COMPARISON OF PHENOTYPIC AND GENETIC DIFFERENTIATION IN SOUTH AMERICAN ANTWRENS (FORMICARIIDAE)

SHANNON J. HACKETT AND KENNETH V. ROSENBERG
Museum of Natural Science and Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803 USA

ABSTRACT.—We examined patterns and levels of genetic, morphometric, and plumage variation in *Myrmotherula* antwrens and related antbirds (Formicariidae). We derived matrices of distances independently for proteins, qualitative plumage traits, and measurements of study skins. Our goals were to assess concordance between the morphological (morphometric and plumage) and genetic patterns, to estimate phylogenetic relationships, and to test monophyly of *Myrmotherula*. *Myrmotherula* is not monophyletic. Analysis of allozymes shows that although the "gray" and "streaked" plumage types are closely related, the "checker-throated" *Myrmotherula* species are not closely related to the gray/streaked clade. Plumage divergence exceeds divergence in protein genes, whereas change in external morphology has been relatively conservative. Antwren speciation has been accompanied by differentiation of plumage types rather than by entry of species into new regions of morphometric space. Relatively large genetic distances among and within *Myrmotherula* species imply that these tropical taxa are older and more geographically subdivided than are most North American species. Neither plumage nor morphometrics alone correctly predicted the genetic relatedness among the taxa. We urge that taxonomic revisions be accompanied by molecular data, especially in species-rich tropical families. *Received 5 June 1989, accepted 26 December 1989.*

RECONSTRUCTION of the history of lineages is a central aim of evolutionary biology. Comparative biochemical techniques provide powerful methods to assess genetic relationships among species. Questions remain about the relative utility of molecular versus morphological approaches (Felsenstein 1985; see papers in Patterson 1987). In particular, it is important to establish if molecules and morphology give concordant pictures of past evolutionary events, and to define the problems and limitations of these methods. Few studies have integrated both morphological and molecular approaches in analyses of the evolutionary history of organisms, and the empirical relationship between morphological variation and genealogy remains poorly understood.

As a case study using multiple data sets, we analyzed levels of genetic, morphometric, and plumage similarity among members of a single large genus of antwrens (*Myrmotherula*) and among other antbirds of uncertain relatedness to *Myrmotherula*. Several features of this genus facilitate comparison of genealogy and morphology. The species in *Myrmotherula* are similar in size and shape, but occur as three distinct plumage groups: checker-throated, gray, and streaked (Table 1). Some of these plumage traits are shared with other small antbirds in a number of related genera; indeed, no unique morphological traits support *Myrmotherula* as a monophyletic group. The natural history and vocal behavior suggest the division of *Myrmotherula* into several ecological groups (e.g. Gradwohl and Greenberg 1984, Remsen and Parker 1984, Rosenberg in press). Questions concerning the pattern of appearance of phenotypic innovations (e.g. the various plumage types or foraging modes) can be addressed only in the context of an estimate of genealogical relationships among species (Felsenstein 1985). The diversity of plumage types and ecological habits makes this avian group a test case for the comparison of patterns of morphologic and genetic evolution.

METHODS

We analyzed tissue samples of 18 *Myrmotherula* species, and 11 other formicariid genera (mostly small antwrens). *Grallaria quitensis* was selected as the outgroup because *Grallaria* is part of a distantly related subgroup of formicariids that may deserve familial status (Sibley and Ahlquist 1985).

Specimens, scientific names, and collecting localities of the 29 species examined are listed in Appendix 1. We used four individuals of *M. haematonota* (one

TABLE 1. Plumage types, weight, and tail measurements of 5 males for each *Myrmotherula* species. Species with * are considered in this study; PS, PD, NB, and SB indicate sample locality (see Appendix 1).

Species	Plumage type	Weight (g)	Tail (mm)
*M. brachyura**	streaked	7.3	17.6
*M. obscura**	streaked	7.1	18.0
*M. sclateri**	streaked	8.6	23.6
M. klagesi	streaked		
*M. surinamensis**	streaked	8.7	24.8
M. ambigua	streaked		
M. cherriei	streaked		
*M. longicauda**	streaked	8.3	36.0
M. gularis	checker-throated		
M. gutturalis	checker-throated		
*M. fulviventris**	checker-throated	10.2	34.3
*M. leucophthalma**	checker-throated	9.3	39.5
M. haematonota (NB)*	checker-throated	8.0	36.8
M. haematonota (SB)*	checker-throated	8.5	34.9
M. haematonota (PD)*	checker-throated	8.7	36.7
M. haematonota (PS)*	checker-throated	9.4	41.5
*M. ornata**	gray[a]	9.6	33.5
*M. erythrura**	checker-throated	10.8	42.5
M. guttata	gray		
*M. hauxwelli**	gray	10.3	25.7
M. erythronotus	gray		
*M. axillaris**	gray	6.8	35.4
*M. schisticolor**	gray	8.6	36.3
M. sunensis	gray		
*M. longipennis**	gray	8.9	33.2
M. minor	gray		
M. iheringi	gray		
*M. grisea**	gray	8.4	34.9
M. unicolor	gray		
*M. behni**	gray		
M. urosticta	gray		
*M. menetriesii**	gray	8.0	29.3
*M. assimilis**	gray	9.3	29.1

[a] Placement in "gray" group reflects male plumage of *Myrmotherula ornata meridianalis*; males of other races have rufous backs, and female plumage suggests inclusion in "checker-throated" group (see text).

from each of four localities) to assess intraspecific geographic variation. There were a total of 32 operational taxonomic units (OTUs). Nomenclature follows Morony et al. (1975). All tissue specimens were obtained from the Louisiana State University Museum of Natural Science (LSUMNS) Frozen Tissue Collection. Voucher specimens (skin and skeletons) for tissue samples are housed in the LSUMNS.

Electrophoresis.—One individual per OTU was used for the analysis of genetic variation. Gorman and Renzi (1979) suggested that one or few individuals per taxon provide robust estimates of genetic distances as long as the number of loci examined is reasonably high and heterozygosity is low (conditions met by this study). However, Archie et al. (1989) challenged this assertion. We suggest that the conservatism of avian allozyme divergence, fixed or nearly fixed allozymes unique to certain groups in this study, and low heterozygosity may minimize the sample-size bias predicted by Archie et al. (1989). Protein electrophoresis followed standard procedures (Johnson et al. 1984). Samples of heart, liver, and muscle were taken within 3 h of death and stored in liquid nitrogen (−196°C). Tissue extracts were prepared by grinding samples of heart, pectoral muscle, and liver in 1 ml of grinding buffer (6% sucrose, 0.01% NAD, 0.01% NADP, 0.01% DTT, deionized water) with a tissue homogenizer. The mixture was spun in a Sorvall RC-5B centrifuge (Sorvall rotor SM 24) at 16,000 rpm for 30 min, and the resulting supernatant frozen (−70°C) for subsequent electrophoretic experiments. Buffer conditions, running conditions, and buffer recipes are available (from Hackett) on request. All gels were 11.5% starch. Each locus was scored on at least two different buffer types to minimize hidden variation (Hackett 1989).

Protein electrophoresis followed standard procedures (Johnson et al. 1984). Twenty-six enzyme systems representing 32 genetic loci were resolved. Enzymes were assayed using procedures outlined by Harris and Hopkinson (1976), with slight modifications. Ten enzymes were monomorphic and fixed for the same allele across all taxa examined: Malate dehydrogenase-1 and 2 (EC 1.1.1.37), isocitrate dehydrogenase-2 (1.1.1.42), fumarate dehydrogenase (4.2.1.2), glutamate dehydrogenase (1.1.1.47), glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12), diaphorase (1.6.*.*), hemoglobin, glutathione reductase (1.6.4.2), and superoxide dismutase-2 (1.15.1.1). Purine nucleoside phosphorylase (NP; 2.4.2.1) was eliminated from the analysis due to inconsistent banding patterns. Alleles at a locus were coded by their mobility from the origin. The most anodal allele was designated "a," the next most anodal "b," and so on.

The computer program BIOSYS-1 (Swofford and Selander 1981) was used to compute genetic distances (Nei 1978, Rogers 1972), estimate Distance-Wagner trees (Farris 1972, 1981), and derive a UPGMA phenogram (Sneath and Sokal 1973). The "multiple addition criterion" of Swofford (1981) was used in the Distance-Wagner procedure because it generally finds trees of better fit to the original distance matrix (Farris 1981). The maximum number of trees held at each step in the Distance Wagner procedure was set at 20. Trees were rooted at the outgroup *Grallaria quitensis*.

We used PHYLIP (Felsenstein 1986) to analyze cladistically the allele distribution. Alleles were coded as present (1) or absent (0) (Mickevich and Mitter 1981). Only phylogenetically informative alleles were used (alleles present in two or more taxa). The data set, which consisted of 56 characters (alleles), was analyzed with the MIX program of PHYLIP. PENNY, a program to find all most parsimonious trees implied

by the data, could not be used because of the large number of taxa in this study.

There is no generally accepted method for coding alleles for cladistic analysis. The use of presence/absence coding of alleles has been criticized for a number of reasons (but see Rogers and Cashner 1987 for a defense of this type of coding), and a more appropriate method of coding may be to treat the locus as a character with its alleles as unordered character states (Buth 1984, Swofford and Berlocher 1987). We chose not to order alleles, because ordering of alleles implies knowledge of evolutionary relationships among specific alleles that is usually lacking in most electrophoretic studies (Mickevich and Mitter 1983). One of the difficulties in coding loci as multistate-unordered characters is the treatment of polymorphisms in taxa, a common result of electrophoretic data (see Appendix 2). We realize the limitations of presence/absence coding of alleles and of using the locus as the character, and we choose to present results treating alleles as characters.

Morphometrics.—On five adult males of each OTU, we measured length of closed wing, wing-tip extension, tarsus length, length of middle toe, exposed culmen length, bill depth, bill width, and tail length (Baldwin et al. 1931); body weight was also used as a morphometric character. From character means, a matrix of taxonomic distances (Sneath and Sokal 1973; available from the authors upon request) was derived and used to construct a UPGMA phenogram to summarize overall morphometric similarity (NTSYS, Rohlf et al. 1974). In addition, we subjected the correlation matrix from character means of all taxa except the outgroup (*Grallaria quitensis*) to principal components analysis (PCA) using SAS (SAS Institute 1982) to identify major axes of morphometric variation. In this analysis, cube root of body weight was used as a morphometric measure and, to assess variation in shape independent of body size, all other measurements were divided by the cube root of body weight. Factors were subjected to varimax rotation, which maximizes the spread of the variable loadings among the factors and thus facilitates interpretation (Johnson and Wichern 1982), but does not affect relative positions of OTUs in PC space.

Plumage.—Variation in male and female plumage characteristics was assessed qualitatively from study skins. Body regions (throat, breast, back, crown, wing, and tail) were treated as characters, with unordered character states being the color or pattern in that body region (e.g. the character "throat" had character states of white, yellow, black, checkered, and so on [see Appendix 3]). Although these character states were somewhat arbitrary (e.g. several subtle shades of gray or brown were considered equivalent), this represents a conservative assessment of plumage variation among these species. From these data, we constructed OTU by OTU pairwise distance matrices separately for males and females, based on simple matching coefficients. This matrix was then used to construct a UPGMA phenogram that summarizes overall plumage similarity (NTSYS, Rohlf et al. 1974). Maximum parsimony analysis of plumage characters (using the computer program PAUP; Swofford 1985) produced the same groupings as the phenetic analysis.

Comparison of genetic, morphometric, and plumage data sets.—We described overall similarity among data sets in three ways. First, Mantel's (1967) test was used to describe the similarity in overall structure between each pair of distance matrices. Second, we performed Spearman rank correlations on paired distances among taxa. Finally, to estimate the magnitude of divergence of morphometric and plumage (male only) characters relative to genetic characters, we compared scaled OTU by OTU distance matrices for each of the three data sets. To scale each matrix, the largest distance in the matrix was assigned a value of 1, and each other distance was then divided by the largest distance. The outgroup was omitted from the scaling process to avoid compression of distances because of the presence of a single large distance.

Results

Plumage.—Distance matrices based on male and female plumage characteristics are available from the authors. Phenetic analyses of male plumage characteristics distributed the *Myrmotherula* species among three major groups (Fig. 1). The first group included five *Myrmotherula* species that are mainly white- or yellow-streaked throughout with black (hereafter, "streaked" *Myrmotherula* group). *Drymophila devillei, Hypocnemis cantator,* and *Herpsilochmus rufimarginatus,* which are also boldly patterned or streaked, were most similar to this group. The second major group consisted of primarily gray species, most with black throats and white-tipped wing coverts, a plumage pattern common in the Formicariidae. Eight *Myrmotherula* species fell within this group (hereafter, "gray" *Myrmotherula* group), along with *Hylophylax poecilonota, Dysithamnus mentalis,* and *Pygiptila stellaris. Myrmotherula axillaris,* along with *Microrhopias quixensis* and *Formicivora rufa,* is aligned with this group but is more extensively black with prominent white markings on the flanks or tail.

A third group consisted of four *Myrmotherula* species (including the four populations of *M. haematonota*) that had brown or rufous upperparts, a checkered throat-patch, and buffy wing spots (hereafter, "checker-throated" *Myrmotherula* group). Finally, *Thamnophilus doliatus* and *Terenura humeralis,* with unique plumage characteristics, formed a fourth group. Analysis of

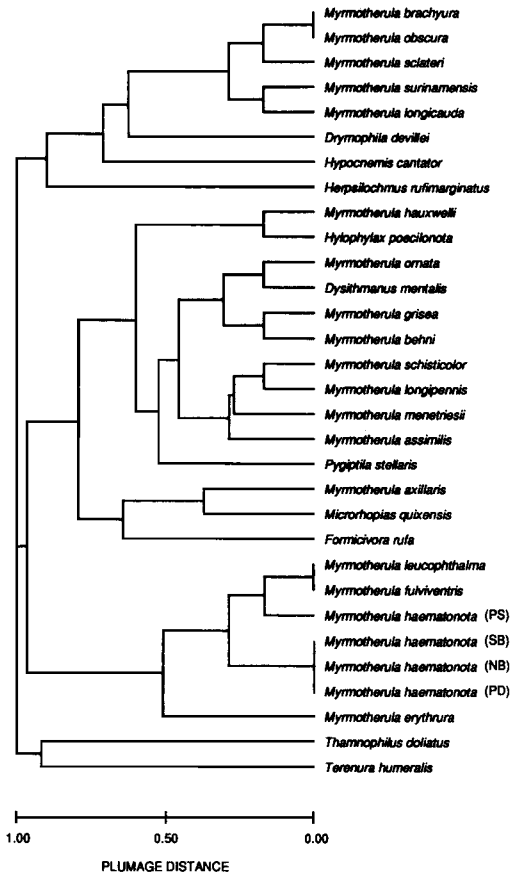

Fig. 1. Phenetic (UPGMA) analysis of variation in male plumage. Cophenetic correlation = 0.898. PS, PD, NB, and SB after *Myrmotherula haematonota* indicate sample locality (see Appendix 1).

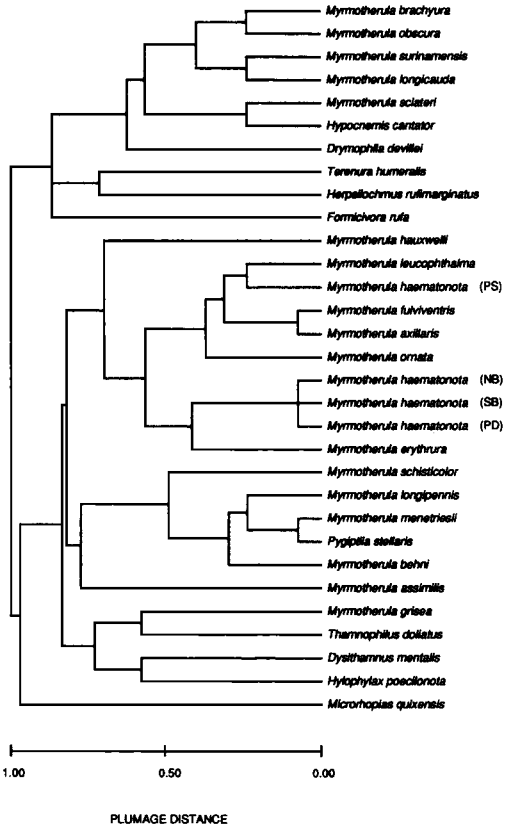

Fig. 2. Phenetic analysis (UPGMA) of variation in female plumage. Cophenetic correlation = 0.841. PS, PD, NB, and SB after *Myrmotherula haematonota* indicate sample locality (see Appendix 1).

female plumage resulted in similar, but less well defined, groups of species (Fig. 2). The first group of streaked *Myrmotherula* and similar species remained nearly the same as in the analysis of males; these species are weakly sexually dimorphic. All remaining species were various shades of brown, buff, or rufous, a typical pattern in female antbirds. The second group, consisting of checker-throated *Myrmotherula* spp. along with *M. hauxwelli*, *M. axillaris*, and *M. ornata*, was defined primarily by the presence of buffy wing spots. Female *M. ornata* have a checkered throat like that of the male checker-throated species. In addition, *M. ornata* exhibits marked geographic variation in male plumage, with some populations having a rufous back; thus, its inclusion in the gray group based on male plumage (Table 1) is tenuous. The remaining species, with the exception of *Microrhopias quixensis*, formed a third, poorly defined group. Females of the gray *Myrmotherula* spp. were most similar to each other, and *Pygiptila stellaris* was identical in all plumage characters to *M. menetriesii*.

Morphometrics.—*Myrmotherula* species varied little in overall mass (7–11 g) and body proportions, especially compared with other antbirds (Figs. 3–5). Most variation among *Myrmotherula* species was in tail length (Table 1). Although the checker-throated species were all relatively long-tailed, this group overlapped with both streaked and gray *Myrmotherula* species (Fig. 3A). Members of the three plumage groups did not differ consistently in tarsus length or wing shape. *M. longipennis* was exceptionally long-winged, and *M. hauxwelli* was long-legged. The only univariate morphomet-

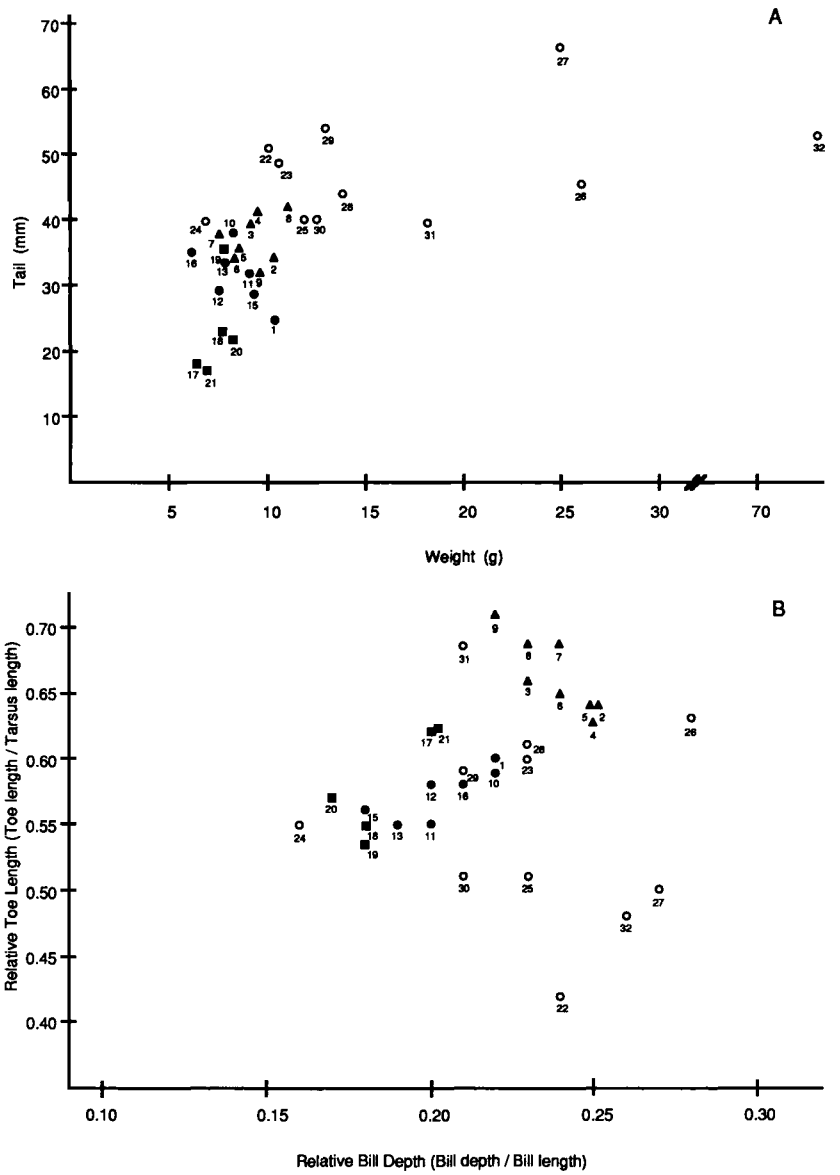

Fig. 3. Morphometric variation in *Myrmotherula* spp. and related antbirds with respect to size and tail length (A), and relative toe length and bill shape (B). Triangles correspond to checker-throated *Myrmotherula* spp., squares correspond to streaked *Myrmotherula* spp., closed circles correspond to gray *Myrmotherula* spp., and open circles correspond to non-*Myrmotherula* species. Numbers for taxa correspond to numbers in Appendix 1.

ric measures that separated the *Myrmotherula* plumage groups were relative bill depth and relative toe length (Fig. 3B). The checker-throated species (including *M. ornata*) had consistently deeper bills and larger feet than other *Myrmotherula*. However, the gray and streaked species overlapped even on these measures.

Principal components analysis for all taxa resulted in five rotated factors with eigenvalues greater than 1.0, which explained 83.0% of the total variance (Table 2). Axes defined by factors 1 and 2 showed relatively clear separation of the three *Myrmotherula* plumage groups (Fig. 4). Factor 1, which represented overall size and

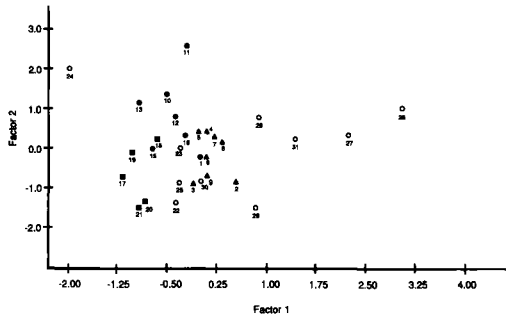

Fig. 4. Distribution of 30 Formicariid taxa in Principal Components space, based on 9 morphometric measures (including weight). See text for interpretation of factors. Numbers and codes as in Fig. 3.

relative bill depth, separated the checker-throated species from all other *Myrmotherula* except *M. hauxwelli*; the gray and streaked groups overlapped completely on this factor. Factor 2 represented relative wing length and shape (long, pointed vs. short, rounded). The gray and streaked *Myrmotherula* species overlapped little along this factor, whereas the checker-throated species all showed intermediate scores. A nearly identical dispersion of the three plumage groups existed along factor 3 (not illustrated), which represented relative tarsus and tail length. Of the 10 outgroup species, 4 overlapped with the *Myrmotherula* species along factor 1, and all overlapped with *Myrmotherula* along factor 2.

The overall morphometric similarity within *Myrmotherula* is reflected in the UPGMA phenogram (Fig. 5) based on the taxonomic distance matrix (available from authors) derived from the raw measurements. Among the other antbird species, *Herpsilochmus rufimarginatus*, *Microrhopias quixensis*, *Hypocnemis cantator*, and *Terenura humeralis*, clustered among the *Myrmotherula* species. The remaining species were either larger in size or longer tailed, and clustered outside the *Myrmotherula* group. Therefore, morphometric measures failed to define clearly *Myrmotherula* apart from other small antwrens.

Genetic.—Of the 32 loci examined, 22 (69%) varied among the species analyzed (Appendix 2). Nei's (1978) average genetic distances within various plumage groups were roughly similar (Table 3): checker-throated *Myrmotherula*, $\bar{D}$ = 0.186; gray *Myrmotherula*, $\bar{D}$ = 0.208; streaked *Myrmotherula*, $\bar{D}$ = 0.136. Distances among single individuals of *Myrmotherula haematonota*

Fig. 5. UPGMA phenogram of 9 morphometric characters (including body weight) based on taxonomic distances for 31 Formicariid taxa. Cophenetic correlation = 0.965. PS, PD, NB, and SB after *Myrmotherula haematonota* indicate sample locality (see Appendix 1).

from allopatric populations averaged 0.060. Average distance within the genus *Myrmotherula* as a whole (0.329) was similar to the average within the family Formicariidae (0.365) as a whole (see Table 3 for a summary of genetic distances in Neotropical birds; matrix of pairwise Rogers' [1972] and Nei's [1978] genetic distances are available from the authors upon request).

The cladistic analysis of alleles based on the maximum parsimony criterion (Fig. 6) divides the *Myrmotherula* species into two major groups. The first major group included all the gray and streaked species, except for *M. assimilis*, which clustered with several non-*Myrmotherula* taxa. There was no clear separation between gray and streaked *Myrmotherula* species. The second major group included all checker-throated *Myrmotherula*, *Microrhopias quixensis*, and *Pygiptila stellaris*. *Myrmotherula ornata* clearly belongs in the checker-throated group.

TABLE 2. Factor loadings of size-transformed morphometric characters of antbirds. Principal components analysis of variable means using Varimax rotation. All variables were divided by the cube root of body weight.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Weight	0.883	0.026	0.160	−0.094	0.080
Wing length	0.130	0.912	0.123	0.089	0.081
Wing-tip extension	−0.165	0.867	−0.254	−0.012	0.005
Tarsus length	0.004	−0.240	0.793	−0.384	0.087
Toe length	0.271	−0.193	−0.042	−0.555	−0.515
Bill length	0.080	0.024	−0.085	−0.054	0.916
Bill depth	0.874	−0.053	0.016	0.366	−0.072
Bill width	0.226	−0.006	−0.037	0.829	−0.059
Tail length	0.226	0.111	0.814	0.331	−0.235
Eigenvalue	2.166	1.968	1.226	1.107	1.003
Proportion of variance	0.241	0.219	0.136	0.123	0.111

The UPGMA phenogram and Distance-Wagner tree (not shown, available from the authors) differed only slightly from the cladogram in topology. The separation of *Myrmotherula* into two major groups remained, although relationships within these clusters and with non-*Myrmotherula* taxa changed slightly. *Myrmotherula fulviventris* was genetically most divergent within the checker-throated group in the distance analyses. Regardless of the type of data analysis performed, the genus *Myrmotherula* was not monophyletic. It would add 21 steps to the phylogeny in Fig. 6 to make *Myrmotherula* monophyletic.

Comparison of genetic, morphometric, and plumage distance matrices.—Mantel's (1967) test (all t-values highly significant, $P < 0.001$) indicated that these matrices have some structure in common, and implied that the pattern of genetic differentiation among these taxa paralleled differences in morphometrics and plumage. Similarly, all pairwise correlations of genetic, morphometric, and plumage distances among these taxa were significant, but correlations were low (from 0.34 between morphometric and genetic distances to 0.58 between genetic and plumage distances).

Scaling of the three distance matrices indicated differences in relative magnitude of character evolution in morphology and protein genes (Fig. 7). In each *Myrmotherula* plumage type and across all taxa examined, plumage distances were relatively higher than genetic distances, whereas morphometric distances were lowest. We suggest that plumage evolution may have been more extensive than protein evolution, whereas morphometric evolution may have been less extensive than protein evolution.

DISCUSSION

The currently recognized genus *Myrmotherula* was defined by the morphological similarity of its members (Sclater 1858). We have shown that this grouping does not reflect a genetic phylogeny and that a systematic revision of the small antwrens is necessary (Rosenberg and Hackett in prep.).

Comparison of evolution in genes and morphology.—Protein evolution in these birds has been paralleled more closely by variation in plumage characteristics than by variation in size and shape. For example, the checker-throated plumage pattern is unique among antbirds; however, the checker-throated species are only weakly differentiated morphometrically from other small antwrens. Our phylogeny suggests that the checker-throated group is a clade separate from all other *Myrmotherula* species. The streaked group also represents a genetic clade, but it probably arose relatively recently from within the gray lineage. As with the gray plumage type, the streaked pattern occurs in other clades (e.g. by our criteria *Hypocnemis cantator* was identical in plumage to *Myrmotherula sclateri*). Without prior knowledge of genetic relationships, these plumage traits could not be used to predict genetic relatedness among the plumage types. For example, we could not have determined that the streaked group was derived from gray ancestors without independent genetic data. Also, the placement of *M. ornata*

TABLE 3. Mean genetic distances ($\bar{D}$; Nei 1978) by taxonomic rank for Neotropical birds. Ranges are given only for taxa examined in this study.

Taxonomic level	Number of comparisons	$\bar{D} \pm$ SD	Range	Reference
Within species				
Myrmotherula haematonota	6	0.060 ± 0.014	0.032–0.066	This study
Mionectes oleagineus	1	0.01		Capparella and Lanyon 1985
Cyclarhis gujanensis	6	0.014 ± 0.009		Johnson et al. 1988
Vireolanius leucotis	3	0.024 ± 0.021		Johnson et al. 1988
Hylophilus ochraceiceps	1	0.00		Johnson et al. 1988
Chiroxiphia pareola	1	0.066		Capparella 1988
Pipra coronata	10	0.019 ± 0.015		Capparella 1988
Glyphorynchus spirurus	10	0.025 ± 0.025		Capparella 1988
Myrmoborus myotherinus	6	0.031 ± 0.034		Capparella 1988
Pithys albifrons	6	0.003 ± 0.002		Capparella 1988
Within genus				
Myrmotherula[a]	210	0.329 ± 0.145	0.032–0.617	This study
checker-throated	28	0.186 ± 0.129	0.032–0.438	This study
gray	28	0.208 ± 0.080	0.049–0.336	This study
streaked	10	0.136 ± 0.068	0.033–0.277	This study
Heliodoxa	21	0.240 ± 0.088		Gerwin 1987
Synallaxis	6	0.19 ± 0.05		Braun and Parker 1985
Cranioleuca	10	0.08 ± 0.03		Braun and Parker 1985
Schizoeaca	1	0.13		Braun and Parker 1985
Mionectes	1	0.08		Capparella and Lanyon 1985
Hylophilus[a]	20	0.302		Johnson et al. 1988
Vireo[a]	142	0.291		Johnson et al. 1988
Pipra	10	0.101		Capparella 1988
Within family				
Formicariidae	496	0.365 ± 0.129	0.032–0.742	This study
Bucconidae[b]	28	0.32 ± 0.09		Lanyon and Zink 1987
Galbulidae[b]	6	0.52 ± 0.11		Lanyon and Zink 1987
Capitonidae[b]	1	0.22		Lanyon and Zink 1987
Ramphastidae[b]	10	0.32 ± 0.07		Lanyon and Zink 1987
Trochilidae	91	0.625 ± 0.215		Gerwin 1987
Vireonidae	322	0.354		Johnson et al. 1988
Cotingidae[b]	3	0.531 ± 0.104		Lanyon 1985
Rupicolidae[b]	28	0.338 ± 0.097		Lanyon 1985
Tyrannidae[b]	105	0.473 ± 0.161		Lanyon 1985

[a] These genera are not monophyletic.

[b] These genetic distances are underestimates because only conservative loci were used in the calculations.

within the checker-throated clade was uncertain. In a similar comparison of genetic and morphological differentiation in Australo-Papuan scrubwrens (*Sericornis*), Christidis et al. (1988) concluded that external morphologic features are not concordant with their genetic phylogeny, and that similarities in plumage represent "unresolved plesiomorphies and homoplasies." Once we have a genetic phylogeny, we can trace the pattern of evolution of plumage and morphometric traits within lineages.

Morphometric conservatism is a characteristic of the family Formicariidae; antbirds seem to share a similar body and bill shape, and vary primarily in overall size. Body weight varied more than other morphometric characters, but morphometric similarity was not simply an effect of similar body sizes. Furthermore, our assessment of plumage variation was conservative. If we recognized more subtle differences in color, the disparity between morphometric and plumage similarities would have been greater. The reason plumage evolution has been

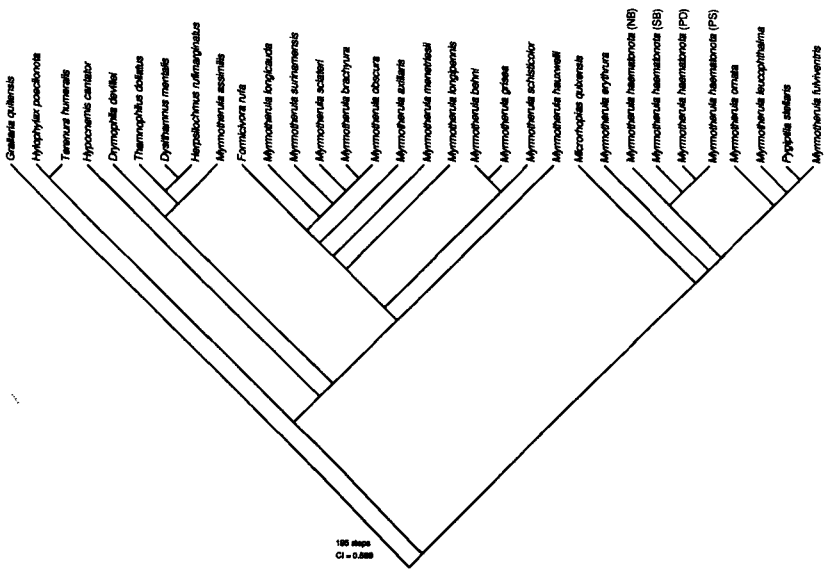

Fig. 6. Parsimony analysis of allelic variation in 32 Formicariid taxa. PS, PD, NB, and SB after *Myrmotherula haematonota* indicate sample locality (see Appendix 1).

more extensive than morphometric evolution is complex. Phenotypic canalization may determine ways in which closely related taxa can diverge, in this case limiting morphometric change in antbirds to size variation on the same basic shape. In the Parulidae, plumage also seems highly variable relative to morphometric variation, whereas plumage variation seems conservative in Furnariidae. Comparison of genetic divergence with plumage and morphometric variation is a way of quantifying "mosaic" evolution (Mayr 1970).

The estimate of genealogical relationships produced from protein electrophoresis is likely a better estimate of phylogeny than one based on comparisons of morphology, although this viewpoint is controversial (Donoghue et al. 1989). Morphological traits are influenced by an unknown number of genes, each with an unknown contribution to the phenotype, and by the effects of natural selection, which can obscure phylogeny through convergence. Also, morphological traits are not likely to be independent, because they are controlled by the same genes (Schaffer 1986, Schluter 1984). The genetic bases of allozyme differences are clearer; the loci are generally independent and relatively free from the effects of natural selection. This does not imply, however, that biochemical estimates are without problems (Lanyon 1988, Pamilo and Nei 1988). For example, if speciation events occurred in bursts with subsequent long periods of independent evolution, then molecular evidence of monophyly may not be retained in extant descendants (Lanyon 1988). In the absence of molecular markers of monophyly, key innovations (behavioral, morphologic, or ecological) may provide evidence of monophyly. In our study, the checker-throated phenotype and specialized dead-leaf foraging were key innovations consistent with our molecular

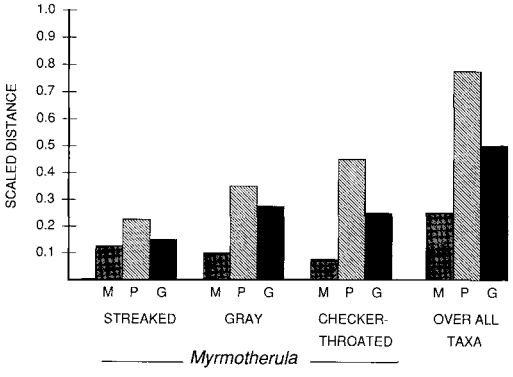

Fig. 7. Comparison of scaled distances within plumage groups and over all taxa for genetic (G), morphometric (M), and male plumage (P) distance matrices.

phylogeny. For data sets that lack molecular support for monophyly, a combination of biochemical and other (morphological, behavioral, ecological) analyses may be necessary to produce robust estimates of phylogenetic relationships.

Disparity between phenetic and cladistic estimates of relatedness exist. In our genetic data set, *M. fulviventris* was the most distinct member of the checker-throated group based on phenetic analysis, whereas the cladistic analysis placed it within this clade (Fig. 6). Several autapomorphies in *M. fulviventris* inflated its genetic distance from other checker-throated species, which placed it outside these species in our phenetic analysis. Such discrepancies may result from variations in the rate of protein evolution or from phenogram instability (Archie et al. 1989). Our cladistic analysis used shared-derived alleles, and autapomorphies have no effect on the species' placement. Thus, in spite of minor discrepancies, both cladistic and phenetic analyses produced similar major groups of species and demonstrated that *Myrmotherula* is not monophyletic.

Levels of genetic differentiation.—Average genetic distance (0.329) in the genus *Myrmotherula* is artificially high because the taxa averaged to get this estimate do not form a monophyletic group. Such overestimates are not unique to this study (Johnson et al. 1988), and caution should be exercised when reporting and comparing genetic distances.

The average genetic distance of 0.060 within *M. haematonota* exceeded the mean value reported between some temperate zone congeneric oscine species ($\bar{D} = 0.0440$; Barrowclough 1980). Genetic distances within other species of tropical birds are of approximately the same magnitude as that within *M. haematonota* (Table 3). We submit that species of Neotropical birds are structured differently from those of temperate birds. Factors that could increase genetic differentiation in Neotropical birds include weak dispersal ability, small long-term effective population sizes, and increased age of the taxa. Capparella (1988) demonstrated the effects of riverine barriers, such as the Amazon and Napo rivers, on the genetic population structure of Neotropical birds. In contrast, there are no known North American avian taxa phenotypically differentiated across even the Mississippi River. The genetic distance (0.066) between two *M. haematonota* populations sampled across the Amazon River is comparable to those found by Capparella (1988) for other taxa.

An implication of the high genetic distances is the probable increased age of tropical forest taxa relative to North American taxa. Even though dating speciation events using molecular data is controversial and requires a number of assumptions (e.g. a molecular clock), such an exercise can be informative. Using a calibration of one unit of Nei's (1978) genetic distance as roughly equivalent to 26 million years of independent evolution (Gutiérrez et al. 1983), we can make a conservative estimate of timing of divergence events within these lineages. With few exceptions, most species-level splits among these antbird taxa occurred at least 2.5 million years before present. Within each plumage type, a few sister taxa probably differentiated during the Pleistocene (e.g. *Myrmotherula longipennis* vs. *M. menetriesii* and *M. obscura* vs. *M. sclateri*). Within the checker-throated clade, all currently recognized species are old, with only population-level differentiation being of Pleistocene origin (i.e. within *M. haematonota*). The checker-throated clade itself has evolved independently from other lineages for approximately 9 million years. Our results, and those of Capparella (1988), contradict Haffer's (1974, 1985, 1987) hypothesis that diversification of Amazonian taxa is largely Pleistocene in origin.

The low levels of genetic differentiation reported for temperate-zone birds have raised questions about the utility of starch-gel electrophoresis of proteins for documenting patterns of population differentiation (see Zink 1986). The comparably greater genetic distances for Neotropical species (at all taxonomic levels) outlined in Table 3 demonstrate that protein electrophoresis permits analyses of Neotropical bird populations.

Our study has implications for several areas of avian systematics. First, current taxonomy, especially of many species-rich tropical groups, may be inadequate, and studies of Neotropical birds may be biased by a taxonomy that does not reflect evolutionary patterns. Morphological analyses should be compared with molecular-based phylogenies in revising classifications, especially at the generic level. Finally, other characteristics of species (ecological, vocal, and behavioral) must be studied to determine how reliable they are as predictors of evolutionary relationships. For example, Christidis et al. (1988) found that patterns of geographical

distribution and foraging niche were consistent with a protein-based phylogeny in cases in which morphological evidence was contradictory. In *Myrmotherula* antwrens, groups based on foraging specialization and vocalizations also correspond to our estimates of genetic relatedness (Rosenberg and Hackett in prep.).

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APPENDIX 1. Scientific names, species codes, and collecting localities of specimens. LSUMNS tissue numbers are in parentheses after scientific name.

Species	Code	Locality
1. *Myrmotherula hauxwelli* (B4128)	MHAUX	Peru: Dpto. Loreto; lower Río Napo region
2. *M. fulviventris* (B2299)	MFULV	Panama: Prov. Darién; E slope Cerro Pirre
3. *M. leucophthalma* (B9223)	MLEUC	Bolivia: Dpto. Pando; S Cobija
4. *M. haematonota* (B1986)	MHPS	Peru: Dpto. Pasco; Villa Rica-Puerto Bermudez Hwy.
5. *M. haematonota* (B9043)	MHPD	Bolivia: Dpto. Pando; S Cobija
6. *M. haematonota* (B4579)	MHSB	Peru: Dpto. Loreto; S bank Río Amazonas, SSW mouth Río Napo
7. *M. haematonota* (B6964)	MHNB	Peru: Dpto. Loreto; N Río Amazonas, 85 km NE Iquitos
8. *M. erythrura* (B5474)	MERYT	Peru: Dpto. San Martín; NE Tarapoto
9. *M. ornata* (B9502)	MORN	Bolivia: Dpto. Pando; S Cobija
10. *M. schisticolor* (B2124)	MSCHI	Panama: Prov. Darién; NW Cana
11. *M. longipennis* (B8877)	MLONP	Bolivia: Dpto. Pando; S Cobija
12. *M. menetriesii* (B9759)	MMENE	Bolivia: Dpto. Pando; S Cobija
13. *M. grisea* (B1132)	MGRIS	Bolivia: Dpto. La Paz; Río Beni
14. *M. behni* (B7453)	MBEHN	Venezuela: TF Amazonas; Cerro de la Neblina
15. *M. assimilis* (B7370)	MASSI	Peru: Dpto. Loreto; Isla Pasto, Río Amazonas
16. *M. axillaris* (B5468)	MAXIL	Peru: Dpto. San Martín; NE Tarapoto
17. *M. obscura* (B4908)	MOBSC	Peru: Dpto. Loreto; S Río Amazonas
18. *M. surinamensis* (B2229)	MSURI	Panama: Prov. Darién; E. slope Ceror Pirre
19. *M. longicauda* (B1091)	MLONC	Bolivia: Dpto. La Paz; Río Beni
20. *M. sclateri* (B9715)	MSCLA	Bolivia: Dpto. Pando; S Cobija
21. *M. brachyura* (B4722)	MBRAC	Peru: Dpto. Loreto; S Río Amazonas
22. *Drymophila devillei* (B9350)	DDEV	Bolivia: Dpto. Pando; S Cobija
23. *Microrhopias quixensis* (B9294)	MQUIX	Bolivia: Dpto. Pando; S Cobija
24. *Terenura humeralis* (B4964)	THUME	Peru: Dpto. Loreto; S Río Amazonas
25. *Herpsilochmus rufimarginatus* (B2162)	HRUFI	Panama: Dpto. Darién; NW Cana
26. *Pygiptila stellaris* (B9703)	PSTEL	Bolivia: Dpto. Pando; S Cobija
27. *Thamnophilus doliatus* (B1488)	TDOL	Bolivia: Dpto. Santa Cruz; S Santa Cruz
28. *Dysithamnus mentalis* (B2003)	DMENT	Peru: Dpto. Pasco; Villa Rica-Puerto Bermudez Hwy
29. *Formicivora rufa* (B6821)	FRUFA	Bolivia: Dpto. Beni; N Yacumo
30. *Hypocnemis cantator* (B9140)	HCANT	Bolivia: Dpto. Pando; S Cobija
31. *Hylophylax poecilonota* (B8905)	HPOEC	Bolivia: Dpto. Pando; S Cobija
32. *Grallaria quitensis* (B357)	GQUIT	Peru: dpts. Piura-Cajamarca; Cerro Chinguela

APPENDIX 2. Allelic frequencies for 22 variable loci. Abbreviations for taxa can be found in Appendix 1. See text for abbreviations for loci.

Locus	MHAUX	MFULV	MLEUC	MHPS	MHPD	MHSB	MHNB	MERYT	MORN	MSCHI	MLONP	MMENE	MGRIS	MBEHN	MASSI	MAXIL	MOBSC
ICD1	d	c	d	d	d	d(0.50) e(0.50)	d	d	d	d	d	d	d	d	e	d	d(0.50) e(0.50)
LGG	d	c(0.50) d(0.50)	d	d	d	d	d	d	d	d	d	d	c	b	c	c	b
LA	b	b	b	b	b	a	b	b	b	a	b	b	a	a	a	b	b
PP	e	e	d	e	c	d	d	d	d	e	e	e	e				
GPI	d	e	e	e	e	e	e	e	e	d	d	d	d	d	e	d	a
MPI	b	a	a	b	b	b	b	b	b	b	b	a	b(0.50) e(0.50)	d b	d a(0.50) b(0.50)	d b	d a(0.50) b(0.50)
GOT1	a	b	c	c	c	c	c	c	c	a	a	a	a				
GOT2	b	b	b	b	b	b	b	b	b	b	b	b	b	a	a	a	a
PGM1	a	a	a	a	a	a	a	a	a(0.50) b(0.50)	a	a	a	a	b a	b a	b a	b a
LDHA	b	b	b	a	a	a	b	b	b	b	b	b	b	b	b	b	b
LDHB	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b
CK1	a	c	a	a	a	a	a	a	a	c	c	c	c	c	b	b	a
CK2	b	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	a(0.50) b(0.50)
α-GPD	f	f	d	d	d	d(0.50) f(0.50)	d	d	b	f	f	f	f	f	e	f	f
GDA	b	c	a	a	a	a	a	a	a	b	b	b	b	b	a	b	b
ESTD	a	c	a	a	a	a	a	a	a	b	b	b	b	b	a	b	b
ADH	a	a	a	a	a	a	a	d	a	f	a	a	e	e	a	a(0.50) d(0.50)	a
MEI	d	b	b	c	c	c	c	a	b	d	d	d	d	d	d	d	d
SDH	b	b	c	b	b	b	b	b	b	b	c	c	b	b	b	c	c
AK	b	b	b	b	b	b	b	b(0.50) c(0.50)	b	b	b	b	b	a	b	b	b
SOD1	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	b
ADA	c	c	d	d	d	d	d	e	d	f	e(0.50) f(0.50)	c	d	d	d	c	c

APPENDIX 2. Continued.

Locus	MSURI	MLONC	MSCLA	MBRAC	DDEV	MQUIX	THUME	HRUFI	PSTEL	TDOL	DMENT	FRUFA	HCANT	HPOEC	GQUIT
ICD1	d	d	d	f(0.50) g(0.50)	a	c	e	d	d	d	d	d	b	b	d
LGG	b	c	a(0.50) b(0.50)	b	f	d	d(0.50) e(0.50)	c	d	b	d	e	d	d	d
LA	b	b	b	d	c	b	a(0.50)	a	b	b	b	b	b	b	e
PP							c(0.50)			g	e	b	f	d	h
GPI	b	b	b	a	e	e	b	e	e	e	a	b	c	e	d
MPI	d	d	d	d	d	e	e	d	e	c	d	b	a(0.50)	b	b
	b	b	a	b	b	b	b	d	a				b(0.50)	c	c
GOT1										a	a	c	a	b	b
GOT2	a	a	a	a	a	c	c	a	c	b	a	b	b	a	c
PGM1	b	b	b	b	b	b	b	b	b	a	a	a	a	b	b
	a	a	a	a	a	a	a	a	a						
LDHA	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b
LDHB	b	b	b	b	b	b	b	b	c	b	a	a	b	b	d
CK1	a	b	a	a	b	a	e	b	e	b	c	c	a	b	d
CK2	b	b(0.50) c(0.50)	b	b	c	c	b	c	c	c	c	b	c	b	c
α-GPD	f	f	f	f	h	d(0.50) f(0.50)	b	b(0.50) c(0.50)	c	g	e	f	g	b	a
GDA	b	b	b	b	b	b	b	b	a	b	b	d	b	b	b
ESTD	b	b	b	b	a	a	a(0.50) b(0.50)	b	a	a	a	a	a	a	a
ADH	a	a	a	a	a	d	a	a	a	a	c	a	a	a	b
ME1	d	d	d	d	d	c	c	d	b	d	d	d	d	c	e
SDH	b	c	c	b	c	b	b	a(0.50) b(0.50)	a	a(0.50) b(0.50)	a	c	a(0.50) c(0.50)	b	b
AK	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b
SOD1	a	c	b	b	f	f	f	f	f	f	f	f	g	d(0.50) e(0.50)	h
ADA	c	a	c	c	b(0.50) c(0.50)	a	c	d	d	d	d	c	a	c(0.50) d(0.50)	f

APPENDIX 3. Male and female plumage characters for taxa studied. Species abbreviations can be found in Appendix 1.

Taxon	Sex	Throat	Breast	Back	Crown	Wing	Tail
MBRAC	m	white	yellow	black streaked	streaked	wing bars	black with white tip
MBRAC	f	white	yellow	black streaked	streaked	wing bars	black with white tip
MOBSC	m	white	yellow	black streaked	streaked	wing bars	black with white tip
MOBSC	f	buffy	yellow	black streaked	streaked	wing bars	black with white tip
MSCLA	m	yellow	yellow	black streaked	streaked	wing bars	black with white tip
MSCLA	f	yellow	yellow & black	black streaked	streaked	wing bars	black with white tip
MSURI	m	black & white	black & white	black streaked	streaked	wing bars	black with white tip
MSURI	f	buffy	buffy	black streaked	rufous with black	wing bars	black with white tip
MLONC	m	white	black & white	black streaked	streaked	wing bars	black with white tip
MLONC	f	buffy	buffy	black streaked	streaked	wing bars	black with white tip
MHAUX	m	gray	gray	gray	gray	spots on secondaries	black with white tip
MHAUX	f	rufous	rufous	brown	brown	spots on secondaries	black with white tip
MLEUC	m	checkered	gray-brown	brown	brown	buffy spots	plain brown
MLEUC	f	rufous	buffy	brown	brown	buffy spots	plain brown
MFULV	m	checkered	gray-brown	brown	brown	buffy spots	plain brown
MFULV	f	buffy	buffy	brown	brown	buffy spots	plain brown
MHNB	m	checkered	gray	rufous	brown	buffy spots	plain brown
MHNB	f	checkered	gray-brown	rufous	brown	buffy spots	plain brown
MHSB	m	checkered	gray	rufous	brown	buffy spots	plain brown
MHSB	f	checkered	gray-brown	rufous	brown	buffy spots	plain brown
MHPS	m	checkered	gray	brown	brown	buffy spots	plain brown
MHPS	f	rufous	rufous	brown	brown	buffy spots	plain brown
MHPD	m	checkered	gray	rufous	brown	buffy spots	plain brown
MHPD	f	checkered	gray-brown	rufous	brown	buffy spots	plain brown
MORN	m	short black	gray-brown	gray	gray	white spots	blackish plain
MORN	f	checkered	rufous	brown	brown	buffy spots	plain brown
MERYT	m	gray	gray	rufous	brown	buffy spots	rufous
MERYT	f	rufous	gray-brown	rufous	brown	buffy spots	rufous
MAXIL	m	extended black	black with white	concealed patch	black	white spots	black with white tip
MAXIL	f	buffy	buffy	brown	brown	buffy spots	plain brown
MSCHI	m	extended black	gray	concealed patch	gray	white spots	blackish plain
MSCHI	f	rufous	rufous	gray	brown	plain	blackish plain
MLONP	m	extended black	gray	concealed patch	gray	white spots	black with white tip
MLONP	f	buffy	buffy	gray	gray	plain	blackish plain
MGRIS	m	gray	gray	gray	gray	plain	blackish plain
MGRIS	f	buffy	rufous	brown	gray	plain	rufous
MMENE	m	extended black	gray	gray	gray	white spots	black with white tip
MMENE	f	buffy	buffy	gray	brown	plain	blackish plain
MASSI	m	gray	gray	concealed patch	gray	white spots	black with white tip
MASSI	f	buffy	buffy	concealed patch	gray	blackish plain	black with white tip
MBEHN	m	extended black	gray	gray	gray	plain	blackish plain

APPENDIX 3. Continued.

Taxon	Sex	Throat	Breast	Back	Crown	Wing	Tail
DMENT	m	gray	gray	gray	gray	white spots	blackish plain
DMENT	f	grayish white	olive	brown	rufous	plain	plain brown
TDOL	m	black & white	black & white bars	black & white bars	black	black & white bars	black & white tip
TDOL	f	rufous	rufous	rufous	rufous	plain	rufous
PSTEL	m	gray	gray	concealed patch	black	white spots	blackish plain
PSTEL	f	buffy	buffy	gray	brown	plain	blackish plain
THUME	m	gray	olive	rufous	black	wing bars	olive
THUME	f	grayish white	olive	rufous	brown	wing bars	olive
HRUFI	m	grayish white	yellow	greenish	black with superciliary	wing bars	white outer tail feathers
HRUFI	f	grayish white	yellow	brown	rufous with superciliary	wing bars	white outer tail feathers
MQUIX	m	extended black	black	concealed patch	black	white spots	white outer tail feathers
MQUIX	f	rufous	rufous	concealed patch	black	white spots	white outer tail feathers
FRUFA	m	extended black	buffy	brown	rufous with superciliary	white spots	black with white tip
FRUFA	f	black & white	black & white	rufous	rufous with superciliary	white spots	black with white tip
DDEV	m	white	unique	concealed white patch	streaked	white spots	black with white tip
DDEV	f	black & white	unique	black streaked	streaked	buffy spots	black with white tip
HCANT	m	yellow	yellow & black	black streaked	streaked	white spots	plain brown
HCANT	f	yellow	yellow & black	black streaked	streaked	wing bars	plain brown
HPOEC	m	gray	gray	black & white bars	gray	spots on secondaries	black with white tip
HPOEC	f	gray	gray	brown	rufous	plain	black with white tip