REVISING SPECIES LIMITS IN A GROUP OF *MYRMECIZA* ANTBIRDS REVEALS A CRYPTIC SPECIES WITHIN *M. LAEMOSTICTA* (THAMNOPHILIDAE)

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Abstract. The magnitude of differentiation in vocal signals examined in a phylogenetic context has helped uncover cryptic diversity in subsocine passerines such as the antbirds (Thamnophilidae). On the basis of variation in 14 acoustic traits of loudsongs and a phylogenetic hypothesis inferred from a mitochondrial gene tree, we investigated species limits in a group of trans-Andean *Myrmeciza* antbirds with a convoluted taxonomic history (*M. l. laemosticta*, *M. l. palliata*, *M. nigricauda*, and *M. berlepschi*). Diagnostic differences in three vocal traits suggest that the two subspecies of the Dull-mantled Antbird (*M. l. laemosticta* and *M. l. palliata*) should be treated as distinct species. We present evidence that only two diagnosable vocal characters may be sufficient for two populations to be considered distinct species in this group. The vocal results are consistent with the molecular phylogenetic analysis, which revealed a long independent history of isolation of *M. l. laemosticta* from *M. l. palliata*, roughly congruent with the divergence observed between species currently recognized in this assemblage. We propose elevating the Magdalena Antbird (*M. palliata*) to species rank and thus that four species be recognized in the *M. laemosticta* complex.

Key words: antbirds, loudsong, Myrmeciza laemosticta, *neotropical birds*, *species delimitation*, *systematics*, *vocal characters*.

La Revisión de los Límites entre Especies en un Grupo de Hormigueros del Género *Myrmeciza* Revela una Especie Críptica en *M. laemosticta* (Thamnophilidae)

Resumen. La magnitud de la diferenciación en señales vocales examinada en un contexto filogenético ha ayudado a descubrir diversidad críptica en Passeriformes subsoscinos como los hormigueros (Thamnophilidae). Investigamos los límites entre especies en un grupo de hormigueros trans-andinos del género *Myrmeciza* con una historia taxonómica confusa (*M. l. laemosticta*, *M. l. palliata*, *M. nigricauda*, y *M. berlepschi*) con base en la variación de 14 rasgos acústicos de los cantos fuertes y una hipótesis filogenética inferida a partir de un árbol de un gen mitocondrial. La existencia de diferencias diagnósticas en tres rasgos vocales sugieren que las dos subespecies de *M. laemosticta* (*M. l. laemosticta* y *M. l. palliata*) deberían tratarse como especies diferentes. Presentamos evidencia que demuestra que sólo dos características vocales diagnósticas podrían ser suficientes para considerar dos poblaciones como especies distintas en este grupo. Los resultados vocales concuerdan con el análisis filogenético molecular, que reveló una larga historia de aislamiento independiente entre *M. l. laemosticta* y *M. l. palliata* que es congruente con el nivel de divergencia observado entre las especies actualmente reconocidas en este grupo. Proponemos elevar al rango de especie a *M. palliata* y, por tanto, reconocer cuatro especies en el complejo de *M. laemosticta*.

INTRODUCTION

Under the biological species concept, the criterion of reproductive isolation is used to set species limits among populations (Mayr 1963, Coyne and Orr 2004). The implementation of this criterion is relatively straightforward for sympatric populations because interbreeding, or lack thereof, can be observed directly. In contrast, applying the biological species concept to assess the status of allopatric populations is problematic because the degree of reproductive isolation between populations that are physically separated cannot be assessed directly and researchers must rely on inference (Sites and Marshall 2004). Inferences about the degree of reproductive isolation in birds are often based on the magnitude of differentiation in attributes implicated in species recognition, mate choice, or mating compatibilities relative to differentiation observed between pairs of sympatric species (Isler et al. 1998, Helbig et al. 2002, Remsen 2005).

Because vocalizations play a crucial role in mate choice and constitute behavioral isolating barriers in birds (Price 2008), vocal traits are often informative in establishing limits among avian species (Remsen 2005). In particular, variation in acoustic traits of the male's loudsong have proven useful in revisions of the taxonomic rank of allopatric populations of birds with largely innate and simple song types like the typical antbirds (Thamnophilidae). Isler et al. (1998) proposed a method of delimiting species of antbirds based on observations of the degree of vocal differentiation between closely related and coexisting species. They reasoned that because syntopic species in this group have diagnostic differences in three or more acoustic variables of the males' loudsongs (sensu Willis 1967), allopatric populations should remain isolated in the hypothetical case of secondary contact if they have reached an equivalent magnitude of diagnostic differentiation in vocalizations. Thus they proposed a minimum of three diagnostic vocal traits as a "yardstick" for ranking of allopatric populations of antbirds as different species. This approach has been applied to a number of groups in the Thamnophilidae, revealing that traditional taxonomy has substantially underestimated species diversity in this family (Isler et al. 1999, 2001, 2005, 2007a, b, 2008, 2009, Zimmer and Isler 2003, Braun et al. 2005). However, only a handful of such evaluations based on vocal data have been coupled with analyses of genetic variation among relevant populations in a biogeographic context (Brumfield 2005, Braun et al. 2005, Isler et al. 2007b, Tobias et al. 2008).

The genus *Myrmeciza* as currently defined is a polyphyletic assemblage comprising 19 species varying greatly in size, morphology, coloration, and vocalizations (Irestedt et al. 2004, Brumfield et al. 2007, Zimmer and Isler 2003, Moyle et al. 2009, Remsen et al. 2010). One subgroup in this genus includes the Dull-mantled Antbird (*M. laemosticta*), Esmeraldas Antbird (*M. nigricauda*), and Stub-tailed Antbird (*M. berlepschi*), which occur in forested lowlands and foothills west of the Andes in northwestern South America and eastern Central America (Fig. 1). The systematics of this group has received little attention despite its convoluted taxonomic history (Robbins and Ridgely 1991). For instance, prior to being placed in *Myrmeciza*, *M. berlepschi* and *M. nigricauda* were classified in *Cercomacra* (and females of *M. berlepschi* were classified in *Thamnophilus*), then were transferred to the genus *Sipia*, now considered invalid (Robbins and Ridgely 1991). Moreover, the female of *M. nigricauda* was long considered to represent a subspecies of *M. laemosticta* (Robbins and Ridgely 1991). On the basis of similarities of the female plumage, Zimmer and Isler (2003) considered *M. laemosticta* to form a superspecies with *M. nigricauda* and to be closely related to *M. berlepschi*.

Current taxonomy recognizes two allopatric populations of *M. laemosticta* at the subspecies level (Robbins and Ridgely 1991, Zimmer and Isler 2003): the Central American nominate form and the subtly paler South American *M. l. palliata*, which
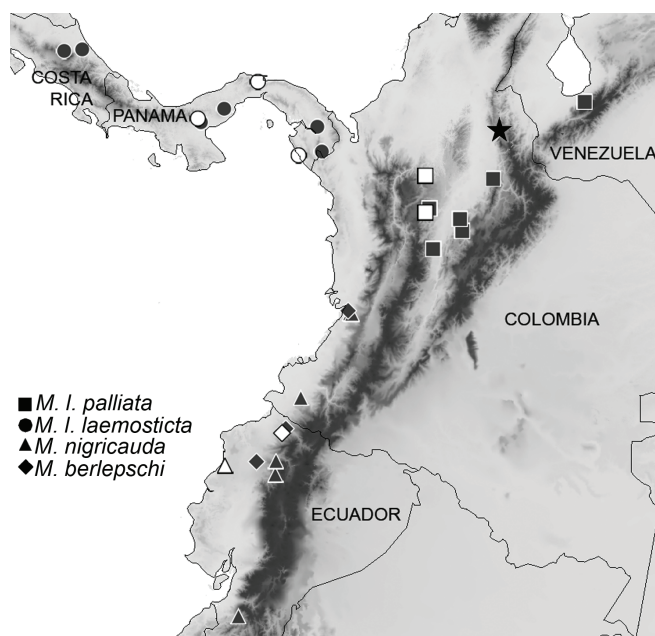

FIGURE 1. Geographic sources of sound recordings (dark symbols) and sequence data (light symbols) of the four taxa of the *Myrmeciza laemosticta* complex. The star represents the type locality of *M. l. palliata*: La Palmita, near Ocaña, Department of Norte de Santander, Colombia.

includes *bolivari* and *venezuelae* synonymized by Robbins and Ridgely (1991). Recent field work in the foothills of the middle Magdalena valley of Colombia revealed that the songs of *M. l. palliata* sound substantially different from those of the Central American *M. l. laemosticta* (Cuervo et al. 2007). These observations raised the question of whether these two taxa deserve species rank. Here, we evaluate species limits in this group of antbirds by integrating the vocal approach of Isler et al. (1998) with molecular phylogenetic data. Specifically, we assess whether *M. l. laemosticta* and *M. l. palliata* are distinct species on the basis of vocal divergence and generate a phylogenetic hypothesis for the group.

METHODS

FIELD SAMPLING

We recorded loudsongs (Willis 1967, Isler et al. 1998) of *M. l. palliata* (Appendix 1) in the middle Magdalena valley of Colombia. Sequences and other recordings already existed in our institutions, were granted by other institutions, or were downloaded from public databases such as www.xeno-canto.org (Appendix 1).

VOCAL ANALYSES

We characterized vocal variation among nominate *M. laemosticta*, *M. l. palliata*, *M. nigricauda*, and *M. berlepschi*. Given its close affinity with this group (see below), we also examined recordings of the Gray-headed Antbird (*M. griseiceps*).

Following the method proposed by Isler et al. (1998), we focused on 12 quantitative and two qualitative vocal characters of males' loudsongs (Appendix 2). We also considered qualitative differences in both calls and females' songs but did not analyze them quantitatively because samples of such vocalizations are scarce. All measurements were made with the program Raven Pro 1.3 (Cornell Laboratory of Ornithology, Ithaca, NY).

For each male recorded, we calculated the mean value of all 12 quantitative variables measured in three bouts of loudsong. To evaluate the difference between any two populations in any of the 12 quantitative variables, we followed the following criteria. First, we considered two continuous and normally distributed variables diagnostic only if their ranges did not overlap and if they met the requirement $x_a + t_a \, \mathrm{SD} \leq x_b - t_b \, \mathrm{SD}$, where x represents the mean and SD the standard deviation of the respective variable, t is the value of t at the 97.5 percentile of the t distribution with $n - 1$ degrees of freedom, n is the number of individuals, and subscripts a and b refer to larger and smaller samples, respectively (Isler et al. 1998). Because all samples were of <30 individuals, we implemented this procedure to assess whether the lack of overlap would remain if sample sizes were larger (Isler et al. 1998).

To evaluate the statistical significance of diagnosability of vocal characters that were not normally distributed, we used a nonparametric bootstrap simulation, following Isler et al. (2007a). The simulation compared the differences between the means of traits of pairs of taxa to a null distribution of differences between means calculated by generating 10 000 sample pairs with replacement. In addition, we ran a discriminant function analysis in the program SPSS 15.0 to determine whether the taxa under study could be diagnosed in a multivariate space (12 variables, Table 1). Finally, we examined qualitative characters through a blind inspection and grouping of printed sonograms followed by an assessment of whether the groupings matched the populations under study.

Diagnostic characters were those unambiguously present in only one population.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Using DNeasy kits (Qiagen, Valencia, CA), we extracted DNA from vouchered tissue samples of *M. l. laemosticta*, *M. l. palliata*, *M. nigricauda*, *M. griseiceps*, and *M. exsul* (Appendix 1). We amplified and sequenced 1041 base pairs of the mitochondrial gene NADH dehydrogenase subunit 2 (ND2) with primers L5216, H6313 (Sorenson et al. 1999) and L5215 (Hackett 1996), and conditions described by Cadena et al. (2007). The sequence of *M. berlepschi* was downloaded from GenBank (EF640029; Brumfield et al. 2007). Sequences were edited and aligned manually with the program Geneious (Drummond et al. 2009) and deposited in GenBank (accession numbers FJ229361–FJ229368, HM486422–HM486425).

PHYLOGENETIC ANALYSIS

We selected taxa for the outgroup (*Terenura sharpei*, *Taraba major*, *Myrmeciza hemimelaena*, and *Hylophylax naevioides*) on the basis of existing phylogenetic analyses of the Thamnophilidae (Irestedt et al. 2004, Brumfield et al. 2007, Moyle et al. 2009). In addition, we included sequences of *M. griseiceps* and *M. exsul* (Chestnut-backed Antbird), as G. Bravo and R. Brumfield (pers. comm.) suggested them to be the closest relatives of the *M. laemosticta* complex. We used maximum likelihood and Bayesian analysis to reconstruct phylogenetic relationships among the 18 sequences of our alignment (Appendix 1).

Maximum-likelihood analyses were run with the program RAxML 7.2.5 on the Cipres Portal version 2.0 (Stamatakis et al. 2008) and the GTR + Γ model of nucleotide substitution. We assessed support for clades on the basis of 10 000 bootstrap replicates. Bayesian analyses were run in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) under the GTR + Γ model selected with the Akaike

TABLE 1. Pairwise diagnosability of loudsongs of antbirds of the *Myrmeciza laemosticta* complex. The number of acoustic variables distinguishing the pair is in parentheses.

	M. l. laemosticta	*M. l. palliata*	*M. nigricauda*
M. l. palliata	(3) Song pace Note structure Change in note structure	—	—
M. nigricauda	(3) Song pace Note structure Change in note structure	(2) Note shape Note structure	—
M. berlepschi	(5) Peak frequency Change in song pace Change in note length Note shape Change in note shape	(3) Change in max. power Note shape Change in note structure	(2) Note shape Change in note shape

information criterion in MrModeltest 2.3 (Nylander 2004). Using one cold and three heated chains for 10 million generations, we ran four replicates, sampling every 1000 generations. We discarded the first two million generations as "burn-in" after finding that stationarity was reached well before this number in each run. To assess the clades' support, we used the remaining 8001 sampled trees to estimate posterior probabilities.

RESULTS

PAIRWISE DIAGNOSABILITY OF LOUDSONGS

We analyzed a total of 144 bouts of loudsong of 42 individuals of *M. l. laemosticta*, *M. l. palliata*, *M. nigricauda*, *M. berlepschi*, and *M. griseiceps* (Appendix 1). Overall, vocal differences among taxa are visually apparent on sonograms of representative loudsongs (Fig. 2). Moreover, we observed diagnostic features in the two qualitative vocal traits (note shape and change in note shape) and in five of the quantitative characters (pace, change in pace, peak frequency, change in note length, and change in maximum power) (Table 2). In the following paragraph, we describe differences between *M. l. laemosticta* and *M. l. palliata* in detail because these taxa were the focus of our taxonomic study. All other pairwise comparisons are described in Appendix 3.

Myrmeciza l. laemosticta and *M. l. palliata* are diagnosable in three vocal characters: song pace, note structure, and change in note structure (Table 1; Figs. 2 and 3). Songs of *M. l. laemosticta* are faster (4.6–5.8 vs. 2.9–3.7 notes sec^{-1}) and consist of two main segments (those of *M. l. palliata* consist of three). In *M. l. laemosticta* the notes of the first segment are rounded and partially modulated; in *M. l. palliata* their initial up-slurred portion and final down-slurred portion are more pronounced. *M. l. palliata* adds a third segment consisting of a high-frequency Λ-shaped note. Whereas in *M. l. laemosticta* the notes change from up-slurred and partially modulated shapes to down-slurred and Λ-shaped, in *M. l. palliata* they change from rounded to Λ-shaped and then to an intermediate Λ-rounded shape (Fig. 2A, B).

MULTIVARIATE VOCAL ANALYSIS

Discriminant function analysis of songs of the four taxa in the *M. laemosticta* complex revealed that each taxon's group centroid differs significantly from the others (Wilks' Λ = 0.003, df = 36, $P < 0.0001$). The analysis classified correctly 100% of individuals to their respective population designation (Table 3; Fig. 4), demonstrating that *M. l. laemosticta*, *M. l. palliata*, *M. nigricauda*, and *M. berlepschi* are all vocally diagnosable with respect to each other in multivariate space.

FEMALES' CALLS AND SONGS

Because of limited sample size, we compared calls and females' songs only qualitatively. Nonetheless, we observed consistent differences between *M. l. laemosticta* and *M. l. palliata*, as well as among these and other related taxa. We observed two different call types in recordings of *M. l. palliata* ($n = 3$). One is a single flattened and rounded note with two poorly defined overtones; the other consists of a well-defined up-slurred note without any overtones (Fig. 5A). Calls of *M. l. laemosticta* differ, having rounded notes but lacking overtones ($n = 3$, Fig. 5B). In *M. nigricauda* we found two types of calls, one with two up-slurred notes and a few overtones, the other Λ-shaped ($n = 5$, Fig. 5C). We found only a single call of *M. berlepschi*, consisting of one flat note with no overtones ($n = 5$, Fig. 5D).
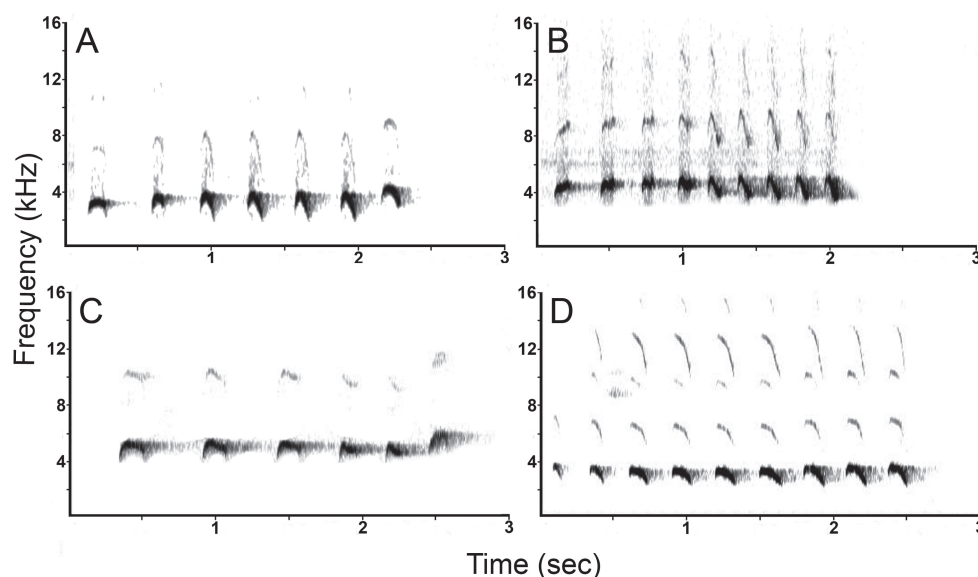

FIGURE 2.   Representative spectrograms of loudsongs of males of *M. l. palliata* (A, xeno-canto 45031), *M. l. laemosticta* (B, BMW 010 11), *M. nigricauda* (C, BMW 035 5), and *M. berlepschi* (D, xeno-canto 3874). For locality information see Appendix and Fig. 1.

TABLE 2. Mean and SD of 12 quantitative vocal variables (left) and pairwise tests of diagnosability in vocal characters across the four taxa of the *M. laemosticta* complex (right).

Vocal variable	Taxon				Pairwise diagnosability test[a]					
	palliata (n = 10)	*laemosticta* (n = 12)	*nigricauda* (n = 10)	*berlepschi* (n = 8)	*palliata* vs *laemosticta*	*palliata* vs *nigricauda*	*palliata* vs *berlepschi*	*laemosticta* vs *nigricauda*	*laemosticta* vs *berlepschi*	*nigricauda* vs *berlepschi*
Note number	7.05 ± 1.28	7.94 ± 0.84	6.00 ± 0.63	8.76 ± 0.69	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 2nd
Duration (s)	2.46 ± 0.32	1.67 ± 0.16	2.13 ± 0.18	2.40 ± 0.13	N.D. 2nd	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st
Pace (notes sec^{-1})	3.10 ± 0.28	5.03 ± 0.45	3.16 ± 0.35	3.97 ± 0.30	b	N.D. 1st	N.D. 1st	b	N.D. 1st	N.D. 1st
Note length (msec)	193.90 ± 40.43	134.14 ± 35.30	205.19 ± 18.50	159.29 ± 21.36	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st
Interval length (msec)	162.68 ± 27.32	98.24 ± 38.46	187.67 ± 56.58	125.32 ± 27.32	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st
Peak frequency (Hz)	4609.3 ± 266.0	4252.3 ± 310.2	5178.3 ± 327.0	3338.8 ± 96.2	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	b	N.D. 1st
Maximum power (dB)	84.76 ± 25.03	78.65 ± 7.10	75.44 ± 9.30	72.57 ± 10.11	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st
Change in pace	0.97 ± 0.10	0.79 ± 0.05	0.98 ± 0.10	1.10 ± 0.05	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	95%[c]	N.D. 1st
Change in note length	1.06 ± 0.14	1.26 ± 0.13	1.05 ± 0.12	0.87 ± 0.07	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	99%[c]	N.D. 1st
Change in interval length	1.60 ± 0.36	1.88 ± 0.32	1.51 ± 0.22	1.14 ± 0.15	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st
Change in peak frequency	0.92 ± 0.04	0.99 ± 0.03	0.96 ± 0.03	1.00 ± 0.02	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st	N.D. 1st
Change in maximum power	1.01 ± 0.02	1.01 ± 0.02	1.01 ± 0.04	0.96 ± 0.01	N.D. 1st	N.D. 1st	95%[c]	N.D. 1st	N.D. 1st	N.D. 1st
Note shape	—	—	—	—	Yes	Yes	Yes	Yes	Yes	Yes
Change in note shape	—	—	—	—	Yes	Yes	Yes	Yes	Yes	Yes
Total differences in vocal characters	—	—	—	—	3	2	3	3	5	2

[a] N.D. 1st, not diagnosable according to the first criterion; N.D. 2nd, diagnosable according to the first criterion but not diagnosable according to the second criterion.

[b] Normally distributed vocal character in which populations differ diagnostically.

[c] Vocal character not normally distributed in which nonparametric bootstrap simulation showed a diagnostic difference at the indicated level (%).
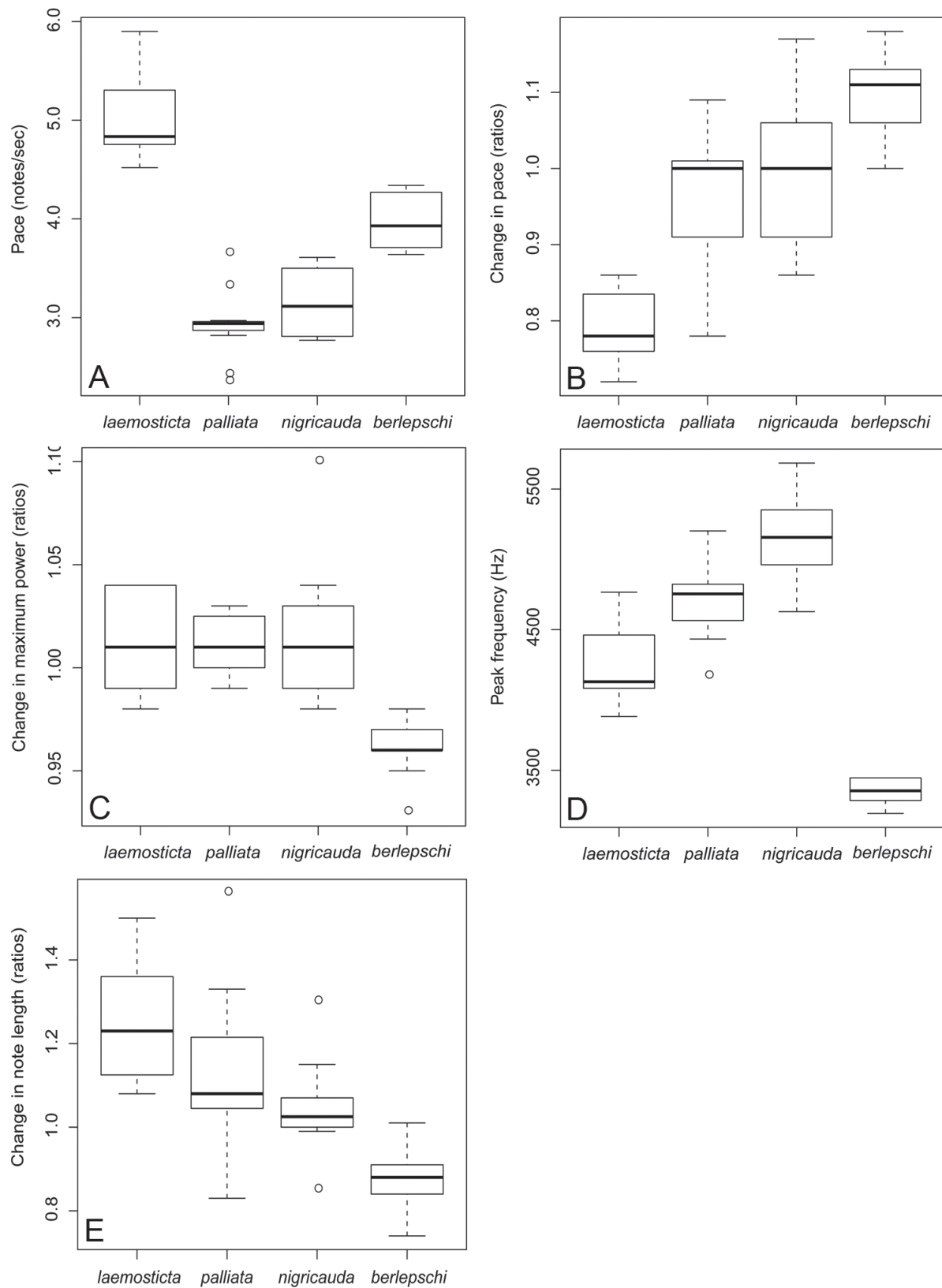
FIGURE 3.   Acoustic variables diagnostic for *M. l. palliata* (*n* = 12)*, M. l. laemosticta* (*n* = 11)*, M. nigricauda* (*n* = 10)*,* and *M. berlepschi* (*n* = 9). A, pace; B, change in pace; C, change in maximum power; D, peak frequency; E, change in note length.

TABLE 3.   Discriminant function analysis of vocal variation in the *Myrmeciza laemosticta* complex based on 12 vocal variables.

	Function		
Variable	1	2	3
Peak frequency (Hz)	0.592[a]	0.104	−0.281
Change in maximum power	0.220[a]	−0.07	0.157
Pace (notes sec^{-1})	−0.226	−0.742[a]	0.132
Duration (sec)	−0.069	0.569[a]	0.205
Note length (msec)	0.134	0.262[a]	0.233
Interval length (msec)	0.156	0.308[a]	0.064
Change in peak frequency	−0.164	−0.202	−0.449[a]
Change in pace	−0.155	0.416	0.483[a]
Change in interval length	0.126	−0.209	0.296[a]
Change in note length	0.108	−0.129	0.390[a]
Maximum power (dB)	0.053	0.031	0.313[a]
Note number	−0.272	−0.124	0.285[a]

[a] Variable with strong loading.

The song of the female of *M. l. palliata* is simple, consisting of a single segment with four flat notes (*n* = 1, Fig. 6A). In *M. l. laemosticta*, however, the female's song has an initial segment with three flat notes and final segment with three down-slurred and chevron-shaped notes (*n* = 4, Fig. 6B). In *M. nigricauda* the female's song consists of a first segment of partially rounded to flat notes with one clear rounded overtone and a final segment comprising three down-slurred rounded notes with a single down-slurred overtone (*n* = 2, Fig. 6C). In females of *M. berlepschi*, the song consists of a single segment with seven flat to down-slurred notes lacking overtones (*n* = 5, Fig. 6D).

SEQUENCE VARIATION AND PHYLOGENETIC ANALYSIS

Topologies recovered by maximum-likelihood and Bayesian-inference methods were identical and provided strong support for the monophyly of the group formed by *M. l. palliata, M. l. laemosticta, M. nigricauda*, and *M. berlepschi* (Fig. 7). Haplotypes of all four members of this clade, the *M. laemosticta* complex, were more distantly related to those of *M. griseiceps* and *M. exsul*. Although a sister relationship between haplotypes of *M. l. laemosticta* and *M. l. palliata* was recovered
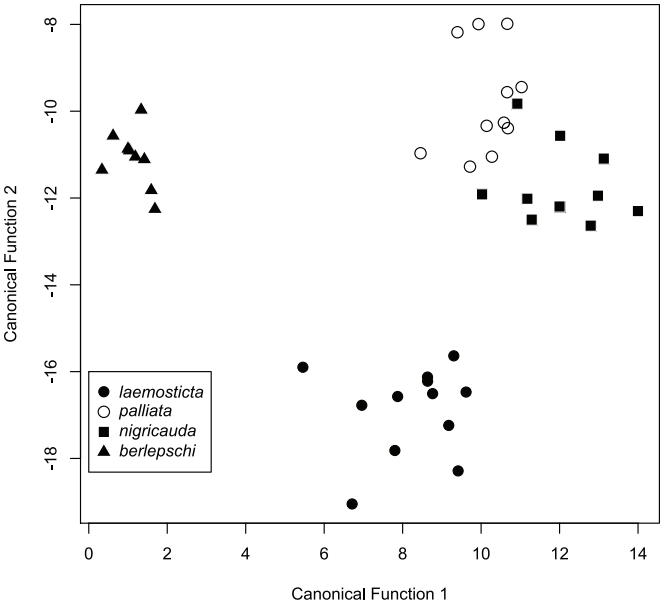

FIGURE 4.   Multivariate space defined by first two canonical functions that discriminate loudsongs of males of the four taxa of the *Myrmeciza laemosticta* complex on the basis of 12 acoustic variables. The first two canonical functions explained 95.2% of the variation in vocal variables (61.2% and 33.0%, respectively), whereas the third function explained the remaining 5.0% (Table 3).

in both reconstructions, it was not strongly supported (61% maximum-likelihood bootstrap and 0.73 Bayesian posterior probability). Among all four taxa, pairwise genetic distances were nearly the same (Table 4), and branches leading to each taxon were relatively long (Fig. 7).

DISCUSSION

SPECIES LIMITS IN THE *MYRMECIZA LAEMOSTICTA* COMPLEX

Multiple lines of evidence support the idea that the species-level diversity in the *M. laemosticta* complex has been underestimated. Specifically, we found that *M. l. palliata* is diagnosably different from *M. l. laemosticta* in three vocal characters, and it merits elevation to species rank according
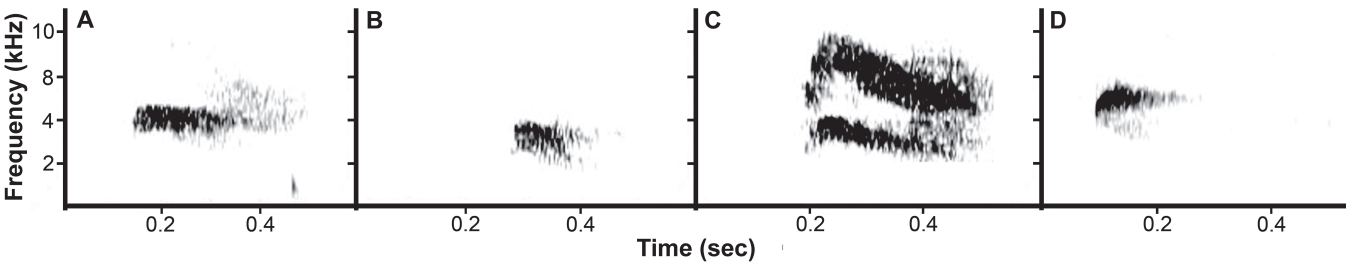

FIGURE 5.   Examples of spectrograms of calls of *M. l. palliata* (A), *M. l. laemosticta* (B), *M. nigricauda* (C), and *M. berlepschi* (D).
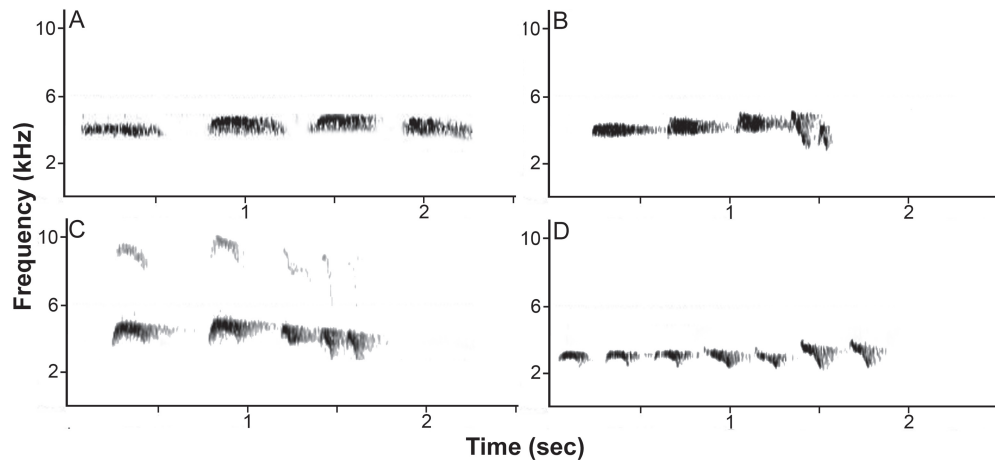
FIGURE 6.   Spectrograms of songs of females of (A) *M. l. palliata*, (B) *M. l. laemosticta*, (C) *M. nigricauda*, (D) *M. berlepschi*.
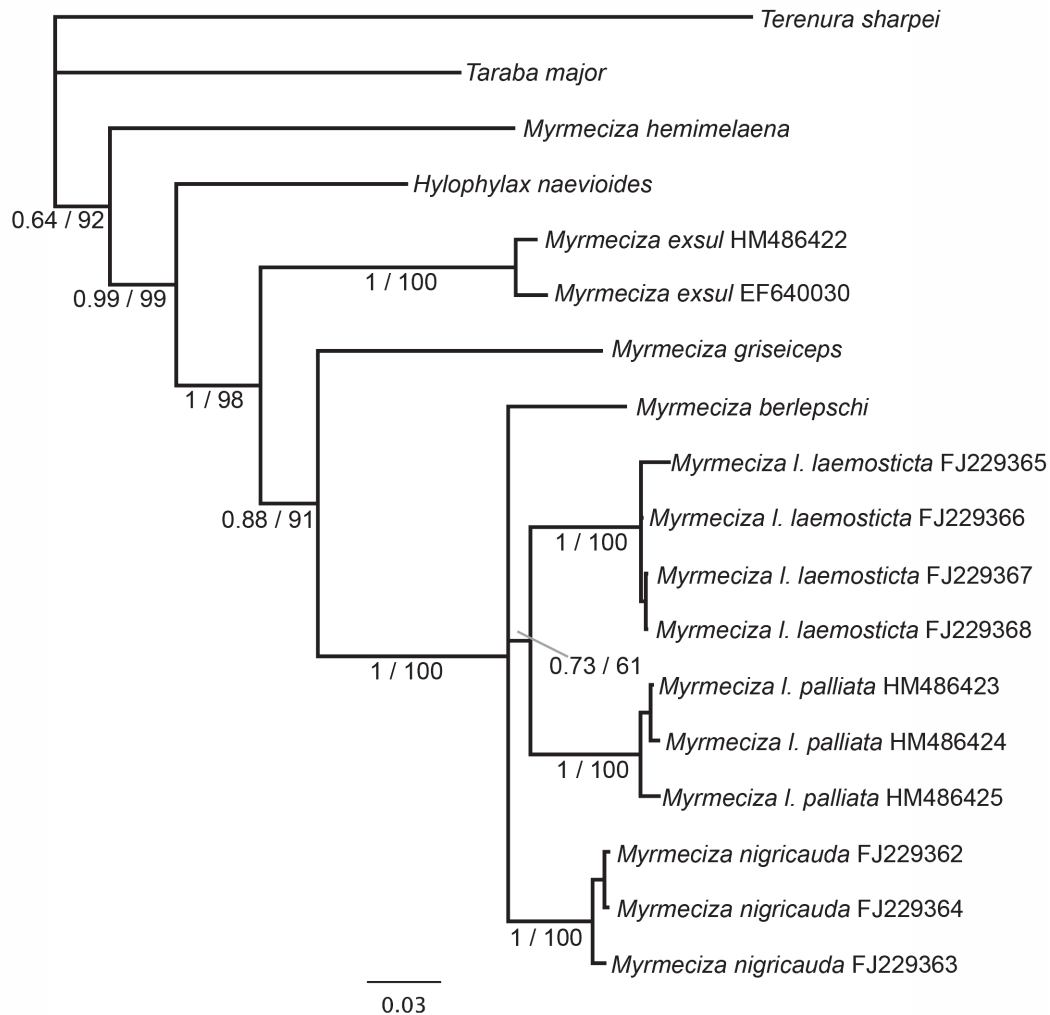

FIGURE 7.   Estimate of the ND2 genealogy of the *Myrmeciza laemosticta* complex, related *Myrmeciza* species, and outgroups. The tree is a 50% majority-rule consensus tree from the Bayesian inference. At each node, values to the left of the slash are the clade's posterior probability, those to the right are bootstrap values from the maximum-likelihood analysis.

TABLE 4.   Average genetic distances (below the diagonal, uncorrected p distances; above the diagonal, GTR $+ I + \Gamma$ model-corrected distances) among taxa of the *M. laemosticta* complex and related species.

	M. exsul	*M. griseiceps*	*M. berlepschi*	*M. nigricauda*	*M. l. laemosticta*	*M. l. palliata*
M. exsul		0.598	0.607	0.556	0.536	0.696
M. griseiceps	0.136		0.657	0.590	0.492	0.353
M. berlepschi	0.129	0.136		0.128	0.154	0.128
M. nigricauda	0.128	0.129	0.067		0.126	0.131
M. l. laemosticta	0.132	0.126	0.078	0.070		0.119
M. l. palliata	0.144	0.118	0.069	0.072	0.070	

to the standards of Isler et al. (1998) for assessing species limits in allopatric populations of antbirds. At the same time, the mtDNA tree suggests a long, independent history of isolation between *M. l. laemosticta* and *M. l. palliata*, roughly congruent with the divergence between species currently recognized in this assemblage. Therefore, our findings demonstrate that *M. l. laemosticta* and *M. l palliata* are best treated as distinct species under criteria serving most species concepts, including the phylogenetic species concept and the more conservative biological species concept.

Before we began this study, we had noted a vocal similarity between *M. l. palliata* and *M. nigricauda* that was borne out by our acoustic analyses; indeed, we found only two diagnostic differences between the loudsongs of these taxa. Likewise, *M. berlepschi* and *M. nigricauda* differ only in two vocal traits. These two species are geographically sympatric (although they largely replace each other elevationally) yet show no evidence of intermediacy or introgression in signals likely related to reproductive isolation. Thus, considering all the evidence, we argue that they are best considered different species. The type locality of *M. l. palliata* is La Palmita (Todd 1917), on the western slope of the eastern Andes of Colombia, near Ocaña, Norte de Santander (Fig. 1 but see Paynter 1997). This name has priority over the synonymized names *bolivari* and *venezuelae* (Robbins and Ridgely 1991). Accordingly, we suggest recognizing four biological species in the *M. laemosticta* complex, as follows:

> *Myrmeciza laemosticta* (Salvin 1865), Dull-mantled Antbird
> *Myrmeciza palliata* (Todd 1917), Magdalena Antbird
> *Myrmeciza nigricauda* (Salvin and Godman 1892), Esmeraldas Antbird
> *Myrmeciza berlepschi* (Hartert 1898), Stub-tailed Antbird

More generally, our results imply that only two diagnostic characters in the loudsong may be sufficient for two populations to be considered distinct species in this complex, as observed between some other sympatric antbirds (*Thamnophilus striatus* and *T. murinus*; *Myrmotherula surinamensis* and *M. cherriei*; Isler et al. 1998). Moreover, we note that the differences in loudsongs we documented are likely associated with diagnostic differences in calls. These vocalizations may

play an important role in species recognition, as seen in other antbirds such as the *Hypocnemis cantator* complex (Isler et al. 2007b, Tobias et al. 2008). Studies evaluating the function and relative importance of species recognition of loudsongs and calls are necessary for the *M. laemosticta* complex and other antbirds.

PHYLOGENY AND BIOGEOGRAPHY OF TRANS-ANDEAN *MYRMECIZA* ANTBIRDS

Our goal was to examine whether vocally distinct populations in this complex correspond to genetically distinct populations, with particular emphasis on *M. palliata*. Our mtDNA phylogeny shows a long branch defining a monophyletic *M. laemosticta* complex including *M .l. laemosticta*, *M. palliata*, *M. nigricauda* and *M. berlepschi*, roughly in agreement with previous hypothesis of species relationships (Robbins and Ridgely 1991, Zimmer and Isler 2003). The ND2 gene tree suggests that *M. griseiceps,* not *M. exsul* (as suggested by Robbins and Ridgely 1991), is the sister taxon of the complex.

Our results do not conform strictly to the biogeographic scenario for this group proposed by Robbins and Ridgely (1991) on the basis of patterns of plumage variation and qualitative assessments of loudsongs. These authors hypothesized that *M. berlepschi* diverged from a proto-*nigricauda*/*laemosticta*/*palliata*, which later, during the Quaternary, differentiated into *M. laemosticta*, *M. palliata*, and *M. nigricauda*. However, the model-corrected ND2 sequence divergence between members of the *M. laemosticta* complex ranges from ~12 to 15%, implying a divergence between 3 and 7.5 million years ago (Pliocene–late Miocene), given a divergence rate of either 2% (Lovette 2004, Weir and Schluter 2008) or 4% per million years (Brumfield and Edwards 2007), respectively. In any case, ND2 substitution rates would have to be substantially higher than reported for avian mtDNA coding genes for these divergences to have occurred in the Pleistocene. Our finding of pre-Pleistocene differentiation agrees with the more ancient divergences found in nearly all groups of antbirds so far examined (Bates et al. 1999, Braun et al. 2005, Brumfield and Edwards 2007). We hope that results from this study serve as working hypotheses for future studies of species relationships in this group.

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APPENDIX 1.   Sources of the sound recordings and sequence data on which this study was based.

Taxon	Collector	Source/catalog number[a]	Locality
Sound recordings			
M. l. laemosticta	K. J. Zimmer	KJZ 012 10	Costa Rica, Braulio Carrillo National Park
	K. J. Zimmer	KJZ 021 01	Costa Rica, Braulio Carrillo National Park
	K. J. Zimmer	KJZ 068	Costa Rica, Braulio Carrillo National Park
	K. J. Zimmer	KJZ 073 04	Costa Rica, Braulio Carrillo National Park
	K. J. Zimmer	KJZ 095 25	Costa Rica, Braulio Carrillo National Park
	D. Ross Jr.	Ross and Whitney (1996)	Costa Rica, Caribbean slope
	B. M. Whitney	BMW 010 11	Panama, Cerro Campana
	B. M. Whitney	Isler and Whitney (2003)	Panama, Darién, Cerro Pirré
	B. M. Whitney	BMW 002 08	Panama, Darién, Darién National Park
	B. M. Whitney	BMW 011 12	Panama, Darién, Darién National Park
	A. M. Cuervo	xeno-canto 44663	Panama, El Coclé, El Copé
	A. M. Cuervo	xeno-canto 44664	Panama, El Coclé, El Copé
M. l. palliata	J. C. Chaves	xeno-canto 45031	Colombia, Antioquia, San Luis
	A. M. Cuervo	IAvH-BSA 30969	Colombia, Boyacá, Otanche, La Ye
	A. M. Cuervo	IAvH-BSA 31064	Colombia, Boyacá, Otanche, La Ye
	A. M. Cuervo	IAvH-BSA 31066	Colombia, Boyacá, Otanche, La Ye
	O. Laverde	IAvH-BSA 17823	Colombia, Boyacá, Otanche, La Ye
	N. Athanas	xeno-canto 10725	Colombia, Boyacá, Pto. Boyacá, Río Ermitaño
	B. M. Whitney	BMW 072 01-1	Colombia, Caldas, La Victoria
	B. M. Whitney	BMW 072 01-2	Colombia, Caldas, La Victoria
	T. Donegan	xeno-canto 24335	Colombia, Santander, Cerro La Paz
	A. Renaudier	xeno-canto 22848	Venezuela, Mérida, Río Frío
M. nigricauda	O. Laverde	IAvH-BSA 24126	Colombia, Nariño, Barbacoas, El Pangán
	B. M. Whitney	BMW 001 11	Colombia, Valle del Cauca, Buenaventura Rd.
	B. B. Coffey	BBC 001 17	Colombia, Valle del Cauca, Río Anchicayá
	B. M. Whitney	BMW 035 5	Ecuador, El Oro, 8–9 km W Piñas
	N. Krabbe	NK 005 14	Ecuador, El Oro, 8–9 km W Piñas
	N. Krabbe	NK 007 57	Ecuador, Esmeraldas, El Placer
	K. J. Zimmer	KJZ 018 19	Ecuador, Pichincha, Chiriboga
	K. J. Zimmer	KJZ 018 20	Ecuador, Pichincha, Chiriboga
	R. Ridgely	Moore et al. (1999)	Ecuador, Pichincha, Chiriboga Rd.
	N. Krabbe	NK 005 15	Ecuador, Pichincha, Hacienda Francisco Rodríguez

(*continued*)

APPENDIX 1.　(*Continued*)

Taxon	Collector	Source/catalog number[a]	Locality
M. berlepschi	B. M. Whitney	BMW 001 10-2	Colombia, Valle del Cauca, Buenaventura
	B. M. Whitney	Isler and Whitney (2003)	Colombia, Valle del Cauca, Buenaventura
	B. M. Whitney	BMW 001 10	Ecuador, Esmeraldas, Alto Tambo
	N. Krabbe	NK 004 62	Ecuador, Esmeraldas, Alto Tambo
	N. Krabbe	NK 004 62	Ecuador, Esmeraldas, Alto Tambo
	N. Krabbe	NK 004 62	Ecuador, Esmeraldas, Alto Tambo
	N. Krabbe	NK 004 62-1	Ecuador, Esmeraldas, Alto Tambo
	N. Athanas	xeno-canto 3874	Ecuador, Pichincha, Río Silanche
Sequences			
M. l. laemosticta	J. Weir	GenBank: FJ229365	Panama, Coclé, El Copé
	R. Faucett	GenBank: FJ229366	Panama, Colón, Upper Rio Chagras
	J. Weir	GenBank: FJ229367	Panama, Darién, Río Piñas
	M. J. Miller	GenBank: FJ229368	Panama, Darién, Río Piñas
M. l. palliata	P. Salaman	GenBank: HM486425	Colombia, Antioquia, Anorí
	A. M. Cuervo	GenBank: HM486423	Colombia, Antioquia, San Luis
	J. C. Chaves	GenBank: HM486424	Colombia, Antioquia, San Luis
M. nigricauda	M. J. Miller	GenBank: FJ229362	Ecuador, Manabí, Cerro Pata de Pájaro
	M. J. Miller	GenBank: FJ229363	Ecuador, Cerro Pata de Pájaro
	M. J. Miller	GenBank: FJ229364	Ecuador, Cerro Pata de Pájaro
M. berlepschi	J. Kennard	GenBank: EF640029	Ecuador, Esmeraldas, El Placer
M. griseiceps	M. Robbins	GenBank: FJ229361	Ecuador, Loja, SE Celica, Río Catamayo
M. exsul	S. Sierra	GenBank: HM486422	Colombia, Caldas, La Miel
	M. J. Miller	GenBank: EF640030	Panama, Panamá
Hylophylax naevioides	G. H. Rosenberg	GenBank: EF640019	Panama, Darién
M. hemimelaena	M. J. Miller	GenBank: EF640032	Peru, Ucayali
Taraba major	S. M. Lanyon	GenBank: EF640053	Peru, Madre de Dios
Terenura sharpei	R. T. Brumfield	GenBank: EF640055	Bolivia, Cochabamba

[a]Acronyms refer to recordists' initials except for sound libraries xeno-canto and IAvH-BSA (Banco de Sonidos Animales, Instituto Alexander von Humboldt, Colombia).

APPENDIX 2.　Description of 14 vocal variables measured on the loudsongs of four taxa of *Myrmeciza* antbirds (*M. l. laemosticta, M. l. palliata, M. nigricauda* and *M. berlepschi*).

Vocal variable	Type	Distribution	Description
Number of notes	Quantitative	Continuous, normal	Number of notes per loudsong
Duration	Quantitative	Continuous, normal	Duration of loudsong in seconds, from the beginning of the first note to the end of the last note
Pace	Quantitative	Continuous, normal	Number of notes over the loudsong's duration, from the beginning of the first note to the end of the last interval
Change in pace	Quantitative	Ratio, not normal	Ratios between the paces of three equally long sections of a loudsong (1/2, 1/3, and 2/3)
Note length	Quantitative	Continuous, normal	Mean duration of notes. Average of notes one, two, middle, penultimate and ultimate in milliseconds
Change in note length	Quantitative	Continuous, not normal	Pairwise ratios of the five notes measured (1/2 . . . 4/5).
Interval length	Quantitative	Continuous, normal	Mean duration of the intervals of silence. Average of intervals of silence that follow the first four notes analyzed on each loudsong, in milliseconds
Change in interval length	Quantitative	Continuous, not normal	Pairwise ratios of the four intervals measured (1/2 . . . 3/4)
Peak frequency	Quantitative	Continuous, normal	Peak frequency in hertz of the five notes measured
Change in peak frequency	Quantitative	Continuous, not normal	Pairwise ratios of the five peak frequencies (1/2 . . . 4/5)
Maximum power	Quantitative	Continuous, normal	Intensity of the note in decibels as defined by Raven, measured in five notes
Change in maximum power	Quantitative	Continuous, not normal	Pairwise ratios of the five maximum power values (1/2 . . . 4/5).
Note structure	Qualitative	—	Shape, syntax, and presence or absence of overtones
Change in note structure	Qualitative	—	Change in shape, syntax, and presence or absence of overtones through the vocalization

APPENDIX 3.   Description of the differences in acoustic characteristics of loudsongs between pairs of *Myrmeciza* antbirds included in this study. See Tables 1 and 2 and Results for a description of the vocal differences between *M. l. laemosticta* and *M. l. palliata*.

Myrmeciza l. palliata vs. *M. nigricauda*: diagnosable in two vocal characters, note shape and change in note structure (Fig. 2a and 2c). Loudsongs of *M. l. palliata* and *M. nigricauda* consists of three segments; in the first segment, notes of *M. nigricauda* are rounded, those of *M. l. palliata* are slurred up–down. The second segment of the loudsong of *M. nigricauda* contains notes that are Λ-shaped and partially modulated, whereas those of *M. l. palliata* are downslurred and Λ-shaped. In the third segment, both taxa emit rounded notes (partially modulated in *M. nigricauda*), but these can be distinguished by the change in frequency across them: the notes are symmetrical in *M. l. nigricauda* but asymmetrical in *M. l. palliata*. Notes of *M. l. palliata* change from rounded to Λ-shaped and finally to an intermediate Λ-rounded shape, whereas those of *M. nigricauda* change from rounded to partially modulated to rounded again.

Myrmeciza l. palliata vs. *M. berlespchi*: Three vocal characters diagnose these two taxa: change in maximum power, note shape, and change in note structure. According to the nonparametric bootstrap test, these two taxa differ significantly in mean change in maximum power (Table 4, Fig. 3e). In terms of note shape, the loudsong of *M. berlepschi* has two segments both with down-slurred rounded notes (second segment includes the last three notes, which are higher in frequency), whereas the loudsong of *M. l. palliata* has three main different segments. Finally, the structure of the notes of loudsongs of *M. berlespchi* do not change, whereas in *M. l. palliata* the notes change from rounded to Λ-shaped and finally to an intermediate rounded Λ shape.

Myrmeciza l. laemosticta vs. *M. nigricauda*: diagnosable on the basis of three vocal characters, song pace, note structure, and change in note structure. The pace of the loudsong of *M. l. laemosticta* (4.61–5.83 notes sec^{-1}) is faster than that of *M. nigricauda* (2.77–3.61 notes sec^{-1}) (Fig. 3a). In terms of note structure, loudsongs of *M. nigricauda* consist of three main segments, those of *M. l. laemosticta* consist of two. In the first segment, the notes of *M. nigricauda* are rounded, those of *M. l. laemosticta* are up-slurred and partially modulated. In the second segment, notes of *M. nigricauda* are Λ-shaped and partially modulated notes, those of *M. l. laemosticta* are Λ-shaped. Finally, *M. nigricauda* adds a third segment consisting of a high-frequency rounded note. Through the loudsong of *M. l. laemosticta*, note structure changes from up-slurred and partially modulated to down-slurred and Λ-shaped, whereas in *M. nigricauda* note structure changes from rounded to partially modulated then back to rounded.

Myrmeciza l. laemosticta vs. *M. berlespchi*: songs differ substantially. Five vocal characters are diagnostic: peak frequency, change in song pace, change in note length, note shape, and change in note shape. Peak frequency is diagnosably lower in *M. berlespchi* (3192.65–3445.31 Hz) than in *M. l. laemosticta* (3881.70–4766.01 Hz), and the change in pace is significantly greater in *M. berlespchi* (Table 4, Fig. 3). In terms of change in note length, *M. berlespchi* has shorter notes at the beginning and longer notes at the end of its loudsong, whereas *M. l. laemosticta* tends to have longer notes at the beginning and shorter notes at the end. The bootstrap test shows that the change in note length of *M. l. laemosticta* is significantly greater than that of *M. berlespchi*. The loudsongs of *M. l. laemosticta* and *M. berlespchi* consist of two distinct segments. Through the song, notes of *M. l. laemosticta* change structure from up-slurred and partially modulated to down-slurred and Λ-shaped, whereas those of *M. berlespchi* are all down-slurred and rounded.

M. nigricauda vs. *M. berlepschi*: loudsongs of these partially sympatric taxa are diagnosable on the basis of two characters, note shape and change in note shape. The loudsong of *M. berlepschi* consists of uniformly down-slurred rounded notes in two segments, whereas that of *M. nigricauda* has three distinct segments definable by note shape. Notes of *M. nigricauda* change from rounded to partially modulated and back to rounded, whereas those of *M. berlespchi* do not change through the song.

M. griseiceps: The loudsongs of this species differ substantially from those of all other taxa in this complex in five quantitative characters (duration, note number, pace, note length and interval length). Multivariate analyses (not shown) place *M. griseiceps* completely separated from the rest of the taxa. Furthermore, in terms of note shape, *M. griseiceps* differs from all other taxa in the complex: its loudsong consists of only one segment of rounded to Λ-shaped notes that decrease progressively in frequency, with a peak at notes 2 and 3.