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STRONG DIVERSIFICATION AT THE TREELINE AMONG *METALLURA* HUMMINGBIRDS

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ABSTRACT.—The hummingbird genus *Metallura* comprises nine species. Six of them live at the treeline and replace each other sharply along the eastern slope of the tropical Andes (*williami, baroni, odomae, theresiae, eupogon,* and *aeneocauda*). Their ranges overlap for 3,000 km along the Andes with *M. tyrianthina,* which lives at lower elevations and is differentiated into several subspecies that show clinal variation. The genus also includes *M. phoebe* in semi-arid western Peru and *M. iracunda* in the Perija Mountains of the northern Andes. The group could be a good model to study relative differences in diversification between montane forest as such and the narrow transition zone toward the barren highlands. Analysis of nucleotide sequences from three different mitochondrial gene fragments (cytochrome *b*, ND2, and ND5) show that *Metallura* forms a monophyletic group whose sister taxon is the genus *Chalcostigma.* The treeline forms of *Metallura,* including the morphologically divergent *M. phoebe,* group in a clade sister to *M. tyrianthina,* confirming the idea that montane forest and treeline forms are sister taxa in a strict sense. Neighboring treeline species show greater morphological and genetic differentiation relative to neighboring montane forest forms. The split between mid-elevation and treeline forms is estimated to have occurred during the Pliocene, suggesting that much of the *Metallura* radiation took place during the Pleistocene. *Received 6 March 1998, accepted 14 December 1998.*

ALTHOUGH MUCH ATTENTION has been devoted to speciation in the lowlands, montane areas may play a central role in biological diversification in the tropics (Vrba 1993, Roy et al. 1997). Among birds of humid Andean forests, two commonly seen distributional patterns are (1) very elongate ranges where a species is continuously present from northern Colombia to Bolivia, with rather weak or clinal subspecific differentiation; and (2) sharp replacement of closely related species along the mountain range, in many cases where the cloud forest is intersected by a deep valley with a hot, dry tropical climate. The boundaries between species could represent initial isolating barriers (O'Neill 1992) or secondary contact zones, which would be maintained most easily where a physical boundary also exists (Fjeldså 1995).

Graves (1985, 1988) demonstrated that range disjunctions and morphological differentiation in the Andes increase with elevation up to the treeline, possibly reflecting the extreme narrowness of ecological zones at high elevations and the consequent vulnerability of local populations in this habitat. Intensive speciation of local remnant populations at the treeline potentially could recruit species into lower elevational zones and contribute to the submontane peak of biological diversity (Rahbek 1997), or possibly even recruit species into the lowlands (Roy et al. 1997).

An ideal situation for studying the role of the treeline zone in diversification would be to compare sister taxa that have widely overlapping ranges but are segregated elevationally. One group that is especially well suited for such an analysis is the hummingbird genus *Metallura,* which comprises nine recognized species (Graves 1980, Heindl and Schuchmann 1998). *Metallura* is part of a species-rich Andean radiation (Bleiweiss et al. 1997) that presumably illustrates speciation events mainly of Pleistocene age (Fjeldså 1992). Members of the genus are characterized by rather small, straight bills and broad tails where the iridescent colors change according to the angle of view. As currently recognized, the genus comprises one species, *M. tyrianthina,* (Tyrian Metaltail), which is widespread in humid montane forests in the tropical Andes region, and one

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species group (the *M. aeneocauda* group; see Graves 1980, Heindl and Schuchmann 1998) that inhabits humid treelines, elfin forests, and adjacent shrubby paramo habitat in the same region. The two overlap for 3,000 km from northern Colombia to Cochabamba in Bolivia (Fig. 1). The genus also includes two rather aberrant, large melanistic species: *M. iracunda* (Perijá Metaltail), which is a local endemic of the isolated Perijá Mountains on the border of Colombia and Venezuela, and *M. phoebe* (Black Metaltail), which inhabits mist vegetation and *Polylepis* woodlands high above the Peruvian coastal desert.

Metallura tyrianthina is common throughout much of the zone of humid montane forest and is differentiated into seven subspecies (Fjeldså and Krabbe 1990, Heindl and Schuchmann 1998) with some broad zones of intergradation (Zimmer 1952). In contrast, the *M. aeneocauda* group inhabits an ecological zone that is usually only a few kilometers wide (Graves 1988) and is sharply differentiated into several species characterized by different combinations of colors of tail and body plumage, scaliness of the plumage, and colors and shape of the gorget. Sharp replacements and non-clinal checkerboard character variation, and indications of marginal sympatry in two places in Ecuador (see below), suggest species rank for several forms (Graves 1980, Fjeldså and Krabbe 1990; see Fig. 1): *M. williami* (Viridian Metaltail), *M. baroni* (Violet-throated Metaltail), *M. odomae* (Neblina Metaltail), *M. theresiae* (Coppery Metaltail), *M. eupogon* (Fire-throated Metaltail), and *M. aeneocauda* (Scaled Metaltail).

It was our *a priori* assumption that *M. tyrianthina* and the *M. aeneocauda* complex of metaltail hummingbirds are sister taxa, and that a comparison of the two would illustrate the effects of the extremely narrow habitats in which treeline species dwell. However, some uncertainty arises because the relationship of *Metallura* with "thornbill" hummingbirds (*Chalcostigma* spp.) is unresolved, because one species, *C. ruficeps* (Rufous-capped Thornbill), combines characters of the two genera (Fjeldså 1992, Schuchmann and Heindl 1997).

In this paper, we make a phylogenetic analysis of hummingbirds of the genus *Metallura* using DNA sequence data from three different mitochondrial gene fragments, for a total of 855 base pairs (bp). We use the phylogeny thus generated to discuss relationships among these birds and possible speciation scenarios, and we use the molecular data to assess the effects of elevation in the diversification process of the group.

Materials and Methods

Samples.—Tissues of eight of the nine *Metallura* species recognized by Graves (1980) were included (Table 1, Fig. 1). Samples of *Metallura iracunda*, as well as samples of the subspecies *Metallura w. williami M. w. recisa, M. theresiae parkeri, M. aeneocauda malagae, Metallura tyrianthina septentrionalis, M. t. oreopola,* and *M. t. districta* were not available for analysis. Tissue samples for taxa analyzed were obtained from the blood collection of the Zoological Museum of Copenhagen and the Louisiana State University Museum of Natural Science frozen tissue collection. To analyze the relationships of metaltails with thornbills, we included samples of *Chalcostigma ruficeps* and *C. herrani* (Rainbow-bearded Thornbill). We used *Eriocnemis nigrivestis* (Black-breasted Puffleg) and *Heliangelus viola* (Purple-throated Sunangel) as outgroups. Both species belong to a different clade than *Metallura* and *Chalcostigma* within the Trochilinae (see Bleiweiss et al. 1997).

DNA extraction.—DNA was extracted from blood samples preserved in EDTA (Arctander 1988) or DMSO (Arctander and Fjeldså 1994). Following Doyle and Doyle (1987), samples were washed in blood buffer (NaCl 0.1 M, Tris-HCl 0.01 M pH 8, EDTA 0.001 M pH 8), incubated in CTAB buffer (Tris-HCl 0.1 M pH 8, NaCl 1.4 M, EDTA 0.02 M pH 8, 0.2% 2-mercaptoethanol and 2% hexadecyl-trimethyl-ammonium-bromide [CTAB]) at 65°C for a minimum of 1 h, and extracted with chloroform/isopentyl alcohol (24:1 v/v). DNA was then precipitated with isopropanol, cleaned twice with ice-cold ethanol, and dissolved in TE buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA).

Alternatively, DNA was extracted from pieces of skin of museum specimens, or tissues other than blood, by soaking in 250 μL of blood buffer, disrupting with a pistil, and incubating overnight at 65°C with Proteinase K (final concentration 1 mg/mL) and SDS (final concentration 1% w/v). The samples were then extracted once with phenol and once with chloroform/isopentyl alcohol (24:1) and precipitated by adding an equal volume of isopropanol.

PCR and sequencing.—PCR products were obtained using primers L14857 (Nunn and Cracraft 1996), H15149 (Kocher et al. 1989), and H15915 (Edwards et al. 1991) for cytochrome *b* (cyt *b*); L5215 and H5578 (Hackett 1996) for ND2; and L13753 (CAG GAA AAT CCG CTC AAT TCG G), L13589 (CCA GCA GCA ATA GAA GGC CC), and H14149 (CCT ATT TTG CGG ATG TCT TGT TC) for ND5 (numbers follow

TABLE 1. Species and area of collection for the samples used in this study.

Species	Country	District (no. of samples)[a]
Metallura tyrianthina	Ecuador	Loja 2 (1)
Metallura tyrianthina	Ecuador	Azuay 1 (2)
Metallura tyrianthina	Ecuador	Pichincha 2 (1)
Metallura tyrianthina	Ecuador	Carchi 1
Metallura tyrianthina	Ecuador	Imbabura 1 (2)
Metallura tyrianthina	Ecuador	Napo 2 (2)
Metallura tyrianthina	Ecuador	Carchi 1
Metallura tyrianthina	Ecuador	Bolivar 0 (1)
Metallura tyrianthina[b]	Peru	Pasco 1
Metallura tyrianthina	Peru	Huancavelica 2 (1)
Metallura tyrianthina	Peru	Apurimac 3 (1)
Metallura tyrianthina	Bolivia	La Paz 1
Metallura tyrianthina	Bolivia	Cochabamba 0 (2)
Metallura williami	Ecuador	Zamora-Chinchipe 2 (2)
Metallura williami	Ecuador	Carchi, Napo, Morona-Santiago 3
Metallura baroni	Ecuador	Azuay 1
Metallura odomae	Ecuador	Loja/Zamora-Chinchipe 2 (2)
Metallura phoebe	Peru	Lima 1 (1)
Metallura theresiae	Peru	Huanuco 2
Metallura eupogon[b]	Peru	Huánuco and Pasco 2
Metallura aeneocauda[b]	Bolivia	La Paz 1
Chalcostigma ruficeps	Peru	Cuzco 2
Chalcostigma herrani	Colombia	Valle 1
Eriocnemis nigrivestis	Ecuador	Pichincha 1
Heliangelus viola	Ecuador	Loja/Zamora-Chinchipe 1 (1)

[a] First number refers to complete sequences; number in parentheses refers to extra individuals that were sequenced only partially (i.e. one or two of the gene fragments only).

[b] Sample provided by LSU collection.

Desjardin and Morais 1990). Double-stranded PCR was performed in 20 μL reaction volumes using equal amounts of primers; 5 μL of the reaction were electrophoresed in a low melting point agarose gel, and the bands were cut out following staining with ethidium bromide and melted in 400 μL of TE buffer. The solution (1 μL) was used as template to generate single-stranded DNA by asymmetric PCR amplification. The conditions were identical to those of the balanced-primer reaction except that the reaction volume was increased to 50 μL, the limiting primer was diluted one hundred fold, and the number of cycles was increased by three. Reaction products were cleaned by washing three times with ddH_2O through Ultrafree-MCf (Millipore Corporation) 30,000-NMWL filters and concentrated to a final volume of 18 μL, of which 8 μL were used for DNA sequencing.

An alternative for generating single-stranded PCR products was to produce double-stranded PCR products using one biotinylated primer in 35-μL reactions. The products were purified using Dynal magnetic beads following the protocol of the supplier. Dideoxy sequencing was performed following the protocol for Sequenase version 2.0 (United States Biochemicals). The products, labeled with S^{35}, were resolved in 6% acrylamide gels. Sequences have been submitted to Genbank under accession numbers AF022659 to AF022711, U85723, U85730, U85731, and U85738.

Analyses.—Sequences were aligned by eye using the program SeqApp 1.9 (Gilbert 1992). They were translated to amino acid sequence using MacClade 3.06 (Maddison and Maddison 1992) to check for possible stop codons. We used the program CS3 (H. Siegismund unpubl.) to calculate the total number of substitutions and the number of transitions (ts) and transversions (tv) between homologous DNA sequences. These figures were compared because it is known that mitochondrial and nuclear sequences exhibit differences in their substitution patterns (Arctander 1995). To assess saturation of the sequences we plotted the observed number of substitutions (p distances) versus Kimura two-parameter distances, which adjust the raw estimate based upon differences in substitution rates between ts and tv. Deviation from the line $y = x$ was taken as an indication that multiple substitutions had taken place at some positions (Burns 1997). Initially, we analyzed the sequences of the cyt *b*, ND2, and ND5 gene fragments separately, and we also made an analysis combining the sequences of the three fragments. Analyses included the use of maximum-likelihood, parsimony, and neighbor-joining algorithms.

For the maximum-likelihood analysis, we used

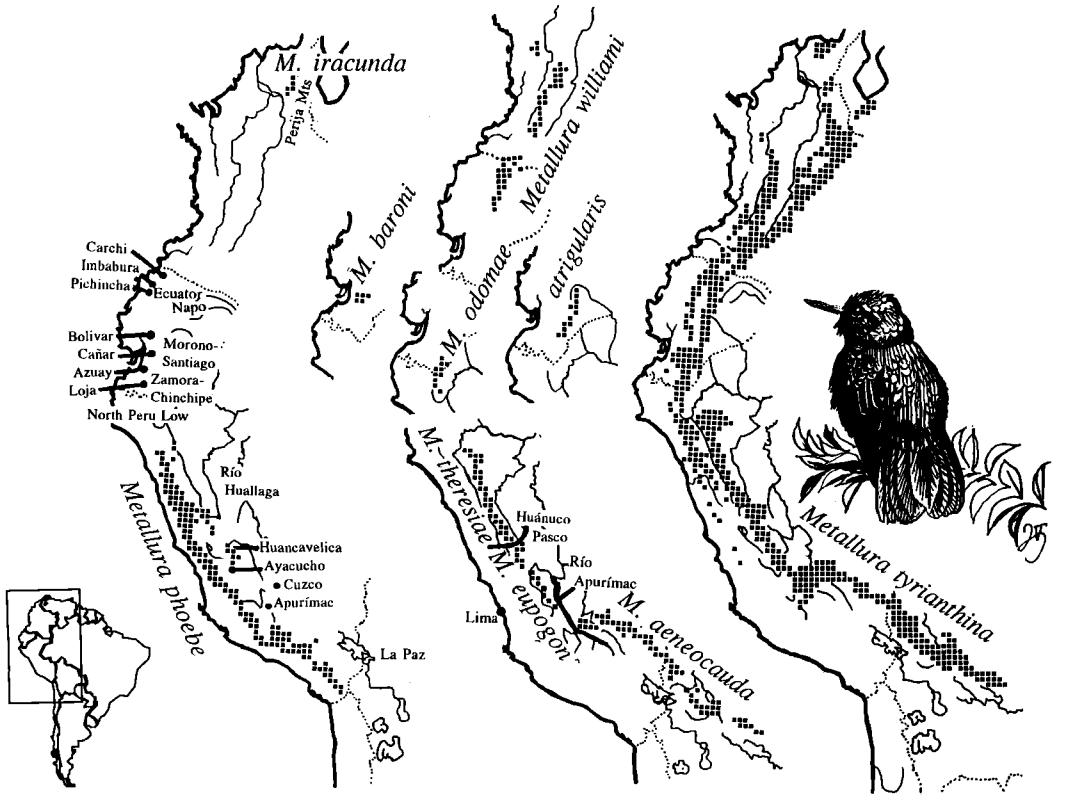

FIG. 1. Distributions of all members of the genus *Metallura* (from the database of the distribution of Andean birds in the Zoological Museum, University of Copenhagen). Each block is 15 minutes of latitude and longitude (ca. 28 × 28 km at this latitude). Species' ranges are made by conservative interpolation between confirmed records (see Fjeldså and Rahbek 1997). Locality names mentioned in the text are given mainly on the left map. Left: the two large melanistic forms *M. iracunda* (Perijá Mountains on the Colombia/Venezuela border) and *M. phoebe* (woodlands and shrub steppe high above the Peruvian coastal desert and locally in intermontane basins). Center: a complex of morphologically distinct forms that replace each other along the Andes in humid treeline habitats, from the north *M. williami* (in Colombia and northern Ecuador, with subspecies *recisa, williami* and *primolinus*), *M. williami atrigularis* (continental divide in central Ecuador), *M. baroni* (Cajas plateau of Azuay, Ecuador), *M. odomae* (along the continental divide from Loja, Ecuador, into extreme northern Peru), *M. theresiae* (the eastern Andes ridge between the North Peru Low and Río Huallaga, with subspecies *parkeri* and *theresiae*), *M. eupogon* (eastern Andes ridge between the Ríos Huallaga and Apurímac), and *M. aenecauda* (Cordillera Oriental from Cuzco, Peru, to Cochabamba, Bolivia, with subspecies *malagae* furthest south). Right: *M. tyrianthina* (widespread in humid montane forest throughout the tropical Andes, including some relict cloud forests on the Pacific slope, with seven subspecies; note that subspecies *chloropogon* inhabits Venezuelan coastal mountains outside the mapped area).

program DNAml in Phylip (Felsenstein 1995). The transition-transversion ratio was estimated empirically. We attempted to accommodate for rate heterogeneity in the sequences (Swofford et al. 1996, Yang 1996). Yang (1996) reported that among-site rate variation in cyt *b* of deuterostomes is best described by a gamma distribution with a parameter of 0.44. We made a coarse approximation to a gamma distribution with a parameter of 0.5 by stating three categories of rates (which do not necessarily correspond to codon positions) with magnitudes of 0.25, 1, and 3, and probabilities of 0.7, 0.25, and 0.05, respectively. For the parsimony analysis, we searched using the branch-and-bound option in PAUP 3.1.1 (Swofford 1991) with the farthest addition sequence. We performed bootstrap replicates with random addition of sequences (500 replicates, but only 200 replicates with maximum likelihood). For the distance analysis a tree was produced using the Neighbor-Joining algorithm based on an uncorrected *p* distance using MEGA 1.01 (Kumar et al. 1993).

We compared our results with a user tree forcing

M. tyrianthina and the *M. aeneocauda* group to be sister taxa with polychotomous population differentiation and equal branch lengths within each, with *M. phoebe* (and *M. iracunda*) representing a deeper branch.

RESULTS

With one exception, the sequences for each fragment from each of the taxa used in the analysis were obtained without problems. The exception was ND2 for *Metallura phoebe*, which yielded sequences that were unreadable due to double bands from a certain base onwards. These PCR products were subsequently cloned using the pCR-Script Amp SK (+) cloning kit (Stratagene). Sequencing of the clones yielded two sequences identical except for the presence of an extra triplet (ATC) at position 5362 (numbers follow Desjardin and Morais 1990). We chose to use the sequence without the extra triplet for our comparisons, since use of the longer sequence would require the insertion of gaps in all other sequences (a less parsimonious option). It is beyond the scope of this work to determine whether the double-band pattern resulting from this insertion is due to heteroplasmy in the individuals sequenced or to a different phenomenon.

Levels and patterns of variation.—All three sequenced fragments showed similar levels of variation, with ND2 showing the highest variation between species. Fewer transversions were detected in ND5 compared with the other two fragments.

Genetic distances among *Metallura* were relatively small (<10%; Fig. 2), a situation in which uncorrected (p) distances are expected to give a good estimate of divergence with the least variance (Kumar et al. 1993, Swofford et al. 1996). Genetic distances among the *M. tyrianthina* subspecies ranged between 0.010 and 0.036 ($\bar{x}$ = 0.027, n = 21), and within the *M. aeneocauda* superspecies, including *M. phoebe*, ranged from 0.002 to 0.085 ($\bar{x}$ = 0.047, n = 28). Despite the small distances, we detected indications of multiple hits in some third- and first-codon positions (as seen from uncorrected vs. Kimura two-parameter distance plots; Burns 1997).

Samples of *M. tyrianthina* from several Ecuadorian localities yielded identical sequences ("Ecuador" in Fig. 3). Birds from Napo, Pichincha, Imbabura, Bolivar, and Azuay showed the same haplotypes for 345 bp of cyt *b*. Similarly, samples from Cochabamba showed no difference from those of La Paz, although they were 200 km apart. The smallest non-zero distance was that between Pasco and Huancavelica (0.011), which are 300 km apart. A larger distance (0.027) was found over 200 km between

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1- *M. w. atrigularis*	-	17/4	10/0	17/4	39/7	22/2	24/2	46/3	57/4	55/5	54/7	60/7	62/6	52/5	53/3	51/7	47/7	79/20	71/14
2- *M. w. primolinus*	0.025	-	20/4	16/3	34/8	39/6	43/6	60/7	60/7	59/9	57/11	62/11	66/8	55/9	56/7	54/11	45/11	84/22	75/16
3- *M. baroni*	0.012	0.029	-	18/5	44/7	33/2	37/2	54/3	59/4	59/5	61/7	63/7	65/6	57/5	58/3	61/6	51/7	87/22	77/14
4- *M. odomae*	0.026	0.023	0.028	-	34/9	32/4	35/5	55/5	55/6	52/8	52/10	56/10	59/8	53/8	51/6	54/9	48/7	78/22	67/15
5- *M. phoebe*	0.056	0.049	0.061	0.051	-	55/5	58/5	65/6	65/7	65/8	65/10	65/10	70/7	64/8	61/6	55/9	45/9	90/22	79/13
6- *M. theresiae*	0.029	0.05	0.043	0.044	0.072	-	2/0	42/1	52/2	52/3	49/5	55/5	57/4	51/3	48/1	52/5	53/5	89/19	72/11
7- *M. eupogon*	0.031	0.057	0.046	0.047	0.074	0.002	-	42/1	53/2	54/3	51/5	56/5	58/4	52/3	49/1	54/5	52/5	90/20	75/12
8- *M. aeneocauda*	0.059	0.080	0.069	0.072	0.085	0.052	0.051	-	55/3	57/4	55/6	54/6	58/5	54/4	53/2	57/6	52/6	91/20	73/13
9- *M. tyrianthina* - Carchi	0.075	0.080	0.076	0.073	0.086	0.066	0.065	0.070	-	8/0	12/2	27/2	26/1	28/0	25/2	56/3	60/3	95/18	85/14
10- *M. tyrianthina* - Ecuador	0.072	0.080	0.076	0.072	0.086	0.066	0.067	0.072	0.010	-	9/2	26/2	26/1	26/0	23/2	56/4	62/3	94/19	78/15
11- *M. tyrianthina* - Loja	0.075	0.081	0.082	0.075	0.089	0.066	0.067	0.073	0.017	0.013	-	26/4	24/3	23/2	23/4	54/6	56/5	95/21	77/17
12- *M. tyrianthina* - Pasco	0.081	0.086	0.083	0.078	0.088	0.072	0.071	0.071	0.034	0.033	0.036	-	6/3	20/2	19/4	59/6	61/5	89/21	79/17
13- *M. tyrianthina* - Huancavelica	0.082	0.087	0.084	0.079	0.090	0.073	0.073	0.075	0.032	0.032	0.032	0.011	-	22/1	21/3	59/5	62/4	91/18	81/16
14- *M. tyrianthina* - Apurimac	0.069	0.075	0.074	0.072	0.085	0.065	0.064	0.069	0.033	0.031	0.030	0.026	0.027	-	15/2	57/4	59/3	86/19	78/15
15- *M. tyrianthina* - Bolivia	0.068	0.074	0.072	0.068	0.079	0.059	0.058	0.065	0.032	0.029	0.032	0.027	0.028	0.020	-	60/6	63/5	87/21	73/13
16- *Chalcostigma ruficeps*	0.071	0.077	0.080	0.075	0.076	0.069	0.070	0.075	0.071	0.071	0.072	0.077	0.076	0.072	0.078	-	39/2	77/19	85/15
17- *Chalcostigma herrani*	0.068	0.071	0.074	0.070	0.068	0.073	0.072	0.073	0.080	0.082	0.078	0.083	0.083	0.078	0.086	0.052	-	80/14	74/14
18- *Eriocnemis nigrivestis*	0.120	0.124	0.129	0.119	0.132	0.130	0.129	0.132	0.134	0.133	0.138	0.129	0.128	0.123	0.126	0.113	0.118	-	103/22
19- *Heliangelus viola*	0.103	0.090	0.108	0.098	0.109	0.100	0.102	0.103	0.118	0.110	0.112	0.113	0.114	0.110	0.101	0.119	0.111	0.147	-

FIG. 2. Pairwise sequence comparisons among the different taxa used in this study. Uncorrected sequence divergence is shown below the diagonal, and the number of transitions/transversions is shown above the diagonal. Based on 855 bp of mtDNA sequence from cyt *b* (345 bp), ND2 (290 bp), and ND5 (220 bp). Boxed comparisons are those between species of the *M. aeneocauda* complex (1 to 8), *M. tyrianthina* from different localities (9 to 15), and *Chalcostigma* species (16 and 17).

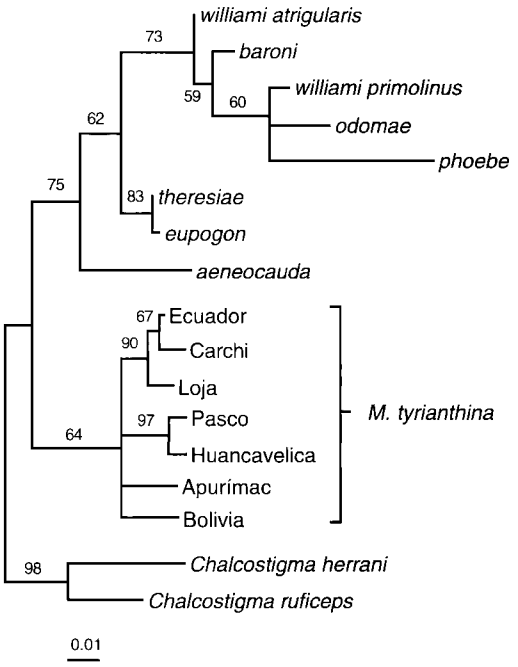

FIG. 3. Maximum-likelihood tree obtained from 855 bp of combined DNA sequences of mitochondrial cyt *b* (345 bp), ND2 (290 bp), and ND5 (220 bp) genes. Figures above the branches represent bootstrap values with the maximum-likelihood algorithm after 200 replicates. Branches are drawn proportionate to the amount of change along the branch, and those with low bootstrap support (<50%) have been collapsed. Names in the *M. tyrianthina* clade refer to localities where the samples originated from and are depicted in Figure 1; Ecuador refers to a widespread haplotype found in all localities other than Carchi and Loja (see Results).

Huancavelica and Apurímac (the even larger distance between Pasco and Loja, 0.036, is less revealing because of the lack of samples from northwestern Peru).

Phylogenetic analyses.—The topologies of the trees based on each of the three fragments varied significantly and were even contradictory. However, in all phylogenies, the *tyrianthina* birds were closely related, as were most of the northern forms of the treeline superspecies (*M. williami atrigularis* and *M. primolinus, M. baroni, M. odomae,* and *M. phoebe*), whereas *M. aeneocauda* was close to the clade formed by *M. theresiae* and *M. eupogon.*

When using a combined data set, all tree-construction algorithms yielded trees with *Chalcostigma* closely associated to *Metallura.* However, both *Heliangelus* and *Eriocnemis* are rather distant from *Metallura* to function as proper outgroups, and they belong to a different group of non-hermit hummingbirds (Bleiweiss et al. 1997). Having confirmed the monophyly of *Metallura,* and its close association with *Chalcostigma,* further phylogenetic analyses were carried out with *Chalcostigma herrani* declared as the outgroup.

A basal dichotomy between *M. tyrianthina* and all treeline forms (including *M. phoebe*; Fig. 3) was found in the optimal trees recovered with all the different algorithms. According to the Kishino and Hasegawa (1989) test, the topologies of the maximum-likelihood tree ($-\log_e$ likelihood (L) = 2,697), the parsimony bootstrap-consensus tree ($-\log_e L$ = 2,719, P = 0.180), and the neighbor-joining tree ($-\log_e L$ = 2,721, P = 0.091) were not significantly different. In contrast, the null model in which we forced *M. tyrianthina* and the *M. aeneocauda* group to be sister taxa, with *M. phoebe* (and *M. iracunda*) representing a deeper branch, was a significantly poorer hypothesis ($-\log_e L$ = 2,939, $P < 0.0001$).

In all trees, *M. phoebe* was grouped with the northern treeline forms, and the two subspecies of *M. williami* that we used (*atrigularis* and *primolinus*) did not group as sister taxa. Unfortunately, we cannot tell where *M. iracunda* fits in, although plumage details and especially the strong sexual dimorphism, would suggest that it is nearest to *M. tyrianthina.*

Elevational effects.—Because of the linearity of the distributional ranges along the eastern slope of the Andes (Fig. 1), one could assume that geographic neighbors were more closely related than non-neighboring species (see Fig. 3). Among the six treeline species that replace each other in a linear fashion along the Andes (*M. williami, M. baroni, M. odomae, M. theresiae, M. eupogon, M. aeneocauda*; Fig. 1), genetic distances between neighboring forms were highly correlated with geographic distances (r^2 = 0.78; Fig. 4). This was not the case among samples of the mid-elevation species *M. tyrianthina* that covered a similar geographic transect (r^2 = 0.32).

DISCUSSION

Phylogenetic relationships.—The DNA divergence data (Fig. 2) highlight the close relation-

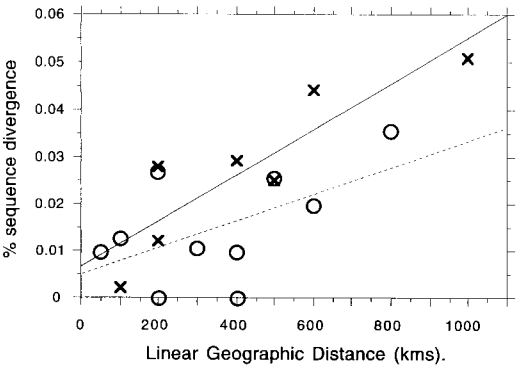

FIG. 4. Relationship between genetic distance (percent sequence divergence) between neighboring taxa and linear geographic distance in *Metallura* hummingbirds. Crosses denote treeline-bound species replacing each other across the eastern slope of the tropical Andes, from Ecuador to Bolivia (*M. williami, M. baroni, M. odomae, M. theresiae, M. eupogon,* and *M. aeneocauda*), and open circles denote *Metallura tyrianthina* from different localities across a similar geographic range (Carchi, Napo, Azuay, Loja, Pasco, Huancavelica, Apurimac, La Paz, and Cochabamba; see Table 1 and Fig. 1 for localities). Continuous line is regression for the treeline species set (r^2 = 0.78), and broken line is regression for the lower elevation *M. tyrianthina* (r^2 = 0.32). Geographic distances are approximate linear distances (km) between samples.

ship between *Metallura* and *Chalcostigma,* which is also evident using different characters (Schuchmann and Heindl 1997). *Chalcostigma ruficeps* is morphologically and ecologically intermediate between *Chalcostigma* and *Metallura,* and the females are difficult to tell apart (even in the hand) from those of *M. t. tyrianthina* and *M. aeneocauda malagae* (Fjeldså and Krabbe 1990, Fjeldså 1992).

Within *Metallura,* two distinct groups were identified: (1) one embracing all representatives of *M. tyrianthina* and (2) one comprising all members of the treeline superspecies. This result confirms the idea that montane forest and treeline forms are sister taxa in a strict sense. *Metallura phoebe,* although morphologically very distinctive, clearly is related to the Ecuadorian treeline taxa and probably originated by successful dispersal of an ancestral form west of the North Peru Low (where the Andes reach their lowest elevation) to the western Andean slope of Peru. Its morphological distinctness does not reflect a particularly early isolation.

Birds from Ecuador (*M. williami, M. baroni,* and *M. odomae*) form a close-knit group. Marginal sympatry may exist between species with slightly different ecological requirements (Fjeldså and Krabbe 1990, Stotz et al. 1996). *Metallura baroni* and *M. w. atrigularis* were found together at Río Mazán in Azuay, Ecuador (Gretton 1986), with *M. baroni* occupying the higher elevational zone; similarly, *M. odomae* and *M. williami primolinus* have both been reported in the same locality in Podocarpus National Park in Loja (Rasmussen and Rahbek 1994). However, this assumed sympatry has not been documented by collection or substantiated by long-term studies (N. Krabbe pers. comm.) and could therefore represent marginal or casual contacts.

The two subspecies of *M. williami* (*atrigularis* and *primolinus*) do not come out as sister taxa in any of the phylogenetic analyses performed. It would be tempting to suggest that the two were separate species, but Moore (1940) stated that birds from Chimborazo show signs of intergradation. Although one of our *M. w. primolinus* samples was from the assumed area of intergradation, its mtDNA was identical to that of samples from Napo and Carchi. The individual variation of birds from Chimborazo and Morona-Santiago should be carefully studied for a better interpretation of what is happening in this area.

Another problem is how to interpret the marked dichotomy of morphological and molecular differences exhibited by the sister species *M. theresiae* and *M. eupogon.* Differences in structural coloration (e.g. gorget) could be the result of minute changes in the arrangement of highly refractive melanin granules in the feather barbules (Dyck 1976, Greenwalt 1991) and therefore may be of slight taxonomic significance. In and of itself, the lack of genetic differentiation between the two forms (Fig. 2) cannot immediately be used to disprove their species status, because the low genetic distance could indicate a very recent and short period of isolation between the two forms.

The groupings within the *Metallura tyrianthina* clade reflect geographic proximity to some extent (Figs. 2, 3). The relatively large genetic change between Huancavelica and Apurimac occurs in an area with several deep gaps in the eastern Andean ridge and cloud cover that is quite variable (Fjeldså 1995), which could act as

a filter barrier. Birds from Ecuador north of Loja show no genetic differentiation regardless of whether they occur on the eastern or western slope of the Andes. Several dispersal corridors connecting eastern and western avifaunas have been postulated in Ecuador (Poulsen and Krabbe 1997), which might explain the lack of genetic differentiation between the seemingly isolated eastern and western populations. The relatively low variation in Ecuador could also suggest a rather recent establishment, although samples from northwestern Peru (*M. t. septentrionalis*) and the extreme northern end of the range would be needed to firmly establish this.

Elevational effects.—The distribution of *M. tyrianthina* broadly overlaps that of the treeline forms, with an elevational segregation in which the former species occupies lower elevations. Even though we lack samples of several of the morphologically most divergent *M. tyrianthina* subspecies, and therefore cannot test this result rigorously, a clear trend emerges from our data. Within a similar geographic transect, stretching from Ecuador to Bolivia (Fig. 1), genetic distances between neighboring forms are highly correlated with linear geographic distances in the treeline species group, but only weakly in the mid-elevation *M. tyrianthina* (Fig. 4). A comparison of genetic differentiation between neighboring taxa suggests that the treeline birds show not only greater morphological differentiation, but also stronger genetic differentiation than the lower-elevation forms. Random morphological variation (Graves 1980, Remsen 1984) suggests that stochastic processes and rapid divergence in small populations (Paterson 1985, Carson 1990) are important elements in the diversification of these birds. Treeline populations may disintegrate easier into tiny isolates because the narrow configuration of the ecotone that they inhabit makes them extremely sensitive to environmental changes (Graves 1988); many potential isolating mechanisms, such as volcanism, glaciation, and forest fragmentation by climate change are prominent in the Andes. Remote sensing data suggest that reduced ecological disturbance occurs in places with peak concentrations of endemism along the Andean treeline (Fjeldså et al. unpubl. data). At least in the southern part of the tropical Andes, these places are characterized by local topographic moderation of the influence of cold south polar winds during winter. Servant et al. (1993) suggest that the Pleistocene vegetational changes in tropical South America were driven by increased effects of these winds. Accordingly, speciation along much of the Andes could be driven by ecoclimatic disturbance in which sensitive species survived periods of stress as local isolates in places where the ecological disturbance was moderated (on various time scales).

Speciation scenario.—Using different substitution rates (2% per million years [see Klicka and Zink 1997]; 10% third-codon position substitutions or 0.5% third-codon position transversions per million years [Irwin et al. 1991]; but see Loewe and Scherer 1997, Hillis et al. 1996), our molecular data suggest that *Chalcostigma* and *Metallura* diverged about 4 million years ago. The early split between mid-elevation and treeline forms of *Metallura* (Fig. 3) appears to have occurred soon afterward, also in the Pliocene, 2 to 4 million years ago, with several speciation events likely to have occurred during the Pleistocene. Figure 3 suggests rather similar area relationships in *M. tyrianthina* and in the treeline group, with the most recent differentiation in the north. Treeline populations in the southern tropical Andes appear to have separated early in the history of the group (in *Metallura* and *Chalcostigma*; Schuchmann and Heindl 1997). It seems that early vicariance events in the south were followed, in both groups, by a relatively more recent establishment in the paramos north of the North Peru Low. However, it is difficult to exclude the alternative interpretation that the most recent opportunities for local differentiation were in the north. In this regard, the lack of samples of *M. t. septentrionalis* and of Colombian populations is regrettable, although morphological variation suggests northward polarity and that both groups originated in the southern part of their range (Heindl and Schuchmann 1998).

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LITERATURE CITED

ARCTANDER, P. 1988. Comparative studies of avian DNA by restriction fragment length polymorphism analysis: Convenient procedures based on blood samples from live birds. Journal für Ornithologie 129:205–216.

ARCTANDER, P. 1995. Comparison of a mitochondrial gene and a corresponding nuclear pseudogene. Proceedings of the Royal Society of London Series B 262:13–19.

ARCTANDER, P., AND J. FJELDSÅ. 1994. Andean tapaculos of the genus *Scytalopus* (Aves, Rhinocryptidae): A study of speciation using DNA sequence data. Pages 205–226 *in* Conservation genetics (V. Loeschke, J. Tomiuk, and S. K. Jain, Eds.). Birkhäuser Verlag, Basel, Switzerland.

BLEIWEISS, R., J. A. W. KIRSCH, AND J. C. MATHEUS. 1997. DNA hybridization evidence for the principal lineages of hummingbirds (Aves: Trochilidae). Molecular Biology and Evolution 14:325–343.

BURNS, K. J. 1997. Molecular systematics of tanagers (Thraupinae): Evolution and biogeography of a diverse radiation of Neotropical birds. Molecular Phylogenetics and Evolution 8:334–348.

CARSON, H. L. 1990. Increased genetic variance after a population bottleneck. Trends in Ecology and Evolution 5:228–230.

DESJARDINS, P., AND R. MORAIS. 1990. Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. Journal of Molecular Biology 212:599–634.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.

DYCK, J. 1976. Structural colours. Pages 426–437 *in* Proceedings of the XVI International Ornithological Congress (H. J. Firth and J. H. Calaby, Eds.). Canberra, 1974. Australian Academy of Science, Canberra.

EDWARDS, S. V., P. ARCTANDER, AND A. C. WILSON. 1991. Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. Proceedings of the Royal Society of London Series B 243:99–107.

FELSENSTEIN, J. 1995. PHYLIP (Phylogenetic Inference Package), version 3.56. Department of Genetics, University of Washington, Seattle.

FJELDSÅ, J. 1992. Biogeographic patterns and evolution of the avifauna of relict high-altitude woodlands of the Andes. Steenstrupia 18:9–62.

FJELDSÅ, J. 1995. Geographical patterns of neoendemic and older relict species of Andean birds: The significance of ecological stability areas. Pages 89–102 *in* Biodiversity and conservation of Neotropical montane forests (S. P. Churchill, H. Balslev, E. Forero, and J. L. Luteyn, Eds.). New York Botanical Garden, New York.

FJELDSÅ, J., AND N. KRABBE. 1990. Birds of the High Andes. Apollo Books, Svendborg, Denmark.

FJELDSÅ, J., AND C. RAHBEK. 1997. Species richness and endemism in South American birds: Implications for the design of networks of nature reserves. Pages 466–482 *in* Tropical forest remnants: Ecology, management, and conservation of fragmented communities (W. F. Laurance and R. O. Bierregaard, Jr., Eds). University of Chicago Press, Chicago.

GILBERT, D. 1992. SeqApp. A biosequence editor and application program, version 1.9a157. University of Indiana, Bloomington.

GRAVES, G. R. 1980. A new species of metaltail hummingbird from northern Peru. Wilson Bulletin 92:1–7.

GRAVES, G. R. 1985. Elevational correlates of speciation and intraspecific geographic variation in plumage in Andean forest birds. Auk 102:556–579.

GRAVES, G. R. 1988. Linearity of geographic range and its possible effect on the population structure of Andean birds. Auk 105:47–52.

GREENWALT, C. H. 1991. Hummingbirds. Dover Publications, New York.

GRETTON, A. 1986. Birds. Pages 33–57 *in* Río Mazán project 1986 report (F. Robinson, Ed.). Oxford University, Oxford.

HACKETT, S. J. 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). Molecular Phylogenetics and Evoluton 5:368–382.

HEINDL, M., AND K. L. SCHUCHMANN. 1998. Biogeography, geographical variation, and taxonomy of the Andean hummingbird genus *Metallura*, Gould 1847. Journal of Biogeography 139:425–473.

HILLIS, D. M., B. K. MABLE, AND C. MORITZ. 1996. Applications of molecular systematics: The state of the field and a look to the future. Pages 515–543 *in* Molecular systematics (D. M. Hillis, C. Moritz, and B. K. Mable, Eds.). Sinauer Associates, Sunderland, Massachusetts.

KISHINO, H., AND M. HASEGAWA. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. Journal of Molecular Evolution 29:170–179.

KLICKA, J., AND R. M. ZINK. 1997. The importance of recent ice ages in speciation: A failed paradigm. Science 277:1666–1669.

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PÄÄBO, S. X. VILLAFRANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proceedings

of the National Academy of Sciences USA 86: 6196–6200.

KUMAR, S., K. TAMURA, AND M. NEI. 1993. MEGA: Molecular Evolutionary Genetic Analysis, version 1.01. Pennsylvania State University, Pennsylvania.

LOEWE, L., AND S. SCHERER. 1997. Mitochondrial Eve: The plot thickens. Trends in Ecology and Evolution 12:422–423.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade: Analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, Massachusetts.

MOORE, R. T. 1940. The nomenclature and habits of the Black-throated Coppertailed Hummingbird. Condor 42:251–254.

NUNN, G. B., AND J. CRACRAFT. 1996. Phylogenetic relationships among the major lineages of the Bird-of-Paradise (Paradisaeidae) using mitochondrial DNA gene sequences. Molecular Phylogenetics and Evolution 5:445–459.

O'NEILL, J. P. 1992. A general overview of the montane avifauna of Peru. Memorias del Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (Lima) 21:47–55.

PATERSON, H. E. H. 1985. The recognition concept of species. Pages 21–29 *in* Species and speciation (E. S. Vrba, Ed.). Transvaal Museum, Pretoria.

POULSEN, B. O., AND N. KRABBE. 1997. The diversity of cloud forest birds on the eastern and western slopes of the Ecuadorian Andes: A latitudinal and comparative analysis with implications for conservation. Ecography 20:475–482.

RAHBEK, C. 1997. The relationship among area, elevation, and regional species richness in Neotropical birds. American Naturalist 149:875–902.

RASMUSSEN, J. F., AND C. RAHBEK. 1994. Aves del Parque Nacional Podocarpus-Una Lista Anotada. Birds of Podocarpus National Park. An annotated checklist. CECIA, Quito, Ecuador.

REMSEN, J. V. 1984. High incidence of "leapfrog" pattern of geographic variation in Andean birds: Implications for the speciation process. Science 224:171–173.

ROY, M. S., J. M. C. SILVA, P. ARCTANDER, J. GARCÍA-MORENO, AND J. FJELDSÅ. 1997. The speciation of South American and African birds in montane regions. Pages 321–339 *in* Avian molecular evolution and systematics (D. P. Mindell, Ed.). Academic Press, San Diego.

SCHUCHMANN, K.-L., AND M. HEINDL. 1997. Biogeographie, geographische Variation und Taxonomie der andinen Kolibrigattung *Chalcostigma* Reichenbach, 1854. Mitteilungen Zoologischer Museum Berlin 73 Supplementum Annales Ornitologici 21:131–153.

SERVANT, M., J. MALEY, B. TURCQ, M.-L. ABSY, P. BRENAC, M. FOURNIER, AND M.-P. LEDRU. 1993. Tropical forest changes during the Late Quaternary in African and South American lowlands. Global and Planetary Change 7:25–40.

STOTZ, D. F., J. W. FITZPATRICK, T. A. PARKER, AND D. K. MOSKOVITS. 1996. Neotropical birds. Ecology and conservation. University of Chicago Press, Chicago.

SWOFFORD, D. L. 1991. PAUP: Phylogenetic analysis using parsimony. Center for Biodiversity of the Illinois Natural History Survey, Champaign.

SWOFFORD, D. L., G. J. OLSEN, P. J. WADELL, AND D. M. HILLIS. 1996. Phylogenetic inference. Pages 407–514 *in* Molecular systematics (D. Hillis, C. Moritz, and B. K. Mable, Eds.). Sinauer Associates, Sunderland, Massachusetts.

VRBA, E. S. 1993. Mammal evolution in the African Neogene and a new look at the great American interchange. Pages 393–432 *in* Biological relationships between Africa and South America (P. Goldblatt, Ed.). Yale University Press, New Haven, Connecticut.

YANG, Z. 1996. Among-site rate variation and its impact on phylogenetic analyses. Trends in Ecology and Evolution 11:367–372.

ZIMMER, J. T. 1952. Studies of Peruvian birds, no. 62. The genera *Patagona, Sappho, Polyonymus, Ramphomicron, Metallura, Chalcostigma, Taphrolesbia* and *Aglaiocercus*. American Museum Novitates No. 1595.

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