributions. Received 3 August 2016. Accepted 22 May 2017.

Key words: Andes, clinal variation, songs, South America, suboscine, taxonomy, vocalizations.

Límites entre especies del complejo *Grallaricula ferrugineipectus*

RESUMEN (Spanish)—*Grallaricula ferrugineipectus* es una especie ampliamente distribuida en los Andes tropicales de América del Sur. Con el fin de establecer los límites de especie, analizamos 73 especímenes, 25 muestras de tejido y 123 grabaciones de cantos que representan su variación geográfica genética, vocal y morfológica. Encontramos que *Grallaricula ferrugineipectus* es una especie polifilética porque las poblaciones de Colombia y Venezuela forman un clado cercanamente emparentado con las poblaciones andinas de *Grallaricula nana*, mientras que las poblaciones de Perú y Bolivia son hermanas de *Grallaricula lineifrons*. Los individuos de Colombia y Venezuela (grupo del norte) compartieron un ancestro común con los individuos de Perú y Bolivia (grupo del sur) hasta hace más de 10 millones de años. Además, los grupos del norte y del sur se diferencian en su canto, lo que sugiere que ya surgió aislamiento reproductivo precigótico entre ellos. Un análisis de función de discriminantes permitió distinguir los cantos de los grupos del norte y del sur en el espacio acústico multivariado, pero los análisis univariados sólo encontraron una variable cuya variación no se sobrepone entre grupos: frecuencia máxima promedio entre notas (y otras medidas del tono correlacionadas). Esto sugiere que el umbral de “tres caracteres”, usado para definir límites de especie en otro grupo de suboscinos (Thamnophilidae), puede ser conservador si se usa en los Grallariidos. Adicionalmente, en el grupo del sur documentamos una aparente variación clinal en la cadencia del canto, hecho que representa un raro ejemplo de variación geográfica clinal en un carácter vocal en suboscinos. Finalmente, mostramos que los grupos del norte y del sur también difieren en morfología. En resumen, los grupos del norte y del sur son divergentes en genética, vocalizaciones y morfología, demostrando que deben ser tratados como dos especies biológicas monofiléticas con distribuciones alopatricas.

Palabras clave: América del Sur, Andes, cantos, suboscinos, taxonomía, variación clinal, vocalizaciones.

¹ Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, USA.

² Edward Grey Institute, Department of Zoology, University of Oxford, Oxford, UK.

³ Cornell Lab of Ornithology, Cornell University, Ithaca, NY, USA.

⁴ Department of Zoology, University of British Columbia, Vancouver, Canada.

⁵ Museum of Natural Science and Department of Natural Sciences, Louisiana State University, Baton Rouge, LA, USA.

⁶ Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Colombia.

⁷ Gimnasio Moderno, Área de Ciencias Naturales, Bogotá DC, Colombia.

⁸ Instituto de Zoología y Ecología Tropical, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela.

⁹ Colección Ornitológica Phelps, Caracas, Venezuela.

¹⁰ Department of Organismic and Evolutionary Biology & Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA.

* Corresponding author: benjamin.vandoren@zoo.ox.ac.uk

The Rusty-breasted Antpitta (*Grallaricula ferrugineipectus*) is a small antpitta in the family Grallariidae distributed from Venezuela to Bolivia, where it inhabits the understory of humid forests in the Andes and other montane systems, such as the Sierra Nevada de Santa Marta and the coastal Venezuela mountains (Hilty and Brown 1986, Krabbe and Schulenberg 2003, Greeney 2013). Like other *Grallaricula* species, *G. ferrugineipectus* is a plump bird with long legs and a short tail, and it is shy and seldom seen (Greeney 2013). Three subspecies are currently recognized, each of which inhabits a distinct montane region: *G. f. ferrugineipectus* is found in northern and western Venezuela and the Sierra Nevada de Santa Marta in adjacent northern Colombia; *G. f. rara* in the Eastern Andes of Colombia and the Sierra de Perijá, which straddles the Colombia-Venezuela border; and *G. f. leymeambae* in the Andean foothills from extreme southern Ecuador to western Bolivia (Greeney 2013).

Current knowledge of the distribution of the species has been improved by recent discoveries of populations outside its traditionally known range. Although its presence in Peru north of the Marañón River had been documented since the mid-1950s based on 2 specimens taken independently by M. Koepcke and T. A. Parker in Cancheque, Piura (Schulenberg and Parker 1981, Parker et al. 1985), there are now recent records in the departments of Piura (Vellinga et al. 2004) and Lambeyeque (Angulo Prato-longo et al. 2012), as well as in the Ecuadorian provinces of Loja and Pichincha (Athanas and Greenfield 2016; P. Coopmans, ornithologist, unpubl. data, collected 1994–2003). Likewise, only in the early 1980s was the species first recorded in Bolivia (Schulenberg and Remsen 1982). More recently, MAR and collaborators discovered a population in the Cauca Valley of the Central Andes in the department of Caldas, Colombia that seems to be geographically isolated from other conspecific populations. Taxonomic affinities of these populations have never been formally assessed, and their taxonomic treatment has been assumed to correspond to that of the geographically closest populations.

Populations differ somewhat in elevational distribution and habitat; subspecies *rara* and *ferrugineipectus* inhabit forested foothills from ~250 to 2200 m.a.s.l. (Krabbe and Schulenberg

2003), and the Cauca Valley antpittas are currently known only from one locality at 1000–1100 m.a.s.l. Hereafter, these 3 populations will be referred to as the ‘northern group.’ By contrast, birds belonging to the ‘southern group’ (populations from Ecuador, Peru, and Bolivia) range substantially higher and inhabit montane forest from 1750 to 3350 m.a.s.l. (Ridgely and Tudor 2009). Southern group birds seem closely tied to bamboo in the genus *Chusquea* (Fjeldsá and Krabbe 1990, Athanas and Greenfield 2016). This habitat association has not been documented for the northern group, which can tolerate some degree of habitat degradation (Hilty and Brown 1986, Niklison et al. 2008; N. Athanas, Tropical Birding Tours, 2017, pers. comm.).

The 3 subspecies of *G. ferrugineipectus* were described based on differences in plumage and morphology. The *rara* subspecies is the most divergent with respect to plumage, with a rich rufous-brown underside and clear rufous tones on the crown and face, in contrast to the dark brown to slate-brown upperside and rufous underside of subspecies *ferrugineipectus* and *leymeambae*. These latter 2 subspecies both lack rufous on the head, have duller underparts, and show an obvious white throat crescent; *G. f. leymeambae* differs from *G. f. ferrugineipectus* in larger size and darker overall coloration (Greeney 2013). Songs of the species also vary across its geographic range. Most noticeably, *G. f. leymeambae* gives slower and higher-pitched songs than *G. f. ferrugineipectus* (Krabbe and Schulenberg 2003). The song of *G. f. rara* is poorly known and not described in recent reference volumes (Krabbe and Schulenberg 2003, Greeney 2013). Finally, recordings from northwest Ecuador demonstrate that this population’s songs seem slower than those of other populations (e.g., see XC35333 on xeno-canto.org).

Because populations of *G. ferrugineipectus* are distributed allopatrically throughout the tropical mountains of northern South America, reproductive isolation between subspecies cannot be directly assessed. Early on, systematists proposed arrangements of species-level taxonomy based on plumage differentiation within this complex. For example, *G. f. rara* was originally described as a species because of its distinctive plumage (Hellmayr and Madarász 1914), while *G. f. leymeambae* was first described as a subspecies (Carriker

1933). More recently, differences in distribution, morphology, and vocalizations between *G. f. leymebambae* and the northern group have led some authors (e.g., Ridgely and Tudor 2009, BirdLife International 2017) to classify it as a distinct species; however, no formal comparative analysis has systematically examined genetic, vocal, and morphological variation within this complex. These data types are complementary: whereas genetic data reveal the evolutionary histories of taxa, vocalizations provide information directly related to the likelihood of premating reproductive isolation (e.g., Isler et al. 1998, Zimmer 2002, Seddon and Tobias 2007, Donegan 2008, Zimmer 2008).

Here, we present an analysis of genetic, vocal, and morphological variation across the range of *G. ferrugineipectus*. We construct a phylogenetic hypothesis including all named taxa therein and their closest relatives to test the monophyly of the species. We then examine intraspecific vocal and morphological variation and assess the diagnosability of vocal traits among populations as a proxy for the level of behavioral premating isolation. With these data, we ask whether populations in the northern and southern groups indeed represent different species and evaluate variation in their constituent subpopulations.

Methods

Molecular data

An ongoing study to elucidate the species-level relationships within the Grallariidae (GAB, unpubl. data) has generated mitochondrial and nuclear sequences for multiple individuals for 52 of the 53 species currently recognized in the family (sensu Remsen et al. 2017). Based on preliminary phylogenetic analyses of this dataset, we selected 25 grallariid individuals of the following species, which include all genera in the family: *Grallaria guatimalensis* (1 sample), *Hyllopezus berlepschi* (1), *Myrmothera campanisona* (1), *Grallaricula flavirostris* (2), *G. lineifrons* (2), *G. nana* (7), and *G. ferrugineipectus* (11; Supplemental Material S1). Of these samples, 5 were derived from toepads of museum specimens (3 samples of *G. ferrugineipectus rara*, 1 sample of *G. flavirostris*, and 1 sample of *G. lineifrons*). This sampling encompasses all currently recognized subspecies within *G. ferrugineipectus* and also includes birds

from the Cauca Valley in Colombia, but it does not yet include samples north of the Marañón River in Peru and Ecuador. For outgroups, we included sequences from the genera *Scytalopus* and *Thamnophilus* (Supplemental Material S1).

We used standard methods described elsewhere (Brumfield and Edwards 2007, Brumfield et al. 2007, Kimball et al. 2009) to extract total DNA and to amplify and obtain sequences for 2 mitochondrial (NADH dehydrogenase subunit 2 – ND2, 1,041 bp; and NADH dehydrogenase subunit 3 – ND3, 351 bp) and 3 autosomal nuclear introns (transforming growth factor- β 2 intron 5 – TGF β 2, 629 bp; muscle-specific kinase receptor intron 3 – MUSK, 651 bp; β -fibrinogen intron 5 – β F5, 568 bp). Because toepad samples came from recent museum specimens collected in the 1990s, DNA extraction from these samples followed the same DNA extraction protocols with the addition of an extended lysis time in dithiothreitol.

We edited sequences and checked that protein-coding sequences did not include stop codons or anomalous residues using Geneious 8.1 (www.geneious.com; Kearse et al. 2012). We aligned sequences for each marker using the MAFFT 7 multiple alignment plugin (Katoh and Standley 2013) implemented in Geneious and obtained a concatenated dataset using Geneious Pro 8.1. In the end, alignments for ND2 and ND3 included sequences for all individuals (27), TGF β 2 (26 individuals), MUSK (25), and β F5 (20). The final alignment included data for 3,223 base pairs. Newly obtained sequences were deposited in GenBank (Supplemental Material S1).

Partition and substitution models

We selected substitution models and the optimal partitioning regime using the greedy algorithm (Lanfear et al. 2012) and PhyML 3.0 (Guindon et al. 2010) implemented in PartitionFinder2 (Lanfear et al. 2017). We evaluated models of molecular evolution available in BEAST (Drummond et al. 2012, Bouckaert et al. 2014), and the maximum number of partitions was set to 5 (each marker treated separately). Using the corrected Akaike information criterion (AICc; Hurvich and Tsai 1989) as a model selection parameter, we were able to partition data in 3 different subsets: ND2, ND3, and the 3 nuclear introns.

Phylogenetic inference

Using the selected partitioned scheme, we conducted a maximum likelihood phylogenetic analysis with the GTR+ Γ model of nucleotide substitution and 999 bootstrap replicates implemented in RAxML 8.2.9 (Stamatakis 2014) on the CIPRES Science Gateway V 3.3 (Miller et al. 2010). We estimated a time-calibrated species tree in a Bayesian framework using the multispecies coalescent model implemented in the program *BEAST2 2.4.4 (Drummond et al. 2012, Bouckaert et al. 2014) on the CIPRES Science Gateway V 3.3. We used unlinked substitution models across partitions and clock models linked by locus (i.e., mtDNA and each intron separately). Gene trees were estimated independently for each of the 4 loci (both mtDNA markers were treated as a single locus). Substitution parameters were based on results previously obtained from Partition-Finder2. Based on the avian mtDNA substitution rate of 2.1%/My (Weir and Schluter 2008), we used a lognormal relaxed molecular clock with a mean rate of 0.0105 for mtDNA. Clock rates for nuclear introns were estimated relative to the rate of mtDNA. We used a Yule prior with no restrictions on tree shape and a randomly generated tree as a starting tree. We ran analyses for 200 million generations with a sampling frequency of 20,000. We determined that replicate analyses converged (effective sample size values >400) using Tracer 1.6 (Rambaut et al. 2014). Using TreeAnnotator 2.4.4 (Drummond et al. 2012, Bouckaert et al. 2014) and a burn-in of 20%, we estimated a posterior distribution of topologies and the maximum clade credibility (MCC) tree.

Morphometric data

We measured 6 morphological variables (wing length, tail length, tarsus length, bill length from nostril to tip, bill gape, and bill depth at nostrils) from 73 study specimens of all subspecies of *Grallaricula ferrugineipectus* (Supplemental Material S2) following Baldwin et al. (1931). We measured 9 specimens of *G. f. ferrugineipectus* (including 6 from the Sierra Nevada de Santa Marta), 6 from the Cauca Valley of Colombia, 10 from *G. f. rara*, and 48 of *G. f. leymebambae*, including one from north of the Marañón Valley. All measurements were taken to the nearest 0.01

mm with a Mitutoyo Digimatic Point Caliper by GAB.

Audio recordings

We compiled recordings of *Grallaricula ferrugineipectus* from the Macaulay Library at the Cornell Lab of Ornithology (macaulaylibrary.org), xeno-canto (xeno-canto.org), the natural sound collection of Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (humboldt.org.co), and private collections of recordists (Supplemental Material S3). We retained recordings that contained high-quality examples of the species' song. Variation in the song of an individual is present in subspecies but is relatively small (e.g., Bard et al. 2002, Kirschel et al. 2011); we confirmed that individual variation was minor (at least within a single recording session) by manually inspecting vocalizations of the same individual. We selected one song per individual for further analysis, typically the best-recorded song in a series or the one with the least background noise. From these recordings, we obtained 110 songs from as many individuals (Fig. 1): 49 of *G. f. ferrugineipectus* (including 13 from the Sierra Nevada de Santa Marta), 4 of *G. f. rara* from the Eastern Andes of Colombia, 5 from the Cauca Valley in the Colombian Central Andes, 12 from northwest Ecuador, 4 from the Andes between the Marañón River and central Ecuador, and 36 of *G. f. leymebambae* south of the Marañón River. To compare vocal variation within the *G. ferrugineipectus* complex to closely related species, we included 7 songs from *G. nana* and 6 from *G. lineifrons* in our analysis, but we did not include *G. flavirostris* because the song of this species seems to consist of only one note and thus cannot be meaningfully compared to the other species, which all have multinote songs.

Acoustic data processing

We used Raven Pro 1.5 (Bioacoustics Research Program 2014) to measure the acoustic properties of vocalizations. *Grallaricula* songs are typically composed of a series of repeated structurally simple notes (Greeney 2013). It is therefore straightforward to capture acoustic variation of *Grallaricula* vocalizations with a series of acoustic measurements. For each song, we manually identified its constituent notes by drawing selec-

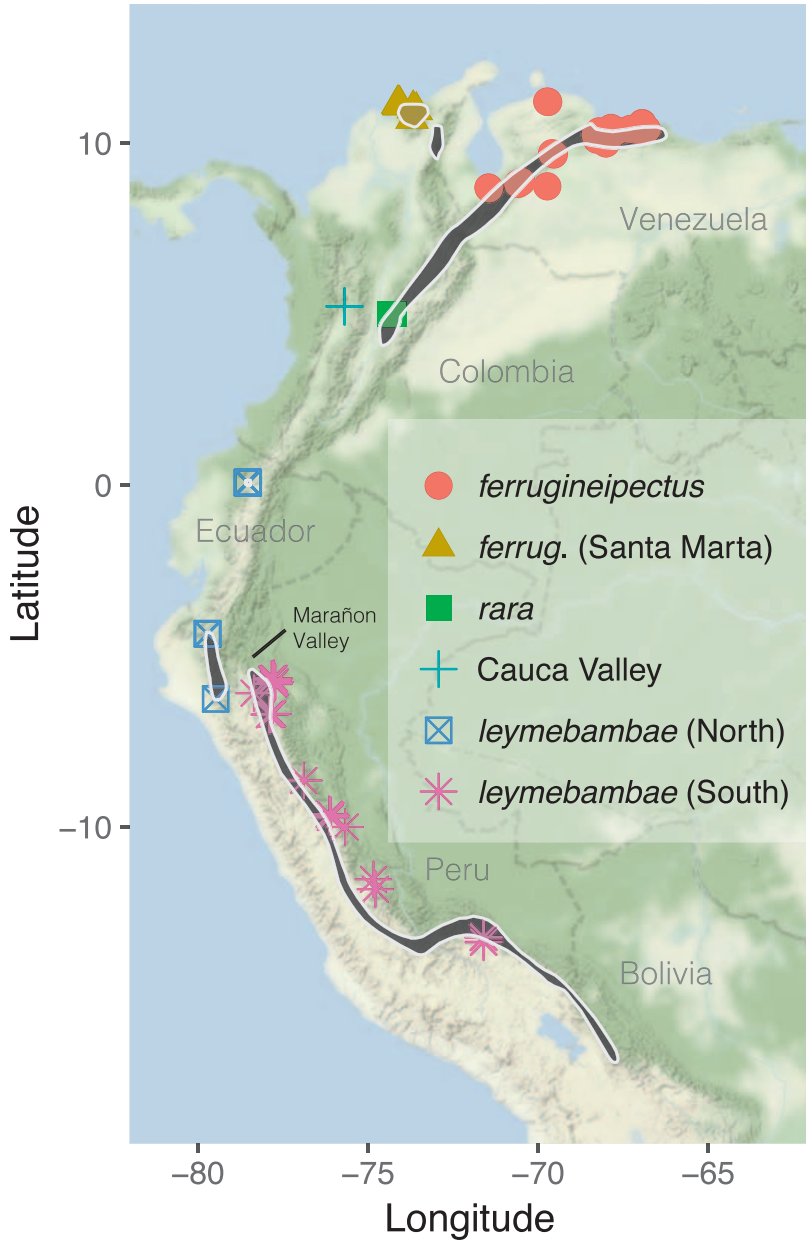


Figure 1. Locations of audio recordings included in this study, colored by population. The range map of this species from BirdLife International (BirdLife International and NatureServe 2015) is indicated in black. Note this map is not fully accurate (recordings exist from sites outside the indicated range).

tion boxes. We then used the Raven software to record the following measurements for each note: the frequency of peak power, the minimum and maximum frequencies of the peak frequency contour, the average slope of the peak frequency

contour, the duration containing 90% of the energy of the note, and the bandwidth containing 90% of the energy of the note. We summarized these note measurements using the following 19 statistics for each song: (1) mean note slope (Hz/ms), (2) mean

note peak frequency (Hz), (3) mean note bandwidth (Hz), (4) mean note peak frequency bandwidth (Hz), (5–6) mean note maximum and minimum frequencies (Hz), (7) number of notes per song (note count), (8) the duration of the song (s), (9) the mean duration of each note (s), (10) the rate of note delivery (note rate or song pace; notes per second), (11) the frequency slope of the song (Hz per note), (12) the song peak frequency bandwidth (Hz), (13–14) the position of the frequency minima and maxima within each note of the song (proportion of note from 0 to 1), (15) the change in note pacing through the song (s per note), (16) the maximum frequency of the song (Hz), (17) the minimum frequency of the song (Hz), (18) the difference between the two (song bandwidth; Hz), and (19) and the position of the maximum frequency note in the song (proportion of song from 0 to 1). We subsequently examined measurements for collinearity and found that measurements 2, 5, 6, 16, and 17 were closely correlated. Of these, we retained only mean note maximum frequency (5) because it showed the highest correlations with the other frequency variables (all $r > 0.95$).

We included recordings made following the use of conspecific playback in our dataset. Although most recordings did not have associated metadata describing whether they followed playback or not, the vast majority of recordings with such metadata were made using playback (see Supplemental Material S3). We therefore have too few recordings made without playback for each taxon to investigate a possible effect of its use at this time; however, we believe the use of playback is unlikely to affect our conclusions regarding acoustic differentiation for 3 reasons. First, the use of playback was relatively consistent among subspecies. Second, we have no a priori reason to believe that playback differentially influences different subspecies. Third, even if playback does induce vocal differences, including recordings made both with and without playback provides a fuller range of vocal variation of each population (i.e., calmer and more agitated individuals), which is a statistically conservative approach to assessing diagnosability.

Statistical analyses

We conducted several analyses to quantify levels of differentiation and diagnosability in

acoustic and morphological traits in our sample. We began by examining differentiation between the northern and southern groups, which have been proposed to represent different species (e.g., Ridgely and Tudor 2009). We first tested for differences in means of vocal and morphological traits using Tukey tests to correct for multiple comparisons. We applied a log transformation when it reduced the skewness of the given trait distribution. We then calculated 95% prediction intervals for all traits, which estimate the spread of observations in a population; non-overlapping prediction intervals indicate that trait distributions are unlikely to overlap even as a larger sample is gathered (Isler et al. 1998). Species delimitation criteria derived for another suboscine group, the antbirds, suggest that allopatric populations should be classified as distinct biological species when they have diagnosable (non-overlapping) differences in at least 3 independent vocal traits (Isler et al. 1998, Isler et al. 2007a, Isler et al. 2007b, Isler et al. 2008). This yardstick approach has also been used in antpittas (Donegan 2008), but its efficacy has not been widely evaluated in the Grallariidae (e.g., by systematically analyzing existing sympatric species pairs). As acknowledged by its proponents, this yardstick approach as a point of reference (Isler et al. 1998) can be relaxed in certain cases, especially when taxa are parapatric or show distinct morphological differentiation (Isler et al. 2007b, Isler et al. 2012).

As an additional measure of diagnosability, we conducted discriminant function analyses on the above groupings. We scaled and centered our data before analysis by subtracting the mean and dividing by the standard deviation. We quantified diagnosability with a cross-validation approach: We withheld one datum, trained the model with the remaining data, and then asked the model to classify the withheld datum. We repeated this procedure 1,000 times and then calculated a misclassification rate for each taxonomic grouping. We analyzed vocal and morphological data separately because our vocal and morphological measurements were made on different individuals.

In addition, we tested for geographic variation within northern and southern groups in vocal and morphological traits. Within the northern group, we examined 4 subpopulations: (1) *G. f. ferrugineipectus* from Venezuela, (2) *G. f. ferrugineipectus* from the Sierra Nevada de Santa Marta, (3) *G.*

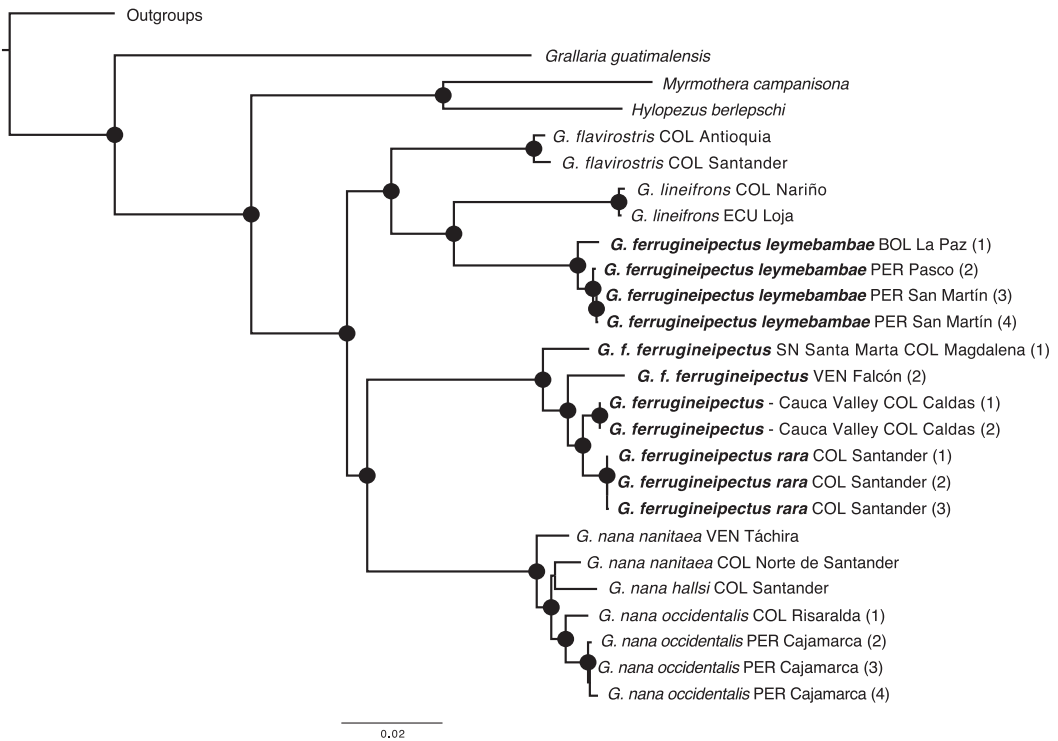


Figure 2. Maximum-likelihood phylogeny of a subset of the Grallariidae. Note that *Grallaricula ferrugineipectus* sensu lato (in bold) is polyphyletic. Black circles at nodes indicate bootstrap support values >70 based on 999 maximum-likelihood replicates.

f. rara from the Eastern Andes of Colombia, and (4) the population from the Cauca Valley of the Colombian Central Andes. We treated the population from the Sierra Nevada de Santa Marta as a distinct group to test for differentiation because of its isolated distribution. Within the southern group, we considered 2 subgroups: (1) antpittas from Ecuador and northwestern Peru, which are vocally similar (P. Coopmans, unpubl. data), hereafter ‘northern *leymeambae*’; and (2) antpittas from northeastern Peru south (hereafter ‘southern *leymeambae*’). These groups are divided by the Marañón River, an important isolating barrier for Andean avifauna (Winger and Bates 2015). We primarily used discriminant function analyses as described above, but we also conducted tests of the ‘75% rule,’ which is satisfied if 75% of the trait values for a population lie outside 99% of the trait values of the other population (Patten et al. 2002). This test has previously been used as a criterion for subspecies classification in the genus *Grallaricula* (Donegan 2008).

Results

Phylogenetic analyses

Maximum-likelihood and Bayesian analyses produced identical topologies supporting the non-monophyly of *Grallaricula ferrugineipectus*. Northern populations (i.e., *G. f. ferrugineipectus*, *G. f. rara*, Cauca Valley, and Sierra Nevada de Santa Marta) form a strongly supported clade that is sister to Andean populations of *G. nana* (albeit with low support in the Bayesian species tree), whereas populations from Peru and Bolivia (i.e., *G. f. leymeambae*) are recovered as sister to *G. lineifrons* (Fig. 2, 3). The time-calibrated species tree estimated that the most recent common ancestor between northern and southern groups occurred between 10.8 and 16.8 million years ago (mya; Fig. 3). Additionally, northern populations of *G. ferrugineipectus* exhibit some degree of geographic structure and differentiation not entirely consistent with current subspecific boundaries. Further insights regarding taxonomic

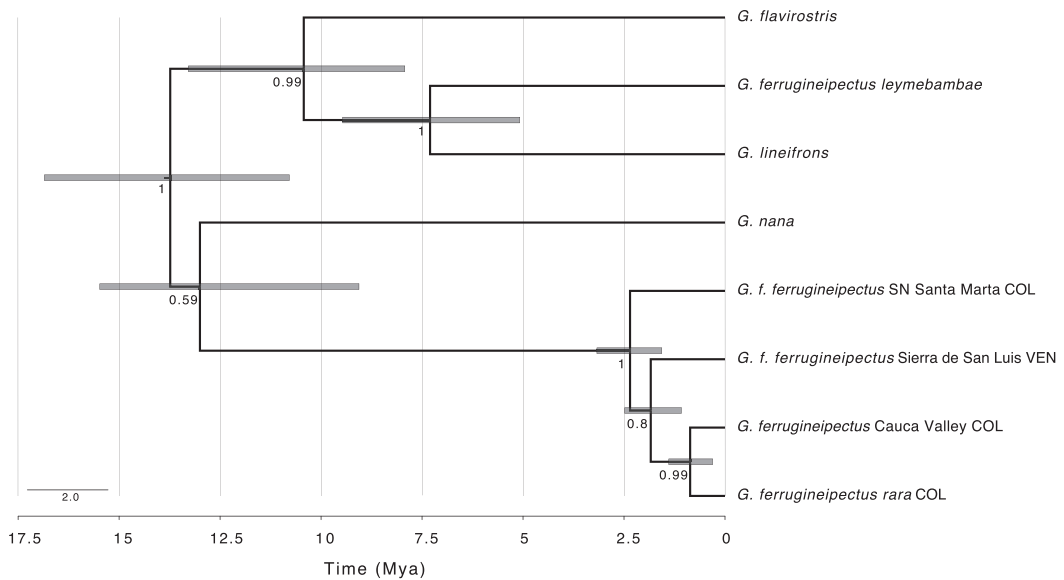


Figure 3. Bayesian estimate of phylogenetic relationships and divergence times among a subset of the Grallariidae. *Grallarica ferrugineipectus* sensu lato is polyphyletic; the northern and southern groups (*G. f. ferrugineipectus* and *G. f. leymebambae*) last shared a common ancestor around 13 million years ago (mya). Bars at nodes indicate the 95% highest posterior density for the inferred divergence time estimates. Numbers at nodes indicate posterior probability support values.

limits of northern populations and their close relationship with *G. nana* will be published elsewhere.

Overall vocal variation

Northern and southern groups show strong divergence in song (Fig. 4). To visualize these differences, we plotted the first 2 factors of a discriminant function analysis run on all populations (Fig. 5). The first factor, which explained 86.7% of between-group variance, loaded strongly for mean note maximum frequency and song pace (Fig. 6). The second factor, which explained 9.3% of the variance, was primarily composed of the number of notes per song and additional variation in song pace (Supplemental Table S1).

Overall morphological variation

We visualized divergence in morphometrics as for vocal variation (Fig. 7). The first factor explained 85.6% of the variance between measured populations and loaded strongly for tarsus, tail length, and wing length. The second factor, which explained 9.3% of the variance, was primarily composed of bill length and gape (Supplemental Table S2).

Tests of species rank

Northern and southern groups significantly differed in the mean values of 13 of 15 vocal traits (Supplemental Fig. S1); however, mean note maximum frequency was the only vocal character for which 95% prediction intervals did not overlap (Table 1). To place this result into context, we considered the number of vocal characters for which 95% prediction intervals did not overlap between currently recognized *Grallarica* species. The southern group differed from *G. nana* in only one vocal character (song pace), whereas populations in the northern group differed from *G. nana* in 3 characters: mean note maximum frequency, the frequency slope of the song, and the position of the maximum frequency in the song. Both northern and southern groups differed from *G. lineifrons* by several vocal traits (7 and 4, respectively), and *G. nana* differed from *G. lineifrons* by 8 vocal traits (Table 1). Although northern and southern groups differed significantly from one another in the mean value of all 6 morphological characters (Supplemental Fig. S2), none was diagnosable at the 95% prediction level.

Discriminant function analysis performed well at separating northern and southern groups based

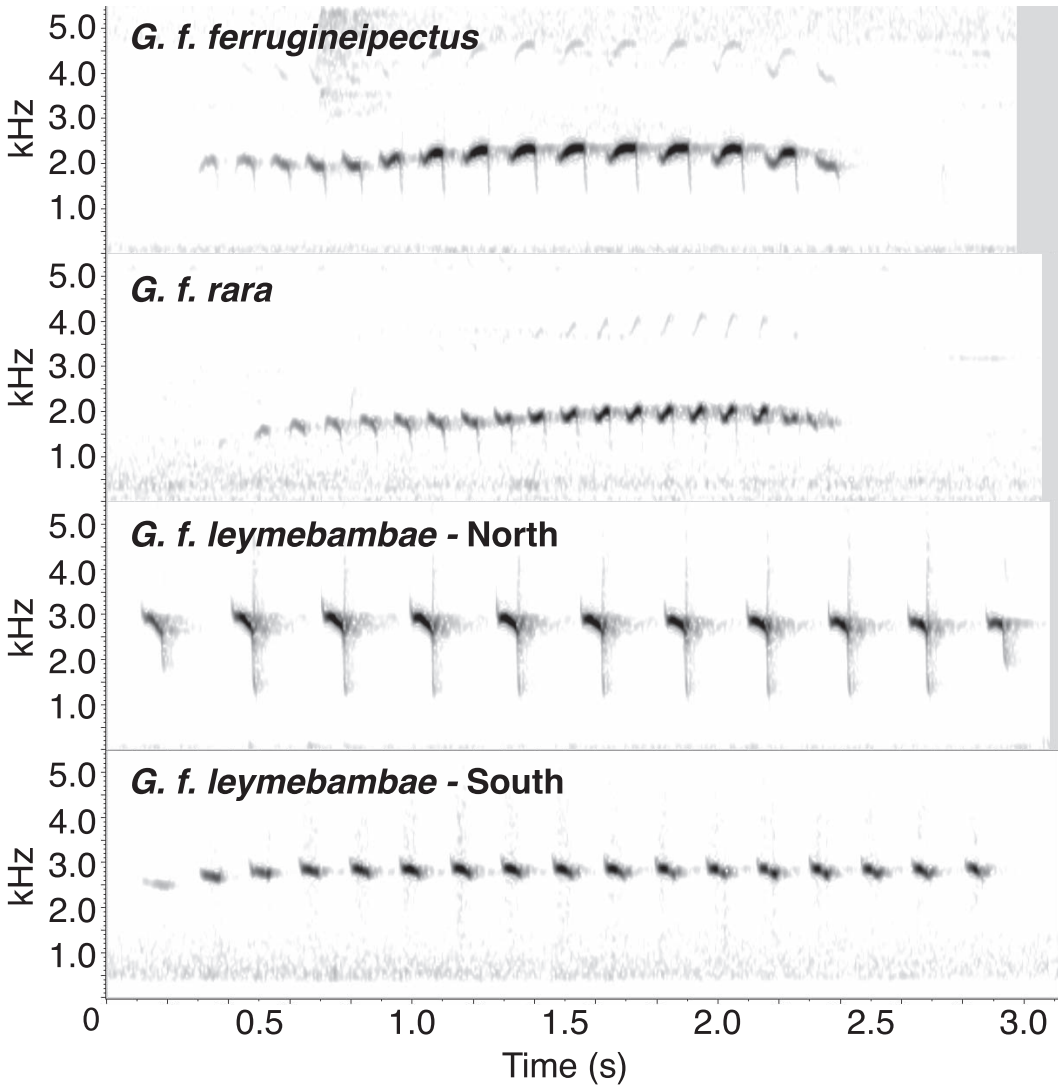


Figure 4. Representative spectrograms of Rusty-breasted Antpitta songs. Spectrograms were made in Raven Pro 1.5 with the following parameters: Hann windows of size 553 samples; hop size 277 samples; DFT size 1024 samples.

on both vocal and morphological traits. For vocal traits, the cross-validated correct classification rate was 100% for the northern group and 97.6% for the southern group. The single discriminant factor loaded most heavily for mean note maximum frequency (Supplemental Table S3). For morphological traits, the cross-validated correct classification rate was 95.5% for the northern group and 100% for the southern group; the discriminant factor was primarily composed of tarsus and tail length (Supplemental Table S4).

Geographic variation within the northern group

Populations in the northern group exhibited significant variation in mean vocal trait values, with 12 of 15 traits showing significant differences among populations (Supplemental Fig. S3); however, discriminant analysis showed a relatively poor ability to distinguish northern populations based on vocal traits. The most distinctive populations were *G. f. ferrugineipectus* from Venezuela and the population from the Cauca

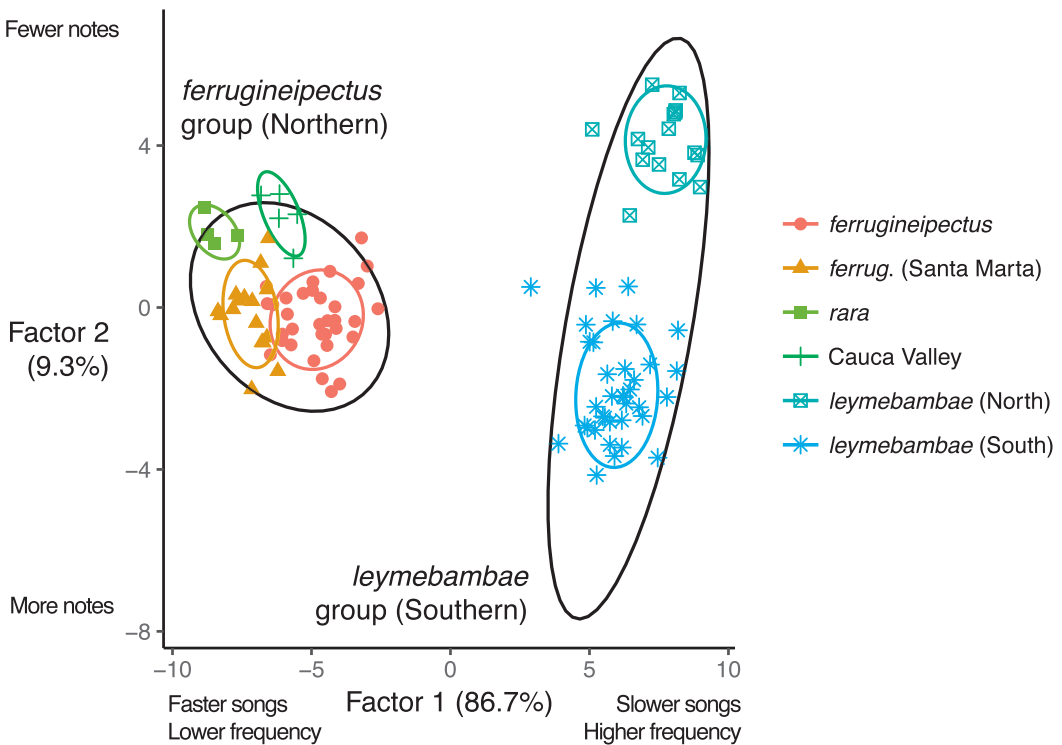


Figure 5. First 2 factors from discriminant function analysis separating Rusty-breasted Antpitta subpopulations by variation in 15 vocal traits. Black ellipses are 95% prediction ellipses for northern and southern groups; colored ellipses are 75% prediction ellipses for subspecies.

Valley of Colombia, which were classified with 85.1% and 82.6% accuracy, respectively. Songs from the Sierra Nevada de Santa Marta were classified with 78.0% accuracy and songs from the subspecies *rara* with 76.9% accuracy. The first discriminant factor (72.4% of variance) loaded strongly for song pace and number of notes, and the second (19.1%) for additional variation in song pace (Supplemental Table S5). By the 75% rule, these 4 populations were generally not diagnosable; *G. f. rara* from the Eastern Andes of Colombia differed from *G. f. ferrugineipectus* from Venezuela in song pace, but this was the only difference among northern populations.

Populations from the northern group showed significant differences in mean values of 4 morphological traits (Supplemental Fig. S4), but there were no differences at the level of the 75% rule. The discriminant analysis of morphological traits performed poorly at distinguishing *G. f. ferrugineipectus* from Venezuela (20.9% correct), *G. f. rara* (51.1% correct), and individuals from

the Cauca Valley (65% correct; Supplemental Table S6), whereas those from the Sierra Nevada de Santa Marta achieved a correct classification rate of 76.8%. Note, however, that the sample sizes for these groups were generally small ($n = 3-8$).

Geographic variation within the southern group

Southern Rusty-breasted Antpitta populations also varied in mean vocal trait values, with significant differences between northern and southern *leymeambae* in 8 of 15 traits (Supplemental Fig. S5). Discriminant analysis performed well in distinguishing the 2 groups, with cross-validated correct classification rates of 95.5% and 97.2% for northern and southern *leymeambae*, respectively. The single discriminant factor (100% of variance) loaded strongly for the number of notes per song, song duration, and song pace (Supplemental Table S7). Considering the 75%

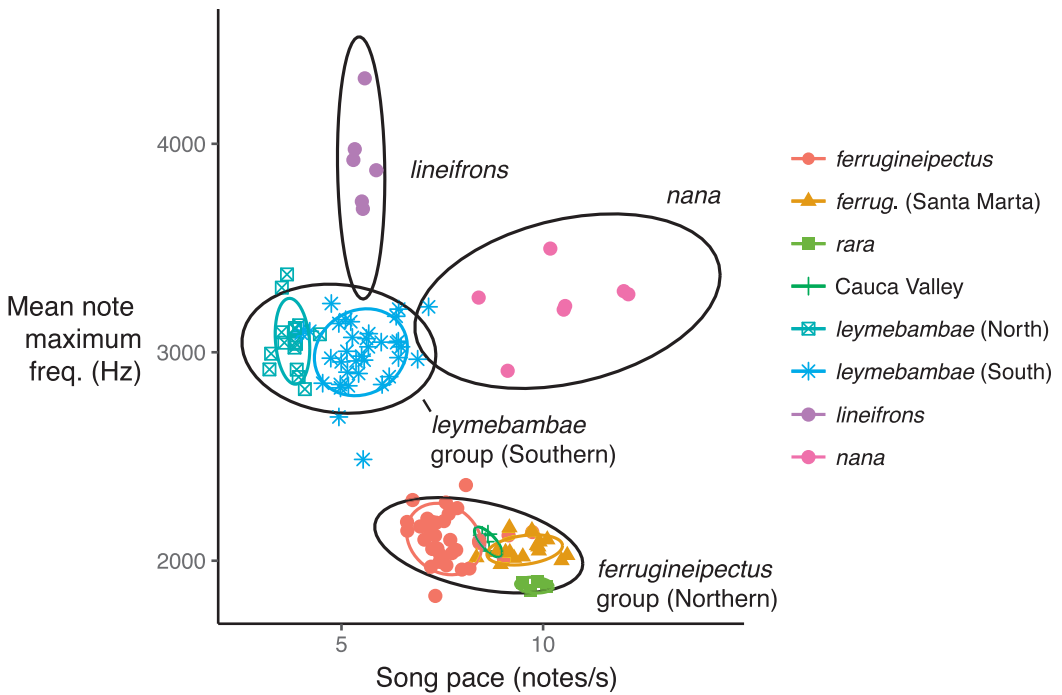


Figure 6. Mean note maximum frequency vs. note rate for all Rusty-breasted Antpitta populations and 2 closely related species, *G. nana* and *G. lineifrons*. These 2 variables generally showed the greatest differentiation across Rusty-breasted Antpittas. Black ellipses are 95% prediction ellipses and colored ellipses are 75% prediction ellipses.

rule, however, the groups showed no diagnosable vocal differences. Morphological tests were not conducted because only one individual was measured from the northern *leymebambae* group.

Discussion

We find that *Grallaricula ferrugineipectus*, as currently recognized, is polyphyletic. The southern subspecies *G. f. leymebambae* is more closely related to *G. lineifrons* and *G. flavirostris* than it is to the northern subspecies *G. f. ferrugineipectus* and *G. f. rara*; in turn, these northern populations are more closely related to *G. nana* than to *G. f. leymebambae*. In fact, the split between the northern and southern clades likely represents the earliest divergence within the genus *Grallaricula* (Fig. 2; GAB unpubl. data), and the age of this split is close to the start of diversification of the *Hylopezus-Myrmothera-Grallaricula* clade, estimated at ~13–21 mya (Ohlson et al. 2013). Hence, *G. ferrugineipectus* as currently defined is polyphyletic and comprises populations that belong to

divergent and distinctive clades. We therefore propose to elevate *G. f. leymebambae* to species rank. We recommend the complex be considered to consist of 2 species and, provisionally, 2 subspecies:

- Species *Grallaricula ferrugineipectus* (Sclater 1857)
- Subspecies *Grallaricula f. ferrugineipectus* (Sclater 1857)
- Subspecies *Grallaricula f. rara* Hellmayr and Madarász 1914
- Species *Grallaricula leymebambae* Carriker 1933

Hereafter, we refer to these taxa by the above naming scheme.

Taxonomic status and nomenclature of the populations in the Cauca Valley of Colombia and north of the Maraón River in Peru and Ecuador will be assessed elsewhere. Here, they are maintained as part of *G. ferrugineipectus* and *G. leymebambae*, respectively, with no defined sub-specific ascription.

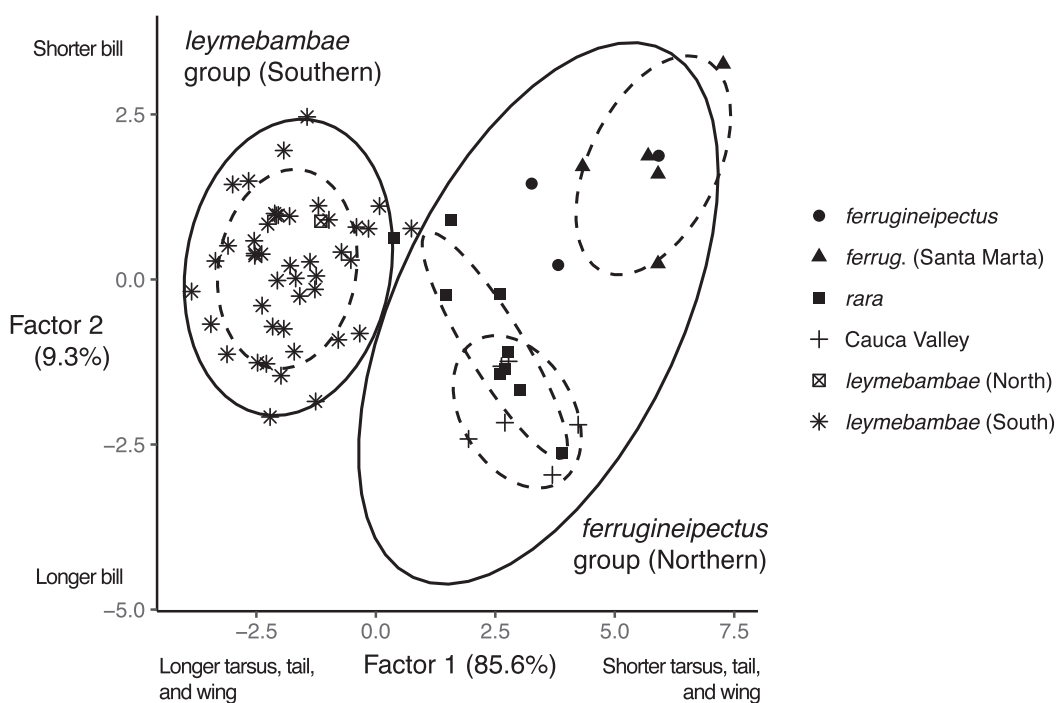


Figure 7. First 2 factors from discriminant function analysis separating Rusty-breasted Antpitta subpopulations by variation in 6 morphological traits. Black ellipses are 95% prediction ellipses for northern and southern groups; colored ellipses are 75% prediction ellipses for subpopulations.

Even though the split between *G. ferrugineipectus* and *G. leymeimbambae* was one of the earliest divergence events in *Grallaricula*, these allopatric taxa were diagnosable at the 95% prediction level in only one measured vocal trait, the mean note maximum frequency, and in no measured morphological traits. In addition, *G. leymeimbambae* differed from *G. nana* by only one vocal character, song pace. By contrast, *G. ferrugineipectus* differs from *G. nana* by 3 vocal characters. A point of reference of diagnosability in at least 3 vocal traits for species status has been frequently used in

suboscine taxonomy, traditionally with antbirds (Isler et al. 1998, Isler et al. 2007a, Isler et al. 2007b, Isler et al. 2008), but also in antpittas (Donegan 2008). The results of the present study suggest that this framework, which does not consider how multiple traits covary among populations, may be conservative when applied to allopatric antpitta taxa. For example, the northern and southern groups did not differ in song pace at the 95% prediction level, but the combination of song pace and note frequency yielded fully distinguishable groups (Fig. 5, 6).

Table 1. Vocal traits that differ between northern and southern Rusty-breasted Antpittas and 2 congeners following the 95% prediction interval test. Numbers refer to the following traits: (1) mean note peak frequency bandwidth (Hz); (2) mean note bandwidth (Hz); (3) mean note maximum frequency (Hz); (4) duration of song (s); (5) mean duration of each note (s); (6) rate of note delivery (notes per s); (7) frequency slope of the song (Hz per note); (8) song peak frequency bandwidth (Hz); (9) song bandwidth (Hz); and (10) position of the highest frequency note.

	<i>G. ferrugineipectus</i> (North)	<i>G. nana</i>	<i>G. lineifrons</i>
<i>G. ferrugineipectus</i> (South)	3	6	3, 7, 8, 9
<i>G. ferrugineipectus</i> (North)		3, 7, 10	3, 4, 5, 6, 7, 8, 9
<i>G. nana</i>			1, 2, 4, 5, 6, 7, 8, 10

Further assessment of the three-trait rule is still required for the Grallariidae. We emphasize that, although diagnosably different in only a single measured vocal trait, songs from northern and southern groups were fully distinguishable in our multivariate discriminant function analyses. Other differences may also be present in calls, which we did not examine here.

Divergence in vocal traits can in part be a by-product of changes in body size, with larger birds emphasizing lower-pitched sounds (Ryan and Brenowitz 1985, Martin et al. 2011). The primary vocal difference between *G. ferrugineipectus* and *G. leymeambae* was in frequency, with *ferrugineipectus* singing lower-pitched songs. Morphological measurements (tarsus, tail, and wing), however, showed that *ferrugineipectus* were generally smaller overall, a difference in the opposite direction than that expected by body size alone. This inconsistency suggests that the primary vocal difference between these groups may be due to selection (e.g., linked to habitat or sexual selection).

We conducted additional analyses to investigate variation in vocalizations and morphology within *G. ferrugineipectus* and *G. leymeambae*. As expected, levels of differentiation were generally lower within than between these groups. We found that northern populations could be vocally distinguished with moderately high accuracy (>75%), but none could be reliably classified with morphological measurements. By the 75% rule, none of these groups showed consistent differences in vocal or measured morphological traits. The small sample sizes of *G. f. rara* and Cauca Valley songs make it difficult to reliably evaluate vocal differences for these groups. Overall, moderate vocal differentiation exists across northern populations, but measured morphological variation is less evident. We note that, although some of these populations (e.g., *G. f. rara*) show divergent plumage, we did not analyze plumage variation. Interestingly, *rara* did not show particularly distinct vocalizations, despite distinctive plumage.

We documented moderate vocal differentiation in *Grallaricula leymeambae*, with northern and southern *leymeambae* distinguishable with 95–97% accuracy. Song pace and number of notes were the most important distinguishing variables; southernmost birds sang almost twice as fast as did

the northernmost birds. This trait seems to vary clinally, with no evidence for distinct jumps in trait value with latitude (Fig. 8). Although previous work has documented clinal variation in morphology in tropical Andean birds (e.g., Graves 1991), examples of clines in suboscine song traits has seldom been noted (but see Isler et al. 2005, who demonstrated clinal variation in song pace in the Variable Antshrike [*Thamnophilus caerulescens*]). Given that vocal traits are presumed to be innate in suboscines, observed vocal clines should reflect underlying genetic clines in the gene(s) and/or regulatory regions that influence the clinal vocal trait (see Isler et al. 2005), and they potentially offer a case example to investigate the genetic basis of suboscine song. Additional potential barriers to dispersal exist, however, through the species' range (e.g., the Huallaga and Apurímac valleys; Winger et al. 2015), and further research is necessary to rigorously evaluate whether latitudinal patterns in song pace in the southern population are truly clinal or instead show a step-wise pattern of variation.

Our results support the well-documented role of Andean geographic features (i.e., valleys and ridges) in driving and maintaining population structure of Andean birds (e.g., Gutiérrez-Pinto et al. 2012, Benham et al. 2015, Winger and Bates 2015). Specifically, features such as the Marañón valley in northern Peru and the Cauca and Magdalena valleys in the Northern Andes have been widely supported as primary barriers to dispersal in the Andes (Vuilleumier 1969, Parker et al. 1985, Fjeldså and Krabbe 1990, Weir 2009, Cuervo 2013, Winger and Bates 2015). These valleys act as pronounced barriers for antpittas and have likely shaped observed patterns of phylogeographic structure within both *G. ferrugineipectus* and *G. leymeambae*. Note also that the apparent disjunct range of *G. ferrugineipectus* sensu lato, with a large gap in distribution between northern Ecuador and central Colombia, is an artifact of taxonomic bias. As this study shows, the populations to the north and south of this 'gap' (i.e., *G. ferrugineipectus* and *G. leymeambae*) are not closely related. In fact, the gap is largely occupied by their respective sister taxa, *G. nana* and *G. lineifrons*. Truly disjunct ranges in Neotropical mountain birds are uncommon, and such distributions may indicate a need for taxonomic revision.

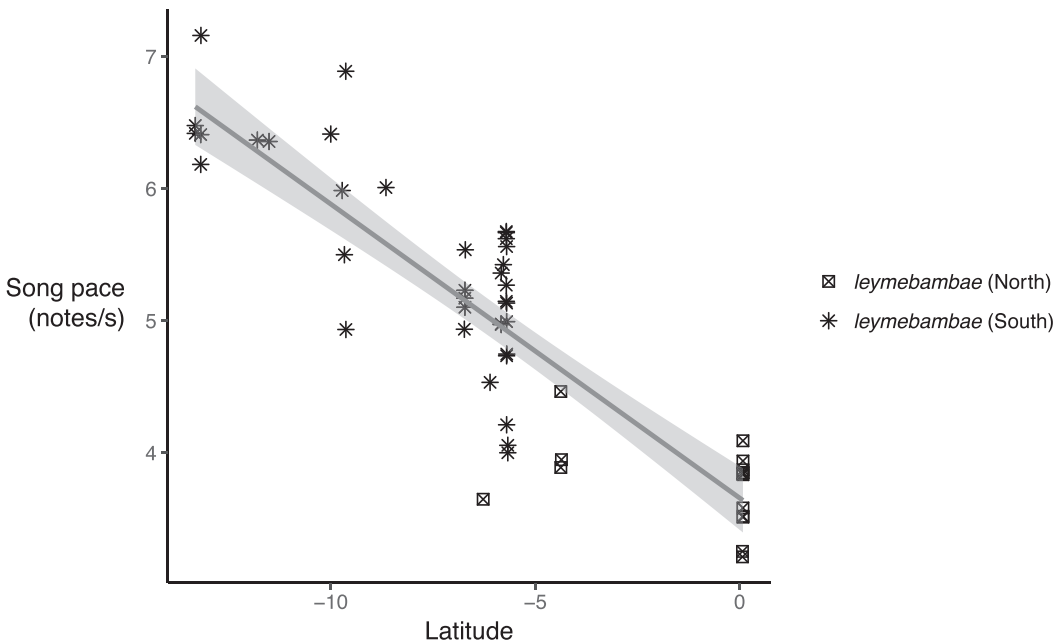


Figure 8. Song pace vs. latitude for southern Rusty-breasted Antpittas.

In sum, we demonstrate that northern and southern populations of *Grallarica ferrugineipectus* sensu lato are (1) deeply divergent genetically and not even sister lineages, (2) differ markedly in song (particularly in song pace), and (3) are morphologically divergent. As such, these 2 groups merit classification as distinct biological species. We further document variation within each group, laying the groundwork for future taxonomic assessments. In particular, further work could incorporate both genetic data and playback experiments to “ask the birds themselves” if the differences in note shape and pacing between southern populations are sufficient to generate pre-mating reproductive isolation based on voice (e.g., Seddon and Tobias 2007, Zimmer 2008, Areta and Pearman 2009, Pegan et al. 2015).

Acknowledgments

This analysis would not have been possible without the efforts of many recordists who have contributed their field recordings of Rusty-breasted Antpitta songs to the Macaulay Library of Natural Sounds and xeno-canto.org and provided private recordings, including: R. Ahlman, F. Angulo, J. Areta, N. Athanas, R. Behrstock, P. Boesman, P. Coopmans, S. Córdoba, O. Cortés, M. Dehling, F. Deroussen, D. Edwards, D. Geale, C. Hanks, M. Harvey, J. Hornbuckle, R. Innes, J. King, N. Krabbe, F. Lambert, D. Lane, H. Lloyd, L.

Macaulay, H. Matheve, J. McGowan, J. Nilsson, M. Robbins, M. Sarver, F. Schmitt, T. Schulenberg, P. Schwartz, D. Stejskal, A. Spencer, H. van Oosten, T. Valqui, B. Whitney, S. Woods, and K. Zimmer. We thank both these recordists and the curators who maintain these collections. Likewise, we are indebted to curators, collection managers, and staff of museum collections (see Supplemental Materials) for allowing access to study specimens and vouchered tissue samples under their care, particularly to D. López, J.D. Palacios, Ana María Umaña, Fernando Forero, Socorro Sierra, and Diego Perico (IAvH), F.G. Stiles (ICN), Miguel Lentino and Margarita Martínez (COP), and S. Cardiff, D. Dittmann, R.T. Brumfield, and J.V. Remsen Jr. (LSUMNS). We especially thank M.L. Isler and N. Athanas for facilitating the access to recordings in private collections and for feedback on our ideas. P. Salaman assisted in providing an unpublished manuscript by P. Coopmans. We thank T. Schulenberg and three anonymous reviewers for helpful feedback. BVD was supported by the Marshall Aid Commemoration Commission. BGF acknowledges support from the National Science Foundation for Graduate Research Fellowship, Award No. 2011083591 and Postdoctoral Fellowship in Biology, Award No. 1523695. Molecular and morphological work were supported by grants from the LSUMNS Big Day Fund, LSU Biograds, and the National Science Foundation (DEB-1011435) to GAB.

Literature cited

Angulo Pratulongo F, Flanagan JN, Vellinga W-P, Durand N. 2012. Notes on the birds of Laquipampa Wildlife

- Refuge, Lambayeque, Peru. Bulletin of the British Ornithologists' Club. 132:162–174.
- Areta JI, Pearman M. 2009. Natural history, morphology, evolution, and taxonomic status of the Earthreeper *Upucerthia saturator* (Furnariidae) from the Patagonian forests of South America. Condor. 111:135–149.
- Athanas N, Greenfield PJ. 2016. Birds of western Ecuador: A photographic guide. Princeton (NJ): Princeton University Press.
- Baldwin S, Oberholser H, Worley L. 1931. Measurements of birds. Volume II. Cleveland (OH): Scientific Publications of the Cleveland Museum of Natural History.
- Bard SC, Hau M, Wikelski M, Wingfield JC. 2002. Vocal distinctiveness and response to conspecific playback in the Spotted Antbird, a Neotropical suboscine. Condor. 104:387–394.
- Benham PM, Cuervo AM, McGuire JA, Witt CC. 2015. Biogeography of the Andean metalltail hummingbirds: Contrasting evolutionary histories of tree line and habitat-generalist clades. Journal of Biogeography. 42:763–777.
- Bioacoustics Research Program. 2014. Raven Pro: Interactive sound analysis software (Version 1.5). Ithaca (NY): Cornell Lab of Ornithology.
- BirdLife International. 2017. Species factsheet: *Grallarica leymebambae*. <http://www.birdlife.org/>
- BirdLife International and NatureServe. 2015. Bird species distribution maps of the world. Version 5.0. BirdLife International, Cambridge (UK), and NatureServe, Arlington (VA), USA.
- Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, et al. 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. PLoS Computational Biology. 10:e1003537.
- Brumfield RT, Edwards SV. 2007. Evolution into and out of the Andes: A Bayesian analysis of historical diversification in *Thamnophilus* antshrikes. Evolution. 61:346–367.
- Brumfield RT, Tello JG, Cheviron Z, Carling MD, Crochet N, Rosenberg KV. 2007. Phylogenetic conservatism and antiquity of a tropical specialization: Army-ant-following in the typical antbirds (Thamnophilidae). Molecular Phylogenetics and Evolution. 45:1–13.
- Carrker MA. 1933. Descriptions of new birds from Peru, with notes on other little-known species. Proceedings of the Academy of Natural Sciences of Philadelphia. 85:1–38.
- Cuervo AM. 2013. Evolutionary assembly of the Neotropical montane avifauna [dissertation]. Baton Rouge (LA): Louisiana State University.
- Donegan TM. 2008. Geographical variation in Slate-crowned Antpitta *Grallarica nana*, with two new subspecies from Colombia and Venezuela. Bulletin of British Ornithology. 128:150–178.
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution. 29:1969–1973.
- Fjeldså J, Krabbe N. 1990. Birds of the high Andes: A manual to the birds of the temperate zone of the Andes and Patagonia, South America. Copenhagen (Denmark): University of Copenhagen, Zoological Museum.
- Graves GR. 1991. Bergmann's rule near the equator: Latitudinal clines in body size of an Andean passerine bird. Proceedings of the National Academy of Sciences. 88:2322–2325.
- Greeney HF. 2013. Rusty-breasted Antpitta (*Grallarica ferruginepectus*). In: Schulenberg TS, editor. Neotropical birds online. Ithaca (NY): Cornell Lab of Ornithology.
- Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Systematic Biology. 59:307–321.
- Gutiérrez-Pinto N, Cuervo AM, Miranda J, Pérez-Emán JL, Brumfield RT, Cadena CD. 2012. Non-monophyly and deep genetic differentiation across low-elevation barriers in a Neotropical montane bird (*Basileuterus tristriatus*; Aves: Parulidae). Molecular Phylogenetics and Evolution. 64:156–165.
- Hellmayr E, Madarász J. 1914. Description of a new Formicarian-bird from Columbia. Annales Musei Nationalis Hungarici. 12:88.
- Hilty SL, Brown WL. 1986. A guide to the birds of Colombia. Princeton (NJ): Princeton University Press.
- Hurvich CM, Tsai C-L. 1989. Regression and time series model selection in small samples. Biometrika. 76:297–307.
- Isler ML, Cuervo AM, Bravo GA, Brumfield RT. 2012. An integrative approach to species-level systematics reveals the depth of diversification in an Andean Thamnophilid, the Long-tailed Antbird. Condor. 114:571–583.
- Isler ML, Isler PR, Whitney BM. 1998. Use of vocalizations to establish species limits in antbirds (Passeriformes: Thamnophilidae). Auk. 115:577–590.
- Isler ML, Isler PR, Brumfield RT. 2005. Clinal variation in vocalizations of an antbird (Thamnophilidae) and implications for defining species limits. Auk. 122:433–444.
- Isler ML, Isler PR, Whitney BM. 2007a. Species limits in antbirds (Thamnophilidae): The Warbling Antbird (*Hypocnemis cantator*) complex. Auk. 124:11–28.
- Isler ML, Isler PR, Whitney BM, Zimmer KJ. 2007b. Species limits in the “Schistocichla” complex of *Percnostola* antbirds (Passeriformes: Thamnophilidae). Wilson Journal of Ornithology. 119:53–70.
- Isler ML, Isler PR, Whitney BM. 2008. Species limits in antbirds (Aves: Passeriformes: Thamnophilidae): An evaluation of Plumbeous Antvireo (*Dysithamnus plumbeus*) based on vocalizations. Zootaxa. 1726:60–68.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution. 30:772–780.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, et al. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28:1647–1649.

- Kimball RT, Braun EL, Barker FK, Bowie RC, Braun MJ, et al. 2009. A well-tested set of primers to amplify regions spread across the avian genome. *Molecular Phylogenetics and Evolution*. 50:654–660.
- Kirschel ANG, Cody ML, Harlow ZT, Promponas VJ, Vallejo EE, Taylor CE. 2011. Territorial dynamics of Mexican Ant-thrushes *Formicarius moniliger* revealed by individual recognition of their songs. *Ibis*. 153:255–268.
- Krabbe NK, Schulenberg TS. 2003. Rusty-breasted Antpitta (*Grallarica ferruginepectus*). In: del Hoyo J, Elliott A, Sargatal J, Christie DA, de Juana E, editors. *Handbook of the birds of the world alive*. Barcelona (Spain): Lynx Edicions.
- Lanfear R, Calcott B, Ho SY, Guindon S. 2012. Partition-Finder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*. 29:1695–1701.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*. 34:772–773.
- Martin JP, Doucet SM, Knox RC, Mennill DJ. 2011. Body size correlates negatively with the frequency of distress calls and songs of Neotropical birds. *Journal of Field Ornithology*. 82:259–268.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Gateway Computing Environments Workshop (GCE)*, 2010. IEEE. p. 1–8.
- Niklison AM, Areta JI, Ruggera RA, Decker KL, Bosque C, Martin TE. 2008. Natural history and breeding biology of the Rusty-breasted Antpitta (*Grallarica ferruginepectus*). *Wilson Journal of Ornithology*. 120:345–352.
- Ohlson JI, Irestedt M, Ericson PG, Fjeldså J. 2013. Phylogeny and classification of the New World suboscines (Aves, Passeriformes). *Zootaxa*. 3613:1–35.
- Parker T, Schulenberg TS, Graves GR, Braun MJ. 1985. The avifauna of the Huancabamba region, northern Peru. In: Buckley P, Foster M, Morton E, Ridgely R, Buckley F, editors. *Ornithological Monographs*, No. 36: Neotropical Ornithology. Washington (DC): American Ornithologists' Union. p. 169–197.
- Patten MA, Unitt P, Sheldon F. 2002. Diagnosability versus mean differences of Sage Sparrow subspecies. *Auk*. 119:26–35.
- Pegan TM, Rumelt RB, Dzielski SA, Ferraro MM, Flesher LE, et al. 2015. Asymmetric response of Costa Rican White-breasted Wood-wrens (*Henicorhina leucosticta*) to vocalizations from allopatric populations. *PLoS One*. 10:e0144949.
- Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. *Tracer v1.6*. Available from: <http://beast.community/tracer>
- Remsen JV Jr, Areta JI, Cadena CD, Claramunt S, Jaramillo A, et al. 2017. A classification of the bird species of South America. American Ornithologists Union. <http://www.museum.lsu.edu/~Remsen/SACCBaseline.htm>
- Ridgely RS, Tudor G. 2009. *Field guide to the songbirds of South America: The passerines*. Austin (TX): University of Texas Press.
- Ryan MJ, Brenowitz EA. 1985. The role of body size, phylogeny, and ambient noise in the evolution of bird song. *American Naturalist*. 126:87–100.
- Schulenberg TS, Parker TA. 1981. Status and distribution of some northwest Peruvian birds. *Condor*. 83:209–216.
- Schulenberg T, Remsen J. 1982. Eleven bird species new to Bolivia. *Bulletin of the British Ornithologists' Club*. 102:52–57.
- Sclater PL. 1857. Descriptions of twelve new or little-known species of the South American family Formicariidae. In: *Proceedings of the Zoological Society of London*. Wiley Online Library. p. 129–133.
- Seddon N, Tobias JA. 2007. Song divergence at the edge of Amazonia: An empirical test of the peripatric speciation model. *Biological Journal of the Linnean Society*. 90:173–188.
- Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 30:1312–1313.
- Vellinga W-P, Flanagan JN, Mark TR. 2004. New and interesting records of birds from Ayabaca province, Piura, north-west Peru. *Bulletin of the British Ornithologists' Club*. 124:124–142.
- Vuilleumier F. 1969. Pleistocene speciation in birds living in the high Andes. *Nature*. 223:1179–1180.
- Weir JT. 2009. Implications of genetic differentiation in Neotropical montane forest birds. *Annals of the Missouri Botanical Garden*. 96:410–433.
- Weir J, Schluter D. 2008. Calibrating the avian molecular clock. *Molecular Ecology*. 17:2321–2328.
- Winger BM, Bates JM. 2015. The tempo of trait divergence in geographic isolation: Avian speciation across the Marañón Valley of Peru. *Evolution*. 69:772–787.
- Winger BM, Hosner PA, Bravo GA, Cuervo AM, Aristizábal N, et al. 2015. Inferring speciation history in the Andes with reduced-representation sequence data: an example in the bay-backed antpittas (Aves; Grallariidae; *Grallaria hypoleuca* s. l.). *Molecular Ecology*. 24:6256–6277.
- Zimmer KJ. 2002. Species limits in Olive-backed Foliage-gleaners (*Automolus*: Furnariidae). *Wilson Bulletin*. 114:20–37.
- Zimmer KJ. 2008. The White-eyed Foliage-gleaner (Furnariidae: *Automolus*) is two species. *Wilson Journal of Ornithology*. 120:10–25.