Evolution in the High Andes: The Phylogenetics of Muscisaxicola Ground-Tyrants

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Phylogenetic relationships within the genus Muscisaxicola, a primarily Andean group of tyrant-flycatchers, were studied using complete sequences of the mitochondrial genes COII and ND3. Relationships among Muscisaxicola species were found to differ substantially from those of previous views, suggesting convergence in traditional avian taxonomic characters within the genus. The 11 species of large, gray, “typical” Muscisaxicola flycatchers (including M. grisea, newly restored to species status) formed a distinct clade, consisting of two major groups: a clade of 6 species breeding primarily in the central Andes and a clade of 5 species breeding primarily in the southern Andes. The other 2 species traditionally placed in this genus, M. fluviatilis, an Amazonian species, and M. maculirostris, were both rather divergent genetically from the typical species, although M. maculirostris may be the sister taxon to the typical clade. The patterns of sympatry exhibited by Muscisaxicola species in the high Andes appear to be the consequence of speciation and secondary contact within regions of the Andes, rather than a result of dispersal between regions. Diversification of the typical Muscisaxicola species appears to have occurred during the middle and late Pleistocene, suggesting generally that taxa of the high Andes and Patagonia may have been greatly influenced by mid-to-late Pleistocene events. There were likely several independent developments of migration within this genus, but migration is probably ancestral in the southern clade, with subsequent loss of migration in two taxa.

Key Words: Andes; systematics; Tyrannidae; biogeography; evolution of migration.

The Andes have provided exceptionally fertile ground for the study of biogeography, geographic variation, and speciation (e.g., Chapman, 1917, 1926; Vuilleumier, 1969; Vuilleumier and Simberloff, 1980; Remsen, 1984; Hillis, 1985; Fjeldså, 1985; Graves, 1985; Patton and Smith, 1992; Bates and Zink, 1994), due in large part to the patterns of elevational zonation shown by many Andean organisms and to the geography of the mountains themselves, which occupy a narrow longitudinal but broad latitudinal range. Inhabitants of the páramo and puna, the distinctive open habitats of the high Andes, have been the focus of much of this research, numerous investigators having undertaken studies of the origins of high Andean organisms, their relationships to lowland faunas of the Neotropics, their adaptive strategies, or their diversification and radiation (see especially papers in Vuilleumier and Monasterio, 1986). Few of these studies, however, have adopted a phylogenetic approach.

Muscisaxicola ground-tyrants (Aves: Passeriformes, Tyrannidae) are a characteristic group of the high Andes and exhibit many of the features typical of high Andean organisms, including patterns of distribution suggestive of a complex speciation history and apparent adaptations to the high Andean environment. Eleven of the 12 traditional species (Hellmayr and Cory, 1927; Traylor, 1979) breed in open scrub and grassland in the high Andes and Patagonia (an isolate of 1 species occurs additionally on the Falkland Islands), but the other species (M. fluviatilis) is an exclusively lowland bird, occupying sandbars along watercourses in Amazonia. Although they occupy structurally simple habitats and are very similar behaviorally and morphologically, as many as 4–5 species of Muscisaxicola can be found breeding in sympatry in both the central Andes of Peru and Bolivia (Vuilleumier, 1971) and the southern Andes of Chile (Cody, 1970; R.T.C., pers. obs.). Several traits of Muscisaxicola species are thought to be adaptations to the extreme environments in which they occur, including inconspicuous plumage (all species are primarily gray or grayish brown, differing mainly in head markings; Table 1), relatively long legs, and simplified vocalizations (Vuilleumier, 1971); their highly terrestrial habitats represent an extreme behavioral type among tyrant-flycatchers. Muscisaxicola is among the most migratory genera of South American birds (Chesser, 1994); 8 species are austral migrants, breeding in south temperate South America and migrating north, primarily in the Andes, for the southern winter (Chesser, 1995; Table 1).
Muscisaxicola is widely held to be closely related to such other ground-tyrant genera as Agriornis and Xolmis (Vuilleumier, 1971; Traylor, 1977; Lanyon, 1986), but little previous phylogenetic work has been done within the genus. Only Vuilleumier (1971) has published an explicit classification of the genus; this classification was based primarily on plumage patterns, size, shape, and proportions, and it divided the traditional genus Muscisaxicola into five groups of 2–3 species each (Table 1). In addition, Vuilleumier also merged the traditional Muscisaxicola with the monotypic genus Muscigralla, so that his Muscisaxicola was composed of the two subgenera Muscisaxicola (12 species) and Muscigralla (1 species).

Species limits in Muscisaxicola have been generally agreed upon in most recent treatments (e.g., Vuilleumier, 1971; Traylor, 1979; Fjeldså and Krabbe, 1990; Ridgely and Tudor, 1994), although the taxon in the alpina–cinerea group (Vuilleumier, 1971) have been the subject of some controversy. Most detailed considerations of Muscisaxicola (e.g., Hillmayr and Cory, 1927; Vuilleumier, 1971; Traylor, 1979) and modern volumes on South American birds (e.g., Fjeldså and Krabbe, 1990; Ridgely and Tudor, 1994) have considered M. alpina and M. cinerea to be separate species, but in the past they were sometimes considered conspecific (e.g., Hillmayr, 1932; Goodall et al., 1957; Meyer de Schauensee, 1970). Fjeldså and Krabbe (1990) recently suggested that the subspecies M. cinerea argentina may actually be conspecific with M. alpina, and Ridgely and Tudor (1994) noted that M. alpina grisea may be specifically distinct from the remainder of M. alpina (i.e., M. a. alpina, M. a. columbiana, and M. a. quesadae).

In this paper, I derive a molecular phylogenetic hypothesis for the genus Muscisaxicola and use this phylogeny to address the following questions:

(1) Does the traditional genus Muscisaxicola constitute a monophyletic group? Do the individual species of Muscisaxicola appear to be monophyletic? Do phylogenetic relationships among species conform to ideas of relationship based on plumage and morphometric characters? Is Muscigralla closely related to Muscisaxicola?

(2) In what geographical context did the patterns of sympatry arise among Muscisaxicola species? Is the Amazonian isolate M. fluviatilis sister to the rest of the genus? What do the molecular data suggest about the time scale over which Muscisaxicola species have evolved?

(3) How many times and under what circumstances did migration evolve among Muscisaxicola species? Were there many independent developments of migration, or was there a south temperate radiation of migratory taxa from a presumed migratory ancestor?

**METHODS**

Tissues were obtained for individual Muscisaxicola species and outgroups during personal fieldwork in

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**TABLE 1**

<table>
<thead>
<tr>
<th>Species group</th>
<th>Length and dorsal coloration</th>
<th>Distinguishing head markings</th>
<th>Breeding distribution</th>
<th>Migration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15 cm, Gray-brown</td>
<td>None</td>
<td>N, C, S Andes/Patagonia</td>
<td>Polymorphic</td>
</tr>
<tr>
<td>1</td>
<td>13 cm, Gray-brown</td>
<td>None</td>
<td>Western Amazonia</td>
<td>Nonmigratory</td>
</tr>
<tr>
<td>2</td>
<td>15.5 cm, Dark gray</td>
<td>Dark face</td>
<td>S Andes/Patagonia, Falkland Islands</td>
<td>Polymorphic</td>
</tr>
<tr>
<td>2</td>
<td>16.5 cm, Pale gray</td>
<td>Dark face, chestnut crown patch</td>
<td>S Andes/Patagonia</td>
<td>Migratory</td>
</tr>
<tr>
<td>3</td>
<td>16.5 cm, Gray</td>
<td>Rufous crown patch</td>
<td>C Andes</td>
<td>Polymorphic</td>
</tr>
<tr>
<td>3</td>
<td>16.5 cm, Gray</td>
<td>Rufous crown patch</td>
<td>C Andes</td>
<td>Nonmigratory</td>
</tr>
<tr>
<td>3</td>
<td>16.5 cm, Gray</td>
<td>Rufous crown patch</td>
<td>C, S Andes/Patagonia</td>
<td>Migratory</td>
</tr>
<tr>
<td>4</td>
<td>18 cm, Gray (C), gray brown (N)</td>
<td>None</td>
<td>N, C Andes</td>
<td>Nonmigratory</td>
</tr>
<tr>
<td>4</td>
<td>16 cm, Gray</td>
<td>None</td>
<td>C Andes</td>
<td>Polymorphic</td>
</tr>
<tr>
<td>5</td>
<td>20 cm, Gray</td>
<td>Conspicuous white lores</td>
<td>C Andes</td>
<td>Nonmigratory</td>
</tr>
<tr>
<td>5</td>
<td>18.5 cm, Gray</td>
<td>Yellow crown patch, conspicuous white lores</td>
<td>C, S Andes/Patagonia</td>
<td>Migratory</td>
</tr>
<tr>
<td>5</td>
<td>18 cm, Gray-brown</td>
<td>Conspicuous white lores, dark forehead</td>
<td>C, S Andes/Patagonia</td>
<td>Migratory</td>
</tr>
</tbody>
</table>

### TABLE 2

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue number</th>
<th>Date</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. albifrons 1</td>
<td>B-22575</td>
<td>4 July 1993</td>
<td>Bolivia: Dep. La Paz, Zongo Valley, 7 km by road N of summit, 4150 m</td>
</tr>
<tr>
<td>M. albifrons 2</td>
<td>B-22576</td>
<td>4 July 1993</td>
<td>Bolivia: Dep. La Paz, Zongo Valley, 7 km by road N of summit, 4150 m</td>
</tr>
<tr>
<td>M. albiflora 1</td>
<td>RTC 420</td>
<td>20 Nov. 1996</td>
<td>Chile: Region Metropolitana, Prov. Cordillera, ca. 2 km ENE Embalse El Yeso, ca. 2500 m</td>
</tr>
<tr>
<td>M. a. alpina</td>
<td>RTC 421</td>
<td>20 Nov. 1996</td>
<td>Chile: Region Metropolitana, Prov. Cordillera, ca. 2 km ENE Embalse El Yeso, ca. 2500 m</td>
</tr>
<tr>
<td>M. albilora</td>
<td>RTC 377</td>
<td>10 Feb. 1996</td>
<td>Argentina: Prov. Río Negro, Depo. Norquinco, ca. 5 km E Manuel Choique, Ruta Provincial 6, ca. 1100 m</td>
</tr>
<tr>
<td>M. capistrata 1</td>
<td>B-103896</td>
<td>5 July 1983</td>
<td>Peru: Depo. Puno, km 73 on Puno-Desquadero Road, ca. 5 km W Juli, 3800 m</td>
</tr>
<tr>
<td>M. c. cinerea 1</td>
<td>RTC 422</td>
<td>20 Nov. 1996</td>
<td>Chile: Region Metropolitana, Prov. Cordillera, ca. 2 km ENE Embalse El Yeso, ca. 2500 m</td>
</tr>
<tr>
<td>M. cinerea argentina</td>
<td>JAG 1792</td>
<td>4 Oct. 1995</td>
<td>Argentina: Prov. Tucumán, El Infiernillo, 7 km N, 60 km W San Miguel de Tucumán, 3370 m</td>
</tr>
<tr>
<td>M. f. flavinucha 1</td>
<td>RTC 362</td>
<td>8 Feb. 1996</td>
<td>Argentina: Prov. Río Negro, Depo. Bariloche, Cerro Perito Moreno, ca. 20 km N E El Bolson, ca. 1500 m</td>
</tr>
<tr>
<td>M. f. flavinucha 2</td>
<td>RTC 433</td>
<td>21 Nov. 1996</td>
<td>Chile: Region Metropolitana, Prov. Cordillera, ca. 15 road km ENE Embalse El Yeso, ca. 3400 m</td>
</tr>
<tr>
<td>M. f. flavinucha</td>
<td>B-1188</td>
<td>6 July 1981</td>
<td>Bolivia: Depo. La Paz, Río Beni, ca. 20 km by river N Puerto Linares, 600 m</td>
</tr>
<tr>
<td>M. frontalis 1</td>
<td>RTC 416</td>
<td>20 Nov. 1996</td>
<td>Chile: Region Metropolitana, Prov. Cordillera, ca. 2 km ENE Embalse El Yeso, ca. 2500 m</td>
</tr>
<tr>
<td>M. frontalis 2</td>
<td>RTC 432</td>
<td>21 Nov. 1996</td>
<td>Chile: Region Metropolitana, Prov. Cordillera, ca. 15 road km ENE Embalse El Yeso, ca. 3400 m</td>
</tr>
<tr>
<td>M. juninensis</td>
<td>B-1203</td>
<td>19 Jul 1981</td>
<td>Bolivia: Depo. La Paz, 5.4 km by road W hydroelectric dam on Zongo Valley Road, 4600 m</td>
</tr>
<tr>
<td>M. madoviana mentalis 1</td>
<td>RTC 363</td>
<td>8 Feb. 1996</td>
<td>Argentina: Prov. Río Negro, Depo. Bariloche, Cerro Perito Moreno, ca. 20 km N E El Bolson, ca. 800 m</td>
</tr>
<tr>
<td>M. madoviana mentalis 2</td>
<td>PRS 1137</td>
<td>9 Feb. 1996</td>
<td>Argentina: Prov. Río Negro, Depo. Bariloche, Cerro Perito Moreno, ca. 20 km N E El Bolson, ca. 1500 m</td>
</tr>
<tr>
<td>M. m. maculirostris 1</td>
<td>B-103851</td>
<td>18 June 1993</td>
<td>Peru: Depto. Arequipa, Cerro Cosnatire, 5 km E Chala, 425 m</td>
</tr>
<tr>
<td>M. m. maculirostris 2</td>
<td>JAG 1793</td>
<td>4 Oct. 1995</td>
<td>Argentina: Prov. Tucumán, El Infiernillo, 7 km N, 60 km W San Miguel de Tucumán, 3320 m</td>
</tr>
<tr>
<td>M. rufivertex occipitalis</td>
<td>PRS 1121</td>
<td>19 Aug. 1994</td>
<td>Peru: Depto. Arequipa, 37 km E Arequipa by road, 11700 ft</td>
</tr>
<tr>
<td>Acriornis montana</td>
<td>RTC 423</td>
<td>20 Nov. 1996</td>
<td>Chile: Region Metropolitana, Prov. Cordillera, ca. 2 km ENE Embalse El Yeso, ca. 2500 m</td>
</tr>
<tr>
<td>Lesonia rufa</td>
<td>RTC 456</td>
<td>29 Nov. 1996</td>
<td>Chile: Regio IX (de La Araucania), Prov. Malleco, ca. 2 km by road from SE end of Lago Gualletué, near origin of río Bió-Bío, 1050 m</td>
</tr>
<tr>
<td>Muscicralla brevicauda</td>
<td>ANSP 4244</td>
<td>18 June 1992</td>
<td>Ecuador: Depto. Guayas, 5 km N Playa, 50 m</td>
</tr>
<tr>
<td>Xolmis pyrope</td>
<td>RTC 393</td>
<td>15 Nov. 1996</td>
<td>Chile: Region Metropolitana, Prov. Chacabuco, 4 km SSW by road from peak of Cerro de El Roble, ca. 1600 m</td>
</tr>
</tbody>
</table>

Note. Voucher specimens for RTC and PRS numbers are housed at the American Museum of Natural History; B- numbers at the Museum of Natural Science, Louisiana State University; ANSP numbers at the Academy of Natural Sciences of Philadelphia; and JAG numbers at the Burke Museum, University of Washington.

Chile and Argentina and from the frozen tissue collections of the Museum of Natural Science, Louisiana State University; the Academy of Natural Sciences of Philadelphia; and the Burke Museum, University of Washington (Table 2). To reduce the possibility of phylogenetic errors due to incomplete lineage sorting, two individuals of each Muscisaxicola species were sampled, with the exception of M. juninensis and M. fluviatilis, for which only single individuals were available. Conspecific individuals were selected from localities as distant as possible, and from different subspecies when available, to provide simple tests of
monophyly of individual species; however, in several instances conspecifics from distant localities were unavailable and individuals from the same or nearby localities were used (Table 2). Outgroups sampled included single individuals of Muscicola brevicauda, Xolmis pyrope, Agriornis montana, and Lessonia rufa, members of ground-tyrant genera postulated to be closely related to Muscisaxicola, and a single individual of Tyrannus melancholicus, member of a tyrannid genus more distantly related to the ground-tyrants (Sibley and Ahlquist, 1985, 1990).

DNA was extracted using a 5% Chelex solution (Walsh et al., 1991). Two complete, protein-coding mitochondrial genes, cytochrome oxidase II (COII; 684 bp) and NADH dehydrogenase subunit 3 (ND3; 351 bp), were amplified via the polymerase chain reaction, using standard protocols (Chesser, 1999). Primers used for COII were (1) L8263 (5'-GCCACTCATGCCTTCTTTATGGG-3'; Chesser, 1999), (2) L8740 (5'-GGCCTCCTGGACTACTAGAAGT-3; courtesy of J. Cracraft and J. Feinstein), and (4) H9085 (5'-CAGGGTGGTCTGTAGTGGTGATTTAGTCGTCC-3'; Lee et al., 1997). "H" and "L" refer here to the heavy and light strands of the mitochondrial genome, respectively, and reference numbers are for the 3' base corresponding to the chicken sequence of Desjardins and Morais (1990). Primers used for ND3 were (1) L10755 (5'-GACCTTCCAATCTTTAAAATCTGG-3'; Chesser, 1999), (2) L11151 (5'-GATTTGTTGAGCCGAAATCAAC-3'; Chesser, 1999), and (3) H11289 (5'-GATAGTATTATGCTTCTAGGGCA-3'; courtesy of G. Barrowclough and J. Groth). Sequencing was conducted using dye-terminator chemistry on an ABI 377 automated sequencer (Applied Biotechnologies Inc., Foster City, CA). Both heavy and light strands were sequenced for all analyzed sequences. Sequences were aligned using the computer program Sequencer 3.0 (GeneCodes, 1995). All sequences used in this study have been deposited in GenBank (Accession Nos. AF 132614–132640 for COII sequences; AF 132641–132667 for ND3 sequences).

Analysis of sequence data was performed using the computer program PAUP* 4.0d64 (Swofford, 1998), with maximum-parsimony as the primary method of data analysis. T. melanochilus was designated the outgroup in all analyses. Parsimony analyses using branch-and-bound searches were conducted with equal character weighting and with downweighting of transitions (by 5:1, the observed transition/transversion ratio in the dataset, as estimated from the most-parsimonious tree). Character support for phylogenies was assessed via bootstrapping (Felsenstein, 1985), computed for 100 branch-and-bound replicates, and branch support (Bremer, 1988, 1994), calculated using the program TreeRot (Sorenson, 1996).

Sequence data were also analyzed using alternative methods, because simulations have shown that agreement among phylogenies estimated using more than one method can be an index of the reliability of the resultant phylogenies (Kim, 1993). Data were analyzed using distance methods, which have been shown to find the proper tree in some instances in which parsimony fails (Hendy and Penny, 1989), and a simplified maximum-likelihood approach. Neighbor-joining analyses were conducted using uncorrected distance and Kimura two-parameter distance, and support was assessed using bootstrapping. The maximum-likelihood analysis was performed on the "typical" Muscisaxicola species (see below) as delineated by both parsimony and distance analyses, with M. maculirostris designated the outgroup. Heuristic searches were conducted with 10 random addition replicates, using a likelihood model employing empirical base frequencies, a transition/transversion ratio estimated from a neighbor-joining tree, and equal rates at all sites.

Two statistical tests were used to assess whether alternative topologies, in which M. alpina grisea and M. a. alpina were constrained to be sister taxa (see below), were significantly different from the best trees found using maximum-parsimony and maximum-likelihood, respectively. The single most-parsimonious tree was tested against the shortest constrained tree using the Wilcoxon signed ranks test (or Templeton test; Templeton, 1983). The single most likely tree was tested against the most likely constrained tree using the Kishino-Hasegawa test (Kishino and Hasegawa, 1989).

Biogeographic analysis of the genus was performed using DIVA 1.1 (Ronquist, 1996), a computer program that parsimoniously infers ancestral distributions based on phylogenetic and current distributional data, without making assumptions about general biogeographic patterns. DIVA is based on the variable assessment of costs for events such as vicariance, dispersal, and extinction. In brief, speciation events are assumed to divide ranges into vicariant components; DIVA reconstructs ancestral distributions based on minimizing the number of dispersal and extinction events implied by the ancestral distributions (see Ronquist, 1997 for complete details). Continental breeding distributions were classified as either northern Andean (from Ecuador north), central Andean (from central Argentina and Chile north to Peru), southern Andean/Patagonian (from central Argentina and Chile south), or some combination of these. Divisions between these areas correspond to natural barriers to gene flow proposed by Vuilleumier (1969): the "Northern Peruvian Low" (separating the Ecuadorian and Peruvian Andes) and the "Central Chilean-Argentine Andes" (separating the southern Chilean-Argentine Andes from the central Andes). The Falkland Islands, inhabited by M. m. madoviana, constituted a fourth area of classification. Because DIVA does not handle trees with polytomies,
reconstructions were conducted on all possible fully resolved most-parsimonious trees.

The evolution of migration was analyzed using the computer program MacClade 3.05 (Maddison and Maddison, 1993). Traditionally recognized species of Muscisaxicola either are migratory, are nonmigratory, or consist of multiple subspecies, some of which are migratory and some of which are nonmigratory (Table 1). Species in these groups were coded as “migratory,” “nonmigratory,” and “polymorphic” for the character state reconstructions in DIVA and MacClade, respectively. The phylogenies used for all characters were migratory, “nonmigratory,” and some of which are nonmigratory (Table 1). Patterns of sequence divergence were similar for both COII and ND3 (see below) as reconstructed by maximum-parsimony, test, $P = 0.001$, with relatively fewer nonsynonymous substitutions in COII (19 nonsynonymous and 177 synonymous substitutions in COII, and 26 and 97, respectively, in ND3), consistent with the known greater selective constraint on cytochrome oxidase genes (e.g., Simon et al., 1994; Nachman et al., 1996).

Uncorrected sequence divergence (Table 3) ranged from 10.2 to 15.5% in comparisons between ingroup (Muscisaxicola, as traditionally defined) and outgroup taxa. Mean interspecific sequence divergence within the traditional Muscisaxicola ranged from 0.3% (between M. cinerea and M. flavinucha) to 12.3% (between M. fluvatilis and M. maculirostris). Excluding M. fluvatilis and M. maculirostris, both of which were highly divergent from the rest of the genus, interspecific sequence divergence was low, varying from 0.3 to 2.9% (between M. capistrata and M. juninensis). Intraspecific sequence divergence was likewise low, varying from 0.0% between the two M. albifrons individuals and between the two M. macloviana individuals to 0.5% between the two M. Rufivertex individuals (excluding the 2.2% divergence between the two M. alpina individuals, which are actually representatives of two different species, as discussed below). Patterns of sequence divergence were similar for both COII and ND3, when analyzed separately.

**RESULTS**

Sequence variation. Of 1035 bp sequenced, 319 sites (30.8%) were variable, and 216 of these were phylogenetically informative. The COII and ND3 sequences provided similar proportions of informative sites. First, second, and third positions varied greatly in their variability: 51 first position sites were variable (16.0% of variable sites), 19 second position sites (6.0%), and 249 third position sites (78.1%); these percentages are very similar to previous data on variability in these two genes in suboscine birds (Chesser, 1999). The two genes differed significantly ($\chi^2 = 8.69$, df = 2; $P = 0.013$) in their distribution of site changes, with relatively low first and especially second position variability in COII (29, 6, and 161 variable sites, respectively, compared to 22, 13, and 88 in ND3). Ratio of synonymous to nonsynonymous substitutions was likewise significantly different (Fisher’s Exact test, $P = 0.001$), with relatively fewer nonsynonymous substitutions in COII (19 nonsynonymous and 177 synonymous substitutions in COII, and 26 and 97, respectively, in ND3), consistent with the known greater selective constraint on cytochrome oxidase genes (e.g., Simon et al., 1994; Nachman et al., 1996).
Muscisaxicola formed a monophyletic group, with the exception of *M. fluviatilis*, which was the sister to *Muscigralla brevicauda* and only distantly related to the other *Muscisaxicola* species. Within *Muscisaxicola*, *M. maculirostris* was sister to the other 10 species (hereafter, the “typical” species or “typical” clade), which formed two monophyletic groups, a clade consisting of *albifrons*, *cinerea*, *flavinucha*, *rufivertex*, *juninensis*, and *alpina grisea* (hereafter, “clade 1”) and a clade consisting of *albilora*, *macloviana*, *alpina alpina*, *capistrata*, and *frontalis* (hereafter, “clade 2”). Relationships within clade 2 were fully resolved: *albilora* and *macloviana* were sister species, as were *capistrata* and *frontalis*, with *alpina alpina* sister to *albilora + macloviana*. Clade 1 contained a polytomy involving *M. albifrons*, *M. cinerea*, *M. flavinucha*, and *M. rufivertex*, with *juninensis* sister to this group and *alpina grisea* sister to *juninensis + the polytomy species*. All individual species were monophyletic, except for *M. alpina*, which included individuals from clades 1 and 2, and possibly *M. cinerea*, for which the relationship of the two sequenced individuals was unresolved. Bootstrap support for and within the typical *Muscisaxicola* clade was strong for the most part, especially in clade 2, whereas support outside this clade was weak.

Differentially weighted parsimony analysis, with transversions weighted five times transitions, resulted in a single most-parsimonious tree (length 1071, CI excluding uninformative characters = 0.58, RI = 0.68). This tree was identical to the equally weighted tree, except that *Lessonia*, rather than *Muscigralla*, was the sister taxon to *M. fluviatilis*.

Both neighbor-joining analyses resulted in the same phylogeny (Fig. 2), which was consistent with the phylogeny obtained using equally weighted parsimony (Fig. 1), except for the positions of *M. fluviatilis* and the outgroups. Both clades of typical *Muscisaxicola* species

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**FIG. 1.** Single most-parsimonious tree based on the equally weighted analysis of combined sequences of COII and ND3. Numbers above branches are percentages of time that the branch was recovered in 100 branch-and-bound bootstrap replicates, and those below branches are branch support values. Symbols following names of *Muscisaxicola* species (abbreviated using the generic initial *M.*) represent the species group designations of Vuilleumier (1971), as follows: asterisk, species group 1; striped square, species group 2; cross, species group 3; circle, species group 4; black square, species group 5.

**FIG. 2.** Phylogram of the neighbor-joining tree constructed using combined sequences of COII and ND3. Numbers above branches are percentages of time that the branch was recovered in 1000 bootstrap replicates; branches lacking numbers were recovered less than 50% of the time. Branch lengths are proportional to the amount of character change along each branch. Approximate percentage sequence divergence is presented at the bottom of the phylogram.
were recovered in the neighbor-joining trees. Relationships within clade 2 were the same as those in the parsimony tree, and relationships within clade 1 were consistent with, although more fully resolved than, those in the parsimony tree. M. maculirostris was sister to the typical Muscisaxicola clade, and M. fluviatilis was sister to maculirostris + the typical clade.

The maximum-likelihood analysis resulted in a single most likely tree (not shown), which was similar to the phylogenies obtained using parsimony. The two clades of typical Muscisaxicola species were recovered in this tree, as well as sister species relationships in clade 2 between M. albilora and M. madoviana and between M. capistrata and M. frontalis; the position of M. a. alpina within this clade was unresolved. Relationships within clade 1 were again poorly resolved. M. flavinucha was found to be sister to a polytomy consisting of the following five taxa: M. al bifrons, M. rufivertex, M. juninensis, M. c. cinerea, and M. alpina grisea/M. cinerea argentina, which were sisters in this tree.

Alternative topologies in which M. alpina grisea and M. a. alpina were constrained to be sister taxa were found to be significantly longer or less likely than the shortest and most likely trees, respectively (P < 0.01, Wilcoxon signed ranks test; P < 0.05, Kishino–Hasegawa tests).

Biogeography and the evolution of migration. Breeding ranges of Muscisaxicola species in clade 1 are primarily central Andean, whereas breeding distributions of species in clade 2 are primarily southern Andean and Patagonian (Table 1, Fig. 3). Reconstruction of ancestral distributions, using DIVA 1.1, suggested that the ancestor of clade 1 was central Andean in distribution and that M. flavinucha later dispersed to the southern Andes. It also suggested that the ancestor of clade 2 was southern Andean/Patagonian in distribution and that M. albilora and M. frontalis later dispersed to the central Andes, M. madoviana to the Falkland Islands, and M. alpina alpina to the northern Andes. The ancestor of the typical Muscisaxicola species, according to DIVA, was distributed in both the central and the southern Andes, and the ancestor of M. maculirostris (distributed throughout the Andes) and the typical species was distributed throughout the Andes.

Excluding M. fluviatilis, treating M. alpina and M. grisea as separate species, and separating M. c. cinerea from M. c. argentina, five Muscisaxicola taxa are migratory, five are nonmigratory, and three are polymorphic (Table 1, Fig. 4). Reconstruction of ancestral conditions using MacClade 3.05 (Fig. 4) suggested that the ancestor of clade 1 (the central Andean clade) was nonmigratory, with migration evolving independently in M. c. cinerea argentina, M. flavinucha, and M. rufivertex. The migratory condition of the ancestor of clade 2 (the southern Andean clade) was equivocal, as was that of the ancestor of the entire typical Muscisaxicola clade. Resolving the character ambiguity to minimize the number of developments of migration resulted in migratory ancestors both to the entire typical clade and to clade 2, with the subsequent loss of migration in clade 2 in M. a. alpina and M. m. madoviana. Resolving the character ambiguity to maximize the number of developments of migration resulted in nonmigratory ancestors both to the typical clade and to clade 2, with three independent developments of migration in clade 2 (in M. albilora, M. madoviana mentalis, and the ancestor of M. capistrata/M. frontalis).

**DISCUSSION**

Phylogenetics. Although the genus Muscisaxicola is generally considered to consist of 12 species (Hellmayr and Cory, 1927; Traylor, 1979), 2 of these species, M. maculirostris and M. fluviatilis, differ phenotypically from the rest of the genus (they are smaller than other Muscisaxicola species, are browner overall, and show rufous wing edgings in adult plumage; Table 1) and
Relationships among typical Muscisaxicola species were found to differ substantially from previous ideas of relationship within the genus. Little support was found for the species groups of Vuilleumier (1971). Three of his four species groups (excluding species group 1) contain taxa from both major clades of typical Muscisaxicola species (Fig. 1). Species group 2, which consists of M. madoviana and M. capistrata, is wholly contained within the southern clade of Muscisaxicola species, but even here the two species are not sister taxa. Thus, the genetic data suggest that the plumage and other phenotypic characters used for designating the species groups have been subject to convergence. For example, rufous crown patches and conspicuous white lores have apparently evolved independently in both major clades of typical Muscisaxicola species (Fig. 1, Table 1). This finding is of potential importance because these types of characters are those that have traditionally been used in lower level avian taxonomic and systematic research, particularly in studies of passerine birds, which are notoriously conservative in morphology.

Sequences of two individuals of most species of Muscisaxicola were consistent with monophyly of those species (Fig. 1). However, M. alpina and M. cinerea were found to be somewhat problematic. M. alpina, as presently constituted, appears to be polyphyletic: alpina grisea is a member of the central Andean clade, whereas alpina alpina belongs to the predominantly southern Andean clade. The correlation of the genotypic differences with phenotypic differences (the dorsal plumage of alpina grisea is much grayer than that of alpina alpina or the other northern subspecies [co-lumbiana and quesedae], which are grayish-brown) and the fact that the constrained topologies were significantly worse than the best trees strongly support the polyphyly of M. alpina. Ridgely and Tudor (1994) noted that alpina grisea differs phenotypically as much from the northern subspecies of alpina (alpina, columbiana, and quesedae) as it does from M. cinerea and suggested that it may warrant species status. This suggestion is supported by the data presented above, and it is recommended that M. grisea (Taczanowski, 1884) once again be recognized as a species distinct from M. alpina (which would continue to include columbiana and quesedae). Although not shown here to be polyphyletic, M. cinerea was not demonstrably monophyletic; that is, no synapomorphies were found to uniquely unite the two individuals sequenced. Fjeldså and Krabbe’s (1990) suggestion that the species M. alpina should perhaps include the subspecies M. cinerea argentina receives some support from the data presented here (M. alpina grisea and M. cinerea argentina are sisters in the phylogeny based on the maximum-likelihood analysis), but it is recommended that these taxa be kept distinct pending more detailed studies.
Biogeography and radiation. Perhaps the most notable results of this study concern the spatial and temporal patterns of the radiation of the typical Muscisaxicola species. Members of the two major clades of Muscisaxicola occupy substantially different regions of the Andes, one clade being found primarily in the central Andes of Peru, Bolivia, and northern Argentina and Chile and the other breeding primarily in the southern Andes and Patagonian region of southern Argentina and Chile. Reconstruction of ancestral geographical ranges indicated that the ancestors of these two clades were likewise distributed in the central and southern Andes, respectively. Thus, the primary division among typical Muscisaxicola species is a biogeographic one, and the patterns of sympathy that we see among these species developed primarily within single biogeographic regions, rather than as the result of repeated extraregional invasions of taxa.

Although such patterns are seemingly consistent with nonallopatric modes of speciation, they are presumably the result of allopatric speciation and secondary sympathy, because the similarities among Muscisaxicola species in morphology, display behavior (Smith, 1971), and overall ecology (Vuilleumier, 1971), together with their homogeneous habitat, make them highly unsuitable candidates for sympatric speciation (to the extent that birds or other terrestrial vertebrates are candidates at all—cf. Futuyma and Mayer, 1980; Kondrashov and Mina, 1986; Grant and Grant, 1989). There presumably has been ample opportunity for allopatric speciation within Muscisaxicola, given the extensive glaciations, habitat change, and tectonic and volcanic activity in the high Andes over the past several million years (see Clapperton, 1993).

The precise location of the presumed former barrier between the central and the southern Andean regions is uncertain but would likely have been close to the current break in distribution between central and southern Andean species, at a latitude of roughly 38–40°S. This is the area in which the geological and topographic character of the Andes alters dramatically and mean elevation declines by some 2000 m (Rabassa and Clapperton, 1990), evidently coincident with a thinner and younger portion of continental crust (Clapperton, 1993). The elevational decline allows moist westerly winds to penetrate from the Pacific, the associated increased humidity favoring the development of glaciers (Rabassa and Clapperton, 1990). Evidence of former glaciations has been found in this area, in the Alumine Valley (Schleider, 1989; cited in Rabassa and Clapperton, 1990) and to the immediate south, extending east from Lago Nahuel Huapi and Cerro El Tronador (Rabassa and Clapperton, 1990). Although the exact dates and extents of the Alumine glaciations are not available, Mercer (1976) concluded that the most extensive glaciations further south in Patagonia probably occurred some 1–1.2 million years ago, which agrees well with the estimated date of the divergence between the two major clades of Muscisaxicola species (see below).

The most striking deviation from the simple biogeographical pattern outlined above is the presence of M. a. alpina, a taxon restricted to the Ecuadorian Andes, in the clade breeding predominantly in the southern Andes. According to the dispersal–vicariance biogeographic reconstruction (Fig. 3), this represents a dispersal event in the ancestor to the albiloramadoviana/alpina clade, because the ancestor of the southern clade occurred only in the southern Andes (an alternative reconstruction, not favored by DIVA, is dispersal on the terminal branch leading to M. a. alpina). Because one of the current taxa in this clade, M. albilora, regularly winters as far north as Ecuador, a likely explanation for this colonization event would be the establishment of a sedentary breeding population on the wintering grounds of the migratory ancestral species, followed by differentiation and speciation. Beginning stages of this phenomenon have been observed in other passerine birds in recent times (e.g., the establishment of breeding populations of the wintering migrant Hirundo rustica in Argentina; Martínez, 1983).

Levels of sequence divergence among the 11 typical Muscisaxicola species (including M. grisea as a separate species) do not exceed 2.9%, with individuals of different species differentiated by as little as 0.1%. There can be no question of the species status of these taxa, all of which occur sympatrically with other Muscisaxicola species, with no evidence of interbreeding. Although birds in general are recognized as having low levels of genetic variability, these figures are low even among birds. Surveys of mitochondrial sequence divergence between avian sister species and between other congeners (Avise and Zink, 1988; Seutin et al., 1993; Klicka and Zink, 1997; Johns and Avise, 1998) have found levels of sequence divergence as great as 10–15% or more and place Muscisaxicola species at the extreme low end of avian interspecific sequence divergence.

Although Pleistocene events have long been proposed as significant contributors to present biodiversity in both temperate and tropical regions (e.g., Rand, 1948; Haffer, 1969), the importance of Pleistocene events has been challenged in recent years (e.g., Zink and Slowinski, 1995; but see Avise and Walker, 1998). My results suggest that middle and late Pleistocene events greatly influenced the diversification of Muscisaxicola species and that taxa of the high Andes and Patagonia may be prime candidates in general for mid- to late Pleistocene effects on biodiversity. Assuming that the mitochondrial genes used in this study are evolving at roughly 2% per million years, an estimate converged upon by avian mitochondrial studies involving RFLP and cytochrome b sequence data (e.g., Shields and Wilson, 1987; Tarr and Fleischer, 1993; Zink and Blackwell, 1998), the deepest split among
typical Muscisaxicola species, separating the two major clades, occurred roughly 1.2 million years ago (2.4% mean divergence between members of the two clades), and all other speciation events occurred during the past million years, during the middle to late Pleistocene (Fig. 2). The central Andean radiation has been particularly recent, with divergences averaging less than 1%, speciation likely having occurred within the past half-million years. Thus, although my results are not consistent with Vuilleumier’s (1971) Muscisaxicola species groups, they accord well with his earlier (1969, p. 1180) broader analysis of the Andean avifauna: “one bird species having colonized the páramo-puna vegetation before the onset of the [Pleistocene] glaciations might thus have been isolated enough, in optimal conditions, to have given rise to five or even more new species.” The results are also consistent with current estimates of a Pliocene origin of high Andean habitats (Van der Hammen and Cleef, 1986).

Migration. It seems clear that the migratory species of Muscisaxicola do not reflect a radiation from a single migratory ancestor, due to the independent development of migration in the two major clades of typical species (if the ancestor to the typical clade was nonmigratory) or to the loss and subsequent independent development in the central Andean clade (if the ancestor was migratory). Reconstruction of migration in the central Andean clade, consistent with the biogeographic reconstruction, indicated independent development of migration in three species (cinerea, rufi-vertex, and flavinucha), two of which contain a sedentary northerly subspecies and a migratory more southerly subspecies (although, depending on the resolution of the polytomy, there could have been a single development of migration, followed by the loss of migration in the northern subspecies of cinerea and rufi-vertex).

In the southern clade, parsimonious reconstruction of the evolution of migration differs depending on whether the number of developments of migration is minimized or maximized. Minimizing gains of migration results in the evolution of migration in the ancestor to the clade, with the subsequent loss of migration in M. m. madloviana and M. a. alpina (as in Fig. 4), whereas maximizing number of gains results in three independent evolutions of migration (in albilora, madloviana mentalis, and the ancestor of the capistrata/fronalis clade). Although there are no a priori expectations for favoring gains or losses of migration among Muscisaxicola species, the reconstruction favoring a single development of migration, with two subsequent losses, seems more likely, for three reasons. First, this reconstruction is consistent with the biogeographic reconstruction, which postulates a southern ancestor. In contrast, the alternative reconstruction would presumably involve three separate colonizations of the temperate zone from the north. Second, in the case of M. madloviana, which consists of a migratory subspecies on the South American continent (M. m. mentalis) and a sedentary subspecies restricted to the Falkland Islands (M. m. madloviana), it seems more likely that a sedentary island taxon evolved from a migratory continental ancestor than that a migratory continental taxon evolved from a sedentary island ancestor (as would presumably have occurred under the alternative scenario, involving a gain of migration in M. m. mentalis). This has been demonstrated in other avian taxa (e.g., the paraphrystetic migratory continental species Anas platyrhynchos has apparently given rise to sedentary island species, rather than the opposite; Cooper et al., 1996; Omland, 1997). Finally, as mentioned above, the establishment of a sedentary breeding population from wintering individuals of a migratory species, as would presumably have happened to establish the northern M. a. alpina from the migratory ancestor of the southern clade, has been observed in other passerine birds in recent times.

The only previous phylogenetic analysis of the evolution of migration in a New World genus appears to be that of Burns (1998), who found that migration evolved only a single time in the tagner genus Piranga (Passeriformes, Thraupidae). In contrast to the south temperate breeding migrants of the genus Muscisaxicola, most migration in Piranga involves Nearctic-Neotropical migrants, species that breed in North America and winter in the Neotropics. Although Muscisaxicola is unusual among South American genera in being so highly migratory, the difference between the multiple evolutions of migration in Muscisaxicola and the single evolution in Piranga is perhaps representative of general differences between the Neotropical–Nearctic migration system and the South American austral system. Most Nearctic–Neotropical migrant species are wholly migratory and belong to genera that are exclusively or primarily migratory; it is likely that many of these taxa represent Nearctic radiations from migratory ancestors. In contrast, many South American austral migrants have sister species or subspecies resident in northern South America (Chesser, 1995); presumably migration evolved independently in many of these taxa.

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