Phylogeography and Adaptive Plumage Evolution in Central American Subspecies of the Slate-Throated Redstart (Myioborus miniatus)

Authors: Pérez-emán, Jorge L., Department of Biology, University of Missouri-St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121, USA, Mumme, Ronald L., Department of Biology, Allegheny College, 520 North Main Street, Meadville, Pennsylvania 16335, USA, and Jabłoński, Piotr G., Laboratory of Behavioral Ecology and Evolution, School of Biological Sciences, Seoul National University, Seoul 151-742, Korea, 56-1 Sillim-dong, Gwanak-gu, Korea

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CHAPTER 8

PHYLOGEOGRAPHY AND ADAPTIVE PLUMAGE EVOLUTION IN CENTRAL AMERICAN SUBSPECIES OF THE SLATE-THROATED REDSTART (*MYIOBORUS MINIATUS*)

JORGE L. PÉREZ-EMÁN,[1,2,6] RONALD L. MUMME,[3] AND PIOTR G. JABŁOŃSKI[4,5]

[1]*Department of Biology, University of Missouri-St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121, USA;*
[2]*Instituto de Zoología y Ecología Tropical, Universidad Central de Venezuela, Avenida Los Ilustres, Los Chaguaramos, Apartado Postal 47058, Caracas 1041-A, Venezuela;*
[3]*Department of Biology, Allegheny College, 520 North Main Street, Meadville, Pennsylvania 16335, USA;*
[4]*Laboratory of Behavioral Ecology and Evolution, School of Biological Sciences, Seoul National University, Seoul 151-742, Korea, 56-1 Sillim-dong, Gwanak-gu, Korea; and*
[5]*Center for Ecological Studies, PAS, Lomianki 05-092, Poland*

ABSTRACT.—The Slate-throated Redstart (*Myioborus miniatus*) is a common warbler of montane forests from northern Mexico to Argentina. We examined phylogenetic structure and plumage pattern in relation to subspecific taxonomy across the broad geographic range of this species. Phylogenetic analysis of two complete mitochondrial protein-coding genes (subunits 2 and 3 of NADH dehydrogenase) from 36 individuals, representing 10 of the 12 subspecies, revealed four clades, three of which showed general concordance with subspecific classification. However, in a Central American clade, four subspecies (*hellmayri*, *connectens*, *comptus*, and *aurantiacus*) could not be resolved by the molecular phylogenetic analysis, even though populations of *hellmayri* and *connectens* are currently geographically isolated from those of *comptus* and *aurantiacus* by the Nicaraguan lowlands. The genetic homogeneity within this clade suggests a late Pleistocene range expansion at a time when today's montane forest types existed at lower elevations. The Pleistocene hypothesis is supported by both paleoecological reconstructions of Central America and the mismatch distribution of pairwise nucleotide differences among haplotypes in our data set. Despite the genetic homogeneity within the Central American clade, the four subspecies are differentiated in plumage pattern, particularly in the extent of white in the tail. Subspecific variation in the tail pattern is of particular interest because the tails are used in animated displays to startle potential insect prey that are pursued and captured in flight. Field experiments conducted with birds of the Costa Rican subspecies *comptus* and their key prey indicated (1) that a contrasting black-and-white tail is critical to flush-pursuit foraging success and (2) that subspecific variation in the extent of white in the tail reflects evolutionary adaptation to regional prey or habitat characteristics that maximizes flush-pursuit foraging performance. Thus, even though the subspecies of the Central American clade are genetically homogeneous with respect to the mitochondrial genes, analysis of tail pattern and its effect on foraging performance suggests a recent adaptive evolutionary divergence. Our findings serve as a reminder that mitochondrial DNA (mtDNA) gene trees will not always succeed in capturing all evolutionarily significant genetic change, and that manipulative field experiments can provide crucial information on the selective factors that lead to evolution of subspecies-specific morphological traits even in the absence of mtDNA diversification.

[6]E-mail: jorge.perez@ciens.ucv.ve

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Filogeografía y Evolución Adaptativa del Plumaje en Subespecies Centroamericanas de *Myioborus miniatus*

RESUMEN.—*Myioborus miniatus* se distribuye en bosques montanos desde el norte de México hasta Argentina. Examinamos la estructura filogenética y el patrón de plumaje y su relación con la taxonomía subespecífica a lo largo de la distribución geográfica de la especie. Análisis filogenéticos de dos genes mitocondriales (subunidades 2 y 3 de la Nicotinamida Adenina Dinucleótido Dehidrogenasa, NADH) en 36 individuos representativos de 10 de las 12 subespecies actualmente reconocidas mostraron cuatro clados, tres de ellos congruentes con la clasificación subespecífica actualmente aceptada. Sin embargo, el clado centroamericano compuesto por cuatro subespecies (*hellmayri, connectens, comptus* y *aurantiacus*) mostró un sorprendente grado de homogeneidad genética, aún cuando las poblaciones de *hellmayri* y *connectens* hoy están geográficamente aisladas de las de *comptus* y *aurantiacus* por las extensas tierras bajas en Nicaragua. La homogeneidad genética de este clado sugiere una expansión demográfica durante el Pleistoceno tardío a lo largo de Centroamérica durante un período cuando los bosques montanos característicos de hoy día existieron a menores altitudes que en la actualidad, una hipótesis congruente con reconstrucciones paleoecológicas y análisis genético-demográficos. Sin embargo, a pesar de la homogeneidad genética de este clado, las cuatro subespecies se diferencian en los patrones de plumaje, particularmente en la extensión del blanco en la cola. La variación subespecífica en estos patrones es de particular interés porque todas las especies del género *Myioborus* efectúan movimientos rápidos con la cola para ahuyentar presas potenciales (insectos) que luego son perseguidas y capturadas en vuelo. En Costa Rica, experimentos de campo realizados con aves de las poblaciones reconocidas como *comptus* y sus presas clave indicaron que: (1) los patrones contrastantes de negro y blanco en la cola son críticos para el éxito de la estrategia de búsqueda de alimento, y (2) la variación subespecífica en la extensión del blanco en la cola refleja adaptación evolutiva a características regionales de las presas o del hábitat, lo cual maximiza la eficiencia de la estrategia de búsqueda de alimento. Por lo tanto, aún cuando los datos de ADN mitocondrial sugieren que las subespecies del clado centroamericano son homogéneas genéticamente, los patrones del plumaje y su efecto sobre la eficiencia de búsqueda de alimento sugieren divergencia evolutiva adaptativa reciente. Nuestros resultados muestran que los análisis de ADN mitocondrial no siempre reflejan exitosamente cambios genéticos evolutivamente significativos y que los experimentos de campo generan información crucial sobre los factores selectivos que pueden determinar la variación geográfica en caracteres morfológicos, aún en ausencia de diversificación en el ADN mitocondrial.

THE UTILITY AND evolutionary significance of avian subspecies has been a subject of enduring ornithological controversy (Rising 2007). Although the focus of the debate has shifted periodically through the years, it has included considerations of the distinctiveness and diagnosability of subspecies (e.g., Amadon 1949, Wilson and Brown 1953, Patten and Unitt 2002, Remsen 2005, Cicero and Johnson 2006) and whether the concept of subspecies has evolutionary validity in the context of the debate over biological and phylogenetic species concepts (e.g., Cracraft 1983, McKitrick and Zink 1988, Remsen 2005, Zink 2006, Rising 2007, Winker et al. 2007).

In recent years, with the advent of phylogeographic studies (Avise 2000), discussion has often focused on the genetics of subspecies. Genetic data, usually based on mitochondrial DNA (mtDNA) sequence variation, frequently suggest that subspecies originally recognized on the basis of plumage or morphological characters may not represent discrete evolutionary genetic units (Ball and Avise 1992). For example, Zink (2004) examined the degree to which molecular phylogenies and genetic structure within recognized species correspond to traditional subspecific taxonomy, and he found that only 3% of continentally distributed avian subspecies represent discrete evolutionary genetic units based on the population genetic signature in mtDNA. Although a subsequent analysis (Phillimore and Owens 2006) has shown that the degree of concordance between mtDNA phylogenetic structure and traditional subspecific taxonomy increases to 36% when data from insular and tropical taxa are included, it is clear that many subspecies originally designated on the basis of plumage or morphological characteristics are not supported by the pattern of mtDNA genetic structure. However, even in cases where subspecific taxonomy is not concordant

with underlying mtDNA phylogenetic structure, subspecific distinctions can still be valuable if subspecific geographic variation reflects important evolutionary adaptations to regional environmental conditions that have occurred in the apparent absence of mitochondrial genetic divergence (Zink 2004, Remsen 2005, Rising 2007, Winker et al. 2007). Here, we describe an example of such a situation in Central American subspecies of the Slate-throated Redstart (*Myioborus miniatus*).

Redstarts in the genus *Myioborus* comprise 12 species of sexually monomorphic Neotropical warblers that range from the mountains of the southwestern United States to the southern Andes (Curson et al. 1994). The Slate-throated Redstart is the most widely distributed species in the genus, occurring in lower montane forests from northern Mexico to northern Argentina (Curson et al. 1994, Di Giacomo 1995; Fig. 1). Recent phylogenetic analysis indicates that *miniatus* is monophyletic and the sister taxon to a clade of 10 primarily South American *Myioborus* that inhabit higher-elevation upper montane forests (Pérez-Emán 2005).

Within its broad geographic range, 12 subspecies of *miniatus* have been recognized historically (Paynter 1968, Curson et al. 1994; Fig. 1) on the basis of geographic variation in several plumage characteristics. Although subtle subspecific differences exist in coloration of the crown, head, and undertail coverts, the most striking geographic variation is in belly color and the extent of white in the tail. Belly color generally varies along a north–south gradient. Birds of the nominate subspecies *miniatus* of Central Mexico and *molochinus* of the Sierra de Los Tuxtlas of Veracruz have intense vermillion bellies. However, bellies grade from light red, salmon, and red-orange in subspecies of northern Central America (*intermedius*, *hellmayri*, *connectens*) to yellow-orange in southern Central America (*comptus*, *aurantiacus*) and yellow in all five South American subspecies (*sanctamartae*, *pallidiventris*, *ballux*, *subsimilis*, and *verticalis*; Ridgway 1902, Curson et al. 1994).

The pattern of variation in the extent of white in the tail is somewhat more complex (Fig. 1). Two subspecies of northern Central America, *hellmayri* of the coastal mountains of Guatemala and El Salvador and *connectens* of interior El Salvador and Honduras (van Rossem 1936), have the least white in the tail, and the extent of white generally increases both to the north and to the south, reaching its maximum extent

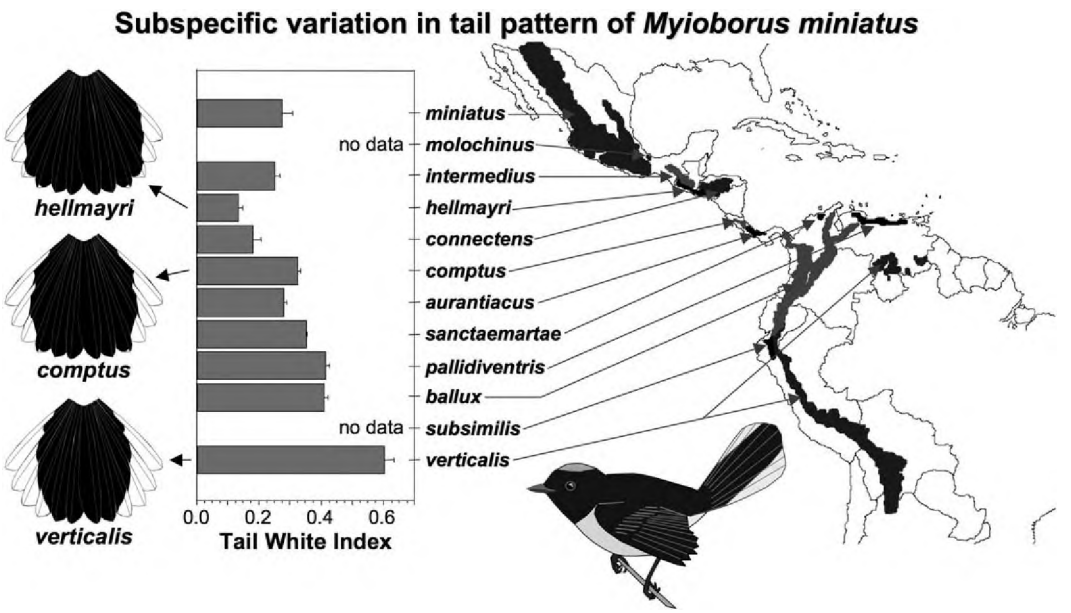

FIG. 1. Range and geographic variation in the extent of white in the tail of subspecies of *Myioborus miniatus*. The tail white index is the average of the maximum linear extent of white in the outer four rectrices divided by rectrix length (based on specimens from the Carnegie Museum of Natural History). Error bars reflect the standard error of the mean.

in *verticalis* of the southern Andes and Guiana Highlands (Fig. 1).

The goals of our study are to (1) examine phylogeographic patterns within *M. miniatus* using sequence information from two mitochondrial genes and focusing mainly on the seven subspecies distributed in Mexico and Central America; (2) contrast the molecular phylogenetic structure to phenotypic variation in plumage characteristics and subspecific taxonomy; (3) illustrate the role of field experiments in understanding the evolution of tail plumage, which is inextricably linked to foraging ecology in this species; and (4) discuss the implications of our results for the long-standing controversies that surround the recognition of avian subspecies

Methods

Specimens.—We obtained tissues of 23 *M. miniatus*, covering most of the distributional range of the species in North America and Central America (from Mexico to Panama), representing all Central American morphologically differentiated populations (Paynter 1968, Curson et al. 1994). We completed the data set with a previously published group of samples (Pérez-Emán 2005) for a total of 36 specimens of this species, including 10 of the 12 currently recognized subspecies (Appendix). We based subspecific designations on locality information, and most tissues were represented by voucher specimens. Only one individual (01541, *M. m. verticalis*) from the Zoological Museum of Copenhagen was not vouchered.

DNA methods.—We isolated whole genomic DNA from small tissue plugs using the PureGene (Gentra Systems, Minneapolis, Minnesota) extraction kit, or the DNeasy Tissue Kit (Qiagen, Valencia, California), following the animal-tissue protocols provided with the kits. We amplified two complete mitochondrial protein-coding genes, subunits 2 and 3 of the NADH (ND2, 1,041 base pairs [bp]; ND3, 351 bp), using the polymerase chain reaction (PCR) and a set of primers previously used in the genus (Pérez-Emán 2005). The PCR conditions were an initial denaturation cycle at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 52°C for 30 s, and an extension phase of 72°C for 60 s. The PCR reactions contained 1–2 μL of DNA template, 0.625U of *Taq* polymerase (PROMEGA), 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, 0.48 μM of each primer, and 80 μM dNTPs, in a total volume of 25 μL. We purified PCR products in 1% low-melting-point agarose gels, excised from the gel under ultraviolet light, with GeneClean III Kit (BIO 101 Systems, Solon, Ohio), and used them as templates for PCR sequencing reactions. We performed sequencing reactions in both directions using Dye Terminator Kits of ABI and the same set of primers used for amplifications. We precipitated PCR products with an ethanol-sodium acetate solution and ran them on an ABI 377 automated sequencer.

Phylogenetic analyses.—We aligned sequences by eye using the program SEQMAN II (DNASTAR, Madison, Wisconsin). To test for incongruent phylogenetic signal between genes, we used the incongruence-length difference (ILD) test (Farris et al. 1995) as implemented in partition-homogeneity tests in PAUP, with 100 replicates, using only informative characters (Cunningham 1997). We examined phylogenetic relationships among haplotypes using both maximum-parsimony and maximum-likelihood methods in PAUP*, version 4.0b10 (Swofford 2003). We performed an equal-weighted parsimony analysis using heuristic searches with starting trees obtained via random stepwise addition of taxa with the tree bisection reconnection (TBR) branch-swapping algorithm for 1,000 replicates. We estimated support for tree nodes with 100 bootstrap pseudo-replicates (Felsenstein 1985) with the full heuristic search and similar previous settings. We estimated a model of molecular evolution that best fits the data using the program MODELTEST, version 3.7 (Posada and Crandall 1998). This software implements a hierarchical comparison of different nested models, based on likelihood ratio tests, using an initial neighbor-joining tree generated with a Jukes-Cantor model of evolution. The model selected is the simplest model that cannot be statistically rejected in favor of a more complex model. Data used in this study fit a general time-reversible model with a substitution rate for variable sites following a gamma distribution (GTR + G) with a shape parameter alpha = 0.1202. Consequently, we implemented this model in a maximum-likelihood analysis, conducting 10 replicate heuristic searches with random stepwise addition of taxa using the TBR branch-swapping algorithm. We estimated bootstrap support with 100 iterations using previous settings. We tested for departures from a molecular clock using a likelihood ratio test comparing likelihoods of phylogenetic hypotheses with

and without a clock assumption. Phylogenetic reconstructions included sequences of *M. pictus* and four other warbler species (*Wilsonia pusilla*, *W. canadensis*, *Ergaticus ruber*, and *Cardellina rubrifrons*) as outgroups, based on a hypothesis of phylogenetic relationships obtained in a previous study (Pérez-Emán 2005; Appendix).

We also performed a Bayesian analysis with all sequences using the program MRBAYES, version 3.1 (Ronquist et al. 2005). We set Bayesian search to four chains running simultaneously for 2 million generations, with trees sampled every 100 generations for a total of 20,000 trees. We conducted four independent runs initiated from different random trees to avoid results depending on initial starting conditions. We plotted results of log-likelihood scores to generation times to identify the point at which log-likelihood values reached an equilibrium state (stationarity) using TRACER, version 1.4 (see Acknowledgments). We also examined convergence of clade posterior probabilities as a function of generation number, as well as the correlation of clade frequencies for pairs of different independent runs using AWTY (Wilgenbusch et al. 2004). We discarded, conservatively, the first 5 million generations and, on the basis of convergence of different runs, combined results of the 15,000 trees of each data set, for a majority-rule consensus tree of 60,000 trees.

We calculated pairwise differences and haplotype and nucleotide diversity indexes, and carried out demographic analyses described below using the program DNASP, version 4.20.2 (Rozas et al. 2003). The haplotype diversity index measures the probability that two randomly chosen haplotypes in the sample are different, whereas nucleotide diversity is a measure of pairwise nucleotide differences per site among haplotypes in the sample (Nei 1987). We derived haplotype networks from statistical parsimony using the program TCS, version 1.13 (Clement et al. 2000). Connections among haplotypes are based on pairwise differences as long as there is a high probability ($P > 0.95$) that the difference in a site between two haplotypes is the product of just one mutation (parsimonious state). We determined mismatch distributions to investigate historical changes (demographic history) in populations (Rogers and Harpending 1992, Rogers 1995). Mismatch distributions are the distribution of pairwise nucleotide differences among haplotypes (Rogers and Harpending 1992) and, when compared to a model of population expansion, can provide insight on the demographic history of species. The shape of these distributions indicates whether populations have gone through episodes of decline or expansion in population growth (unimodal distributions) or, on the contrary, have been characterized by constant population sizes (multimodal distributions; Rogers and Harpending 1992, Rogers 1995). We evaluated statistical significance for population expansion using Ramos-Onsins and Rozas's *R*2 statistic (Ramos-Onsins and Rozas 2002), which is based on the difference between the number of singleton mutations and the average number of nucleotide differences among sequences within a population sample, and Fu's *F* test (Fu 1997), which tests for deviation from neutrality but which is sensitive to departures from population stationarity (excess of young and rare mutations in non-recombining sequences) and can reveal histories of demographic events such as population expansions. We based significance on confidence limits generated under a coalescent model assuming constant population size and the number of segregating sites (Rozas et al. 2003). We also used the McDonald-Kreitman test to check for the effect of natural selection in the combined ND2 and ND3 data, because it has been shown that positive selection can affect mtDNA and bias interpretation of genetic patterns (Ballard and Whitlock 2004). As such, we compared the ratio of synonymous to nonsynonymous polymorphic sites to the ratio of synonymous to nonsynonymous fixed substitutions between pairs of clades obtained in our study, with the assumption that ratios should be the same in the absence of positive selection (Zink 2005, Zink et al. 2005).

The demographic population model described above estimates three parameters of interest—the effective population size, both present and before the expansion, and the time elapsed between the two (t). Consequently, we estimated the time elapsed from population expansion. The actual mutation rate per nucleotide and generation (μ) has to be known in order to estimate the time (number of generations) since population-size change (t), assuming that $\tau = 2ut$ (τ measures time in mutational events) and $u = \mu L$, where L is the length of the sequence of DNA analyzed (Rogers and Harpending 1992). We assumed mutation rates ranging from 0.02 (cytochrome-*b* data; Weir and Schluter 2008) to 0.03 (based on comparisons of genetic divergences between cytochrome-*b* and ND2/ND3 genes in *miniatus* sequences; J. L. Pérez-Emán et al. unpubl. data) substitutions per site per million years. Because 1-year-old birds

regularly acquire territories and breed (R. L. Mumme unpubl. data), we assumed a generation time of one year as the age at which sexual maturity is reached. However, given that birds of this species have a long lifespan (Lentino et al. 2003, R. L. Mumme unpubl. data), we suspect that mean generation time is probably closer to 2 years than to 1 year and, as such, we also considered this age as an estimate of generation time.

Results

Phylogenetic relationships among haplotypes.—We sequenced a total of 1,392 bp (complete ND2 and ND3 genes), all of them clean and unambiguous, with no instances of unusual stop codons, insertions or deletions, which indicates that the sequences were not nuclear copies (pseudogenes). All new sequences have been deposited in GenBank (accession numbers GQ335543–GQ335588). We included the complete sequences of both ND2 and ND3 genes combined in phylogenetic analyses, because a partition homogeneity test failed to find differences in phylogenetic signal between genes ($P = 0.65$).

Phylogenetic reconstructions identified four lineages with high support (bootstrap values > 85% and posterior probabilities = 100%; Fig. 2A). Mexican populations of the subspecies *miniatus* located to the west of the Isthmus of Tehuantepec

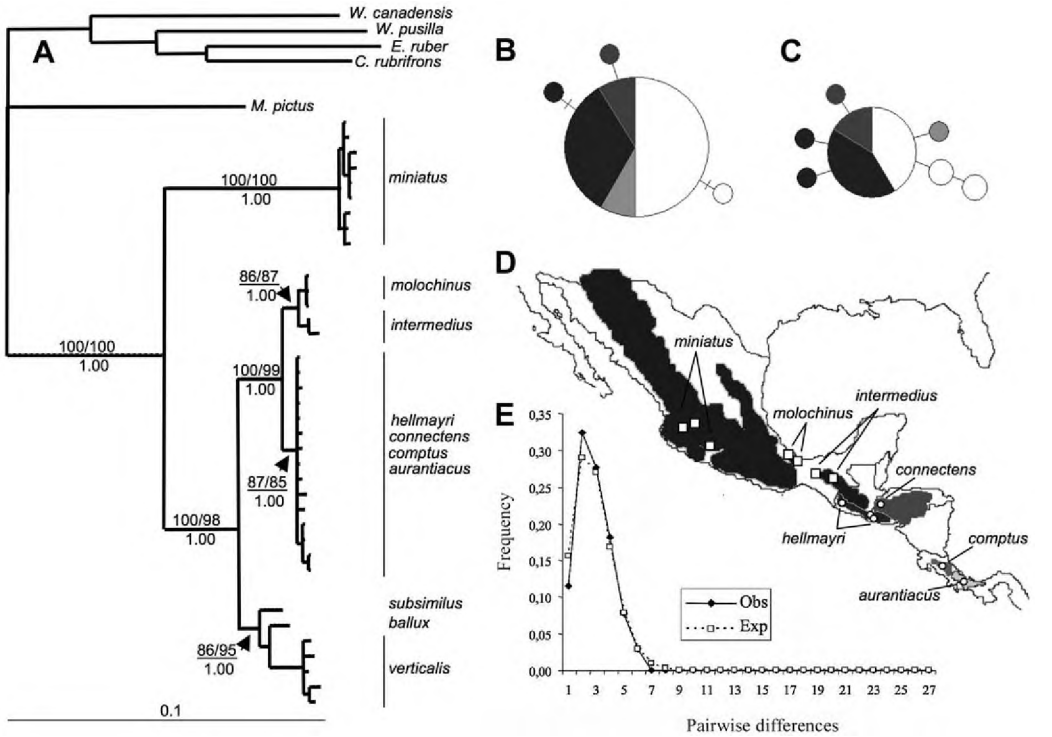

FIG. 2. Phylogenetic relationships and genetic differentiation of *Myioborus miniatus* in North America and Central America. (A) Maximum-likelihood tree topology of *miniatus* haplotypes. Numbers above the nodes represent bootstrap support values from maximum-parsimony and maximum-likelihood analyses. Numbers below the nodes represent posterior probabilities obtained from Bayesian inference. Subspecies names identify each of the major lineages. (B) Central American *miniatus* haplotype networks of both mitochondrial ND2 and (C) ND3 genes. Colors represent each of the subspecies included in this clade: black = *hellmayri*, dark gray = *connectens*, light gray = *comptus*, and white = *aurantiacus*. (D) Distributions of each subspecies of *miniatus* in North America and Central America. Circles represent voucher localities associated with each of the previous four subspecies included in haplotype networks (identified by their names). Squares represent voucher localities of other North American and Central American populations (subspecies). (E) Mismatch distribution of haplotypes included in the Central American clade, assuming a population growth model. Both observed and expected distributions are represented. See text for statistical significance of these results.

represented a basal lineage. A South American clade included populations of the subspecies *ballux* from eastern Panama, and a third lineage represented Mexican populations adjacent to the Isthmus of Tehuantepec, including the isolated subspecies *molochinus* from the Los Tuxtlas mountains of Veracruz and the subspecies *intermedius* from eastern Oaxaca and Chiapas. A fourth clade comprised individuals of four Central American subspecies (*hellmayri*, *connectens*, *comptus*, and *aurantiacus*) from western Guatemala through the Chiriqui Highlands in Panama. Populations from this last clade were very homogeneous, differing by an average of <2 pairwise nucleotide differences ($k = 1.87$) and showing no resolution in the phylogenetic tree, a result that contrasts with the morphological and plumage differentiation shown by populations distributed along this geographic range. A likelihood ratio test failed to reject a clock-like evolution of sequences, which indicates a lack of rate heterogeneity.

Genetic variation in miniatus *populations*.—We found a total of 32 haplotypes among 36 sequences of *M. miniatus* analyzed. Haplotype divergences among lineages were larger than within lineages. Average sequence divergence (*p*-distance) between Mexican populations of *miniatus* was 5.8% when compared to populations adjacent to the Isthmus of Tehuantepec and Central American clades and 5.9% when compared to the South American clade, indicating an old split between the Mexican clade and the other lineages. Sequence divergence between the Isthmus of Tehuantepec and the Central American and South American clades averaged 2.5%. Divergence within clades was low, with the largest value found in the South American clade (0.9%) and the smallest value recorded for the Central American clade including populations from Guatemala to Panama (0.1%). These data are in agreement with haplotype and nucleotide diversities, which were highest for the South American clade and smallest for the Central American clade, highlighting the small genetic divergence in this clade (Table 1).

Demographic history of miniatus *populations*.—The genetic homogeneity found in the Central American clade is consistent with patterns of rapid diversification or rapid expansion of populations forming such a clade. Haplotype networks of both mitochondrial genes (ND2 and ND3) showed a similar star-like structure characterized by one common and frequent haplotype distributed from Guatemala to Panama, and other closely related haplotypes found in low frequencies (Fig. 2B–D). A mismatch distribution of *miniatus* from Central America (Guatemala to highlands of Panama) fit the expected pattern under population expansion (Fig. 2E; $R^2 = 0.086$, $P < 0.05$). Fu's *F*-test of neutrality was also significantly negative ($F_S = -4.80$, $P < 0.05$), which indicates that populations from Central America have not been stationary through time. McDonald-Kreitman tests were not significant in any of the pairwise comparisons of clades (*P* values ranging from 0.67 to 1.00). Estimated times from population expansion ranged from 33,500 years (67,000 years considering a generation time equal to 2 years) with a 2% calibration rate to 22,350 (44,700) years with a 3% rate, consistent with a late Pleistocene temporal framework.

Discussion

Phylogeography of Myioborus miniatus *populations*.— Phylogenetic reconstructions of *M. miniatus* populations included in our study identified four well-supported clades, each of them representing monophyletic groups consistent with

Table 1. Genetic diversity indices for each of the four geographic clades recognized by phylogenetic analyses. Subspecies of *Myioborus miniatus* included in each clade were as follows: Mexican clade, *miniatus*; Isthmus of Tehuantepec clade, *molochinus* and *intermedius*; Central American clade, *hellmayri*, *connectens*, *comptus*, and *aurantiacus*; South American clade, *subsimilis*, *verticalis*, and *ballux* (including populations from eastern Panama).

Clade	*n*	Number of haplotypes	Haplotype diversity	Nucleotide diversity	Nucleotide differences
Mexican	9	8	0.97	0.0024	3.28
Isthmus of Tehuantepec	5	3	0.70	0.0026	3.60
Central American	15	9	0.89	0.0013	1.87
South American	7	7	1.00	0.0088	12.30

geography. The most distinct clade, a basal lineage, comprised populations of the nominate subspecies *miniatus* from northern and central Mexico. This clade was sister to a large group composed of a clade encompassing populations of three South American subspecies (*ballux*, *subsimilis*, and *verticalis*), which was sister to a second clade subdivided into two main lineages: one represented by populations on opposite sides of the Isthmus of Tehuantepec (including two subspecies, *molochinus* from the Sierra de Los Tuxtlas mountains of Veracruz, and *intermedius* of the interior highlands of Oaxaca and Chiapas), and another one comprising Central American populations ranging from Guatemala to the Chiriqui highlands of western Panama. This phylogeographic pattern, a Mexican basal taxon sister to a clade of Central American and South American lineages sister to each other, is largely congruent with those shown by other Neotropical taxa with similar geographic distributions—for example, *Chlorospingus ophthalmicus* (García-Moreno et al. 2004, Weir et al. 2008) and *Arremon brunneinucha* (Cadena et al. 2007, Navarro-Sigüenza et al. 2008). The phylogeographic pattern also suggests a northern origin and subsequent southern dispersal for the *Myioborus miniatus* species complex, which has also been suggested for hummingbirds in the genus *Lampornis* (García-Moreno et al. 2006) and solitaires in the genus *Myadestes* (Miller et al. 2007).

The Central American lineage, representing populations belonging to four different named subspecies (*hellmayri*, *connectens*, *comptus*, and *aurantiacus*), was characterized by a surprising lack of genetic structure. This lack of structure could be the result of either high current levels of gene flow or recent species range expansions (Zink 1997). Although extensive gene flow seems possible in populations that represent subspecies pairs that exist in parapatry (e.g., *connectens* and *hellmaryi*, *comptus* and *aurantiacus*), populations of *connectens* and *hellmaryi* are separated from *comptus* and *aurantiacus* by several hundred kilometers of Nicaraguan lowlands, which makes extensive gene flow between these two subspecies groups exceedingly unlikely, at least at present. Our results are more consistent with the hypothesis of recent population expansion. Population expansions that result from bottleneck episodes or rapid expansions from a leading front are expected to result in homogeneous populations with low levels of genetic diversity (Rogers and Harpending 1992, Hewitt 2000). The observed fit of mismatch distributions to patterns expected with population expansion, particularly when added to the star-like haplotype phylogeny found for this clade, argues for a late Pleistocene range expansion of the Central American lineage across this region.

The proposed late Pleistocene expansion of the Central American lineage now encompasses an area from Guatemala to Panama and includes regions of extensive lowland forest (i.e., Nicaragua) where *M. miniatus* is neither currently distributed nor regularly recorded. Although it is possible that the proposed range expansion may have occurred as the result of long-distance colonization across unsuitable lowland habitat, we think that the expansion was more likely facilitated by a more extensive distribution of montane forest at lower elevations during the late Pleistocene. Paleoecological analysis indicates that the late Pleistocene flora at Lake La Yeguada, Panama, at elevation 650 m, included montane forest elements that are now more characteristic of elevations above 1,500 m (Bush et al. 1992). This analysis suggests the possibility that montane forests suitable for *M. miniatus* occurred at much lower elevations during the late Pleistocene, and this could have facilitated the Central American population expansion throughout the region during this period.

Regardless of the evidence of recent population expansion, the lack of genetic structure within the Central American clade is surprising given the fairly dramatic differences in plumage, particularly belly color and tail pattern, among the four subspecies; *connectens* and *hellmayri* have salmon to red-orange bellies and relatively little white in the tail, whereas *comptus* and *aurantiacus* have yellow-orange bellies and more extensive white in the tail. One possible hypothesis to explain this pattern is that subspecific variation in plumage pattern may reflect phenotypic plasticity rather than underlying genetic variation. In other words, the subspecific variation in belly color and tail pattern within the Central American clade could be a result of geographic variation in environmental conditions during growth and development, and not of subspecific differences in genes affecting growth and development. The plasticity hypothesis is an intriguing possibility particularly for belly color, because environmental variation in dietary carotenoid intake is known to account for many cases of within- and between-population variation in the extent of yellow versus red plumage in birds (Hill 1993, 2006). However, phenotypic plasticity seems unlikely to account

for variation in tail pattern. First, there is generally little evidence that variation in diet or other environmental variables can produce consistent variation in pigment deposition in melanin-based black-and-white plumage patterns (Hill 2006). Second, our preliminary examination of data from museum specimens and recaptures of individual birds between tail molts (R. L. Mumme unpubl. data) suggests that there is relatively little within-population or within-individual variation in tail pattern; if tail pattern were phenotypically highly plastic, one would predict that variation within populations would be considerable and that tail patterns of individual birds might also vary from molt to molt as social status or nutritional condition change. Neither prediction appears to be true. Finally, at least some of the subspecies that differ greatly in tail pattern (e.g, *miniatus* vs. *hellmayri*, *comptus* vs. *verticalis*) are quite different from one another in mtDNA (Fig. 2), which suggests that differences in tail patterns among those subspecies pairs may also have a genetic basis. Nonetheless, even though we believe that phenotypic plasticity is unlikely to explain subspecific variation in tail pattern in *M. miniatus*, it remains a possibility that can be eliminated only by reciprocal transplant (e.g., James 1983) or "common garden" experiments (e.g., Berthold et al. 1992) that, given the geographic distribution of these taxa across several different Central American countries, would be very difficult to conduct.

Assuming that phenotypic plasticity is not involved and that subspecific plumage variation within the Central American clade is based on underlying genetic variation, the results of our mtDNA analyses suggest that plumage divergence evolved recently (since the late Pleistocene) and in the absence of any corresponding neutral genetic divergence at the level of mtDNA. What evolutionary processes drove this rapid plumage evolution? For subspecific variation in belly color, we are unable to answer this question; we have no data that bear directly on the question of why *connectens* and *hellmayri* should have salmon or red-orange bellies whereas *comptus* and *aurantiacus* have yellow-orange bellies, or why the marked north-south gradient in belly color exists generally in the Slate-throated Redstart. In the case of tail pattern, however, we have good reason to suspect that subspecific variation within the Central American clade is a result of divergent natural selection, and we discuss the evidence in the following sections.

The black-and-white tail as foraging adaptation.—Like all redstarts in the genus *Myioborus*, the Slate-throated Redstart uses striking animated displays of its contrasting black-and-white tail to startle potential insect prey, which are then pursued and captured in flight (Jabłoński 1999, Mumme 2002). This is an innate (Jabłoński et al. 2006b) foraging tactic termed "flush-pursuit foraging" (Remsen and Robinson 1990). During flush-pursuit foraging, redstarts hop stiffly from branch to branch, erecting and fanning their contrasting black-and-white tails, drooping their wings, and pivoting and pirouetting from side to side. By experimentally darkening the white feathers of birds in the field, and by testing the response of insects to models of foraging birds, our earlier work has demonstrated that the presence of contrasting black-and-white tail feathers is critical in triggering insect escape behavior during flush-pursuit foraging, in both the Painted Redstart (*M. pictus*) in Arizona (Jabłoński 1999, 2001; Jabłoński and Strausfeld 2000, 2001) and the Slate-throated Redstart in Costa Rica (Mumme 2002, Galatowitsch and Mumme 2004, Mumme et al. 2006).

Foraging displays in *Myioborus* are effective because they exploit the simple sensory and neuromuscular pathways that govern visually evoked insect escape response. Insect escape behavior has been an important model system in insect neurobiology and is generally well understood (Bullock 1985, Holmqvist and Srinivasan 1991, Rind and Simmons 1992, Hatsopoulos et al 1995, Gabbiani et al. 1999, Jabłoński and Strausfeld 2000, Santer et al. 2006, Shin and Jabłoński 2008). Insect escape responses are visually evoked through pathways tuned to looming motion, such as an approaching predator. In particular, these pathways are sensitive to an increased rate of image expansion on the insect retina, accelerated translational movement across the visual field, and increased contrast between the object and the background. Thus, the three most striking visual aspects of foraging displays in *Myioborus*—spreading of the tail and wing to increase stimulus size, side-to-side pivoting motions to increase stimulus movement, and conspicuous display of the black-and-white tail feathers to increase stimulus contrast—exploit the visual sensitivity of escape pathways in insect prey and lead to an increase in the number of potential prey flushed by a foraging bird (Jabłoński and Strausfeld 2000, 2001). Additionally, the side-to-side pivoting promotes foraging success by affecting prey escape direction (Jabłoński 2001, Jabłoński and McInerney 2005).

Having a black-and-white tail is therefore clearly an important foraging adaptation in *Myioborus* (Jabłoński 1999, Mumme 2002). However, this does not necessarily mean that geographic (subspecific) variation within *M. miniatus* in the extent of white in the tail is adaptive and driven by diversifying selection. A legitimate nonadaptive null hypothesis is that although some white is necessary for successful flush-pursuit foraging, subspecific variation in the extent of white in the tail could be entirely inconsequential to foraging performance and simply a result of genetic drift, not natural selection (Mumme et al. 2006). The alternative adaptationist hypothesis, however, is that subspecific geographic variation in the tail pattern of *M. miniatus* has been shaped by natural selection and reflects adaptation to regional prey and habitat characteristics that maximizes flush-pursuit foraging performance.

We have tested these two alternatives with a set of field experiments conducted with the subspecies *M. miniatus comptus* in Monteverde, Costa Rica, in which we either reduced or increased the amount of white in the tail of Monteverde birds to mimic the tail patterns found in *hellmayri* of northern Central America or *verticalis* of Bolivia (Mumme et al. 2006). First, birds with reduced-white *hellmayri*-like tails, although they performed better than birds with no white, had significantly decreased flush-pursuit foraging performance compared with *comptus*-like controls. However, birds with the increased-white *verticalis*-like tail pattern had slightly but not significantly lower foraging performance than *comptus*-like controls (Fig. 3A). These results suggest that the particular black-and-white tail pattern of *comptus* represents a broad but nonetheless distinct locally adaptive peak that maximizes the flushing of insect prey under Costa Rican field conditions. This conclusion is corroborated by a series of field experiments that tested the response of seven different Costa Rican insect prey species, six species of homopterans, and an asilid robber fly, to models of foraging *Myioborus* ssp. Under Monteverde field conditions, insect prey were significantly less responsive to models of *hellmayri* versus *comptus*, and slightly but not significantly less responsive to models of *verticalis* (Fig. 3B; Galatowitsch and Mumme 2004, Mumme et al. 2006).

Collectively, the results of the bird and insect experiments indicate that the tail pattern of Costa Rican birds of the subspecies *comptus* maximizes flush-pursuit foraging performance for the specific prey and/or habitat conditions that exist within its range. Because efficient foraging is known to have direct positive effects on lifetime reproductive success in birds (Lemon and Barth 1992), it is likely that the tail pattern of this subspecies has been shaped by a history of natural selection.

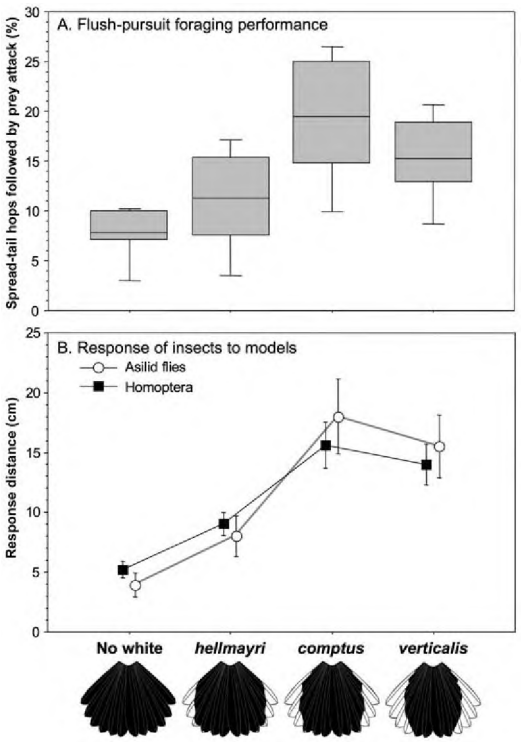

FIG. 3. (A) Flush-pursuit foraging performance of individuals of *Myioborus miniatus comptus* following experimental manipulation of the extent of white in the tail. Flush-pursuit foraging performance was measured by the percentage of hops in the characteristic spread-tail posture that were followed by an attack on a prey item. Median (horizontal line), 25th and 75th percentiles (box), and 10th and 90th percentiles (whiskers) are shown. Modified from Mumme et al. (2006). (B) Mean response distance of homopterans and asilid flies in reaction to a model of a foraging redstart representing four different plumage patterns. Mean and standard errors are shown. Based on data from Galatowisch and Mumme (2004) and Mumme et al. (2006).

Evolutionary divergence of tail pattern: Prey-driven or habitat-driven?—One issue raised by our findings is the nature of the environmental variation that appears to have produced subspecific divergence in tail pattern. Two hypotheses can be proposed: a prey-specific hypothesis and a habitat-specific hypothesis (Jabłoński et al. 2006a, Mumme et al. 2006). The prey-specific hypothesis

suggests that different subspecies of *M. miniatus* may exploit insect prey with different visual sensitivities or escape behavior, leading to selection for subspecific divergence in plumage pattern. However, the neural mechanisms that govern insect escape behavior are simple and highly conserved (Bacon and Strausfeld 1986; Jabłoński and Strausfeld 2000, 2001), and significant geographic variation in how those systems respond to subtly different types of visual signals may not exist (Jabłoński et al. 2006a, Mumme et al. 2006). Detailed studies of the prey base of different subspecies of *miniatus* in different geographic regions would be required to test the prey-specific hypothesis and determine whether geographic variation in insect prey could potentially produce plumage divergence.

The habitat-specific hypothesis (Jabłoński et al. 2006a) proposes that geographic variation in habitat or environmental conditions may select for subspecific divergence in plumage patterns. Under this hypothesis, geographic variation in the physical environment, not the prey base, promotes diversification of plumage pattern by selecting for tail patterns that work best under prevailing ecological conditions. This hypothesis is supported by experiments that showed that the background against which model redstarts are displayed can significantly influence the effectiveness of a particular plumage pattern in startling prey; models with relatively little white were more effective when displayed against relatively light backgrounds, whereas models with more extensive white were more effective when displayed against darker backgrounds (Jabłoński et al. 2006a). Similarly, in field experiments, muscid flies living in very different habitats responded differently to models of redstarts with a particular tail pattern (Jabłoński et al. 2006a). Although these data support the habitat-specific hypothesis, a definitive test will require detailed studies of the physical environment of *M. miniatus* throughout its broad geographic range.

Subspecific taxonomy, plumage pattern, and genetic divergence.—One of the implications of the present study is that the current subspecific taxonomy of the Central American populations of *M. miniatus*, a taxonomy based on decades-old evaluation of plumage variation, is not supported by modern evolutionary genetic analysis of sequence divergence in mtDNA. Although several of the 12 recognized subspecies of *M. miniatus* appear to represent distinct evolutionary lineages (e.g., the nominate subspecies *M. m. miniatus* of central Mexico, as well as the subspecies *molochinus*, *intermedius*, and *verticalis*), four Central American subspecies—*hellmayri*, *connectens*, *comptus*, and *aurantiacus*—cannot be resolved by mtDNA analysis. From an mtDNA perspective, subspecific designations of these taxa do not correspond to independent evolutionary units (Zink et al. 2000).

Should we therefore conclude that the four named Central American subspecies have no genetic or evolutionary validity? This question lies at the heart of much of the recent controversy surrounding the recognition of avian subspecies (e.g., Zink 2004, Phillimore and Owens 2006, Rising 2007). We believe, however, that such a conclusion would be unwarranted. Although our genetic analysis suggests that the subspecies of the Central American clade are indeed genetically homogeneous, at least from the perspective of mtDNA, our analysis of plumage pattern and its effect on foraging performance suggests that the Central American subspecies have undergone recent evolutionary divergence that appears to have been adaptive and driven by natural selection for improved foraging performance. As pointed out by other authors (Rising 2007, Winker et al. 2007) mtDNA gene trees will not always reflect every instance of evolutionarily significant genetic change. In fact, evolutionary change in neutral characters at the intraspecific level is unlikely to result in monophyletic groups; on the contrary, paraphyletic and even polyphyletic patterns are expected as a result of incomplete lineage sorting (Funk and Omland 2003). Moreover, populations that have undergone recent historical demographic expansions will display genetic homogeneity that could confound the identification of evolutionary units. As a consequence, any adaptive evolutionary change that occurred during a short time will cause a lack of concordance among phenotypic characters that have been subject to selection and neutral molecular characters (Moritz 2002). Examples of morphological differences in clearly diagnosable taxa that are uncorrelated with genetic divergence include sparrows in the genus *Passerculus* (Remsen 2005, Zink et al. 2005, Rising 2007), warblers of the *Dendroica coronata* species complex (Milá et al. 2007), gnatcatchers in the genus *Polioptila* (Zink et al. 2000), and grouse species in the subfamily Tetraoninae (Oyler-McCance et al., this volume). In many of these examples, morphologically distinct populations

deserve taxonomic status; lumping different morphological and ecological populations into one name can confound the evolutionary history of the organism and produce taxa that are not predictive of ecology or morphology (Barrowclough 1982, Remsen 2005).

An important point to consider is the diagnosibility of infraspecific categories. Most of the criticism directed toward subspecies categories focuses on the lack of unambiguous criteria to diagnose and identify geographically distinct populations (Wilson and Brown 1953). Expanding and formalizing the historical "75% rule" proposed by Amadon (1949), Patten and Unitt (1992) criticized the normal approach of considering mean differences instead of magnitude of variation. In the case of subspecies of *M. miniatus*, formal descriptions of the four subspecies included in the Central American clade focused on mean differences in some morphological characters as well as variation in plumage coloration, especially in the crown, chest, belly, and tail feathers (van Rossem 1936, Wetmore 1944). At present, we lack sufficient data to apply statistical tests of diagnosibility to these populations. However, *hellmayri–connectens* populations are clearly 100% diagnosable compared with *comptus–aurantiacus* populations, both for belly color and tail pattern, and the differences in tail pattern appear to be adaptive. Moreover, these populations are potentially isolated geographically. Thus, even in the absence of mtDNA genetic divergence, geographic variation of this sort ought to be recognized taxonomically if we want classifications to be predictive. On the other hand, the populations within the two subspecies groups (*hellmayri–connectens* and *comptus–aurantiacus*) are more similar to each other, and there is likely some geographic contact between populations within each group. In the case of *comptus* and *aurantiacus*, there seems to be evidence for a large contact zone and, possibly, intergradation in the Costa Rican central plateau (Slud 1964). Consequently, each of these populations should be studied more thoroughly if we want to recognize or reject subspecies names given to some geographic variation that may be clinal.

Our results highlight the importance of selecting a set of diverse characters when evaluating geographic variation within species and underscore the value of field experiments as tools for understanding and evaluating potential selective mechanisms responsible for evolutionary divergence within a species. We suggest, as some have previously done (Legge et al. 1996, Crandall et al. 2000, Remsen 2005), that subspecies concepts should be critically examined but, at the same time, should be sufficiently broad to include cases where subspecific adaptation has occurred in the apparent absence of overall mitochondrial genetic divergence. Recognizing both phylogenetic and adaptive changes in the evolutionary history of an organism, and correctly naming such geographic variation, will produce classifications that are consistent with evolutionary history.

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APPENDIX. List of taxa, tissue numbers, museum collections, and localities of samples used in the study.

Taxon	Tissue no.	Museum[a]	Locality
Myioborus miniatus			
miniatus	FMNH 4647	FMNH	Mexico: Michoacan, Tancitaro
miniatus	FMNH 4478	FMNH	Mexico: Jalisco, Sierra de Manantlan, Puerto Los Mazos
miniatus	FMNH 4477	FMNH	Mexico: Jalisco, Sierra de Manantlan, Puerto Los Mazos
miniatus	FMNH 5628	FMNH	Mexico: Jalisco, Sierra de Manantlan, Puerto Los Mazos
miniatus	FMNH 2185	FMNH	Mexico: Jalisco, Sierra de Manantlan, Puerto Los Mazos
miniatus	FMNH 1217	FMNH	Mexico: Jalisco, Sierra de Manantlan, Puerto Los Mazos
miniatus	FMNH 1209	FMNH	Mexico: Jalisco, Sierra de Manantlan, Puerto Los Mazos
miniatus	JK03-239	MBM	Mexico: Jalisco, Sierra de Bolaños, 5.8 km N, 9 km W of Bolaños
miniatus	GMS581	MBM	Mexico: Jalisco, Sierra de Bolaños, 5.8 km N, 9 km W of Bolaños
molochinus	PEP 2883	UNAM	Mexico: Veracruz, Sierra de Santa Marta, 700 S Santa Marta
molochinus	PEP 2884	UNAM	Mexico: Veracruz, Sierra de Santa Marta, 700 S Santa Marta
molochinus	PEP 2919	UNAM	Mexico: Veracruz, Volcan de San Martin de Tuxtla, 1 km N La Herradura
intermedius	FMNH 1693	FMNH	Mexico: Chiapas, Cerro Tzontehuitz, 7 km NE San Cristobal de las Casas
intermedius	CHIMA 530	UNAM	Mexico: Oaxaca, San Isidro La Gringa, 1 km SE San Francisco La Paz
connectens	DHB3162	MBM	Honduras: Dpto. Copan, Copan Ruinas, 15 km N
connectens	DHB3163	MBM	Honduras: Dpto. Copan, Copan Ruinas, 15 km N
hellmayri	DHB4569	MBM	Guatemala: Dpto. Quezaltenango, Santa María de Jesús 5 km SSW, Finca de Santa María
hellmayri	DHB4593	MBM	Guatemala: Dpto. Quezaltenango, Santa María de Jesús 5 km SSW, Finca de Santa María
hellmayri	KU 5917	KU	El Salvador: Ahuachapan Department
hellmayri	KU 5931	KU	El Salvador: Ahuachapan Department
hellmayri	KU 6009	KU	El Salvador: Sonsonate Department
comptus	B 27304	LSUMNS	Costa Rica: San Jose Province; 0.5 km NNE San Ramon
aurantiacus	B 26421	LSUMNS	Panama: Chiriquí Province, District Gualaca, Cordillera Central
aurantiacus	B 01561	LSUMNS	Panama: Chiriquí Province, District Gualaca, Cordillera Central
aurantiacus	B 05337	LSUMNS	Panama: Chiriquí Province, District Gualaca, Cordillera Central
aurantiacus	B 05475	LSUMNS	Panama: Chiriquí Province, District Gualaca, Cordillera Central
aurantiacus	B 29046	LSUMNS	Panama: Chiriquí Province, District Gualaca, Cordillera Central
aurantiacus	B 28167	LSUMNS	Panama: Chiriquí Province, District Gualaca, Cordillera Central
aurantiacus	B 28175	LSUMNS	Panama: Chiriquí Province, District Gualaca, Cordillera Central
ballux	B-1423	LSUMNS	Panama: Darién Province; ca. 9 km NW Cana on slopes Cerro Pirre
subsimilis	B-178	LSUMNS	Peru: Piura Dept.; km 34 on Olmos-Bagua Chica Hwy
verticalis	O 1541	ZMUC	Ecuador: Zamora-Chinchipe, near Chinapinza
verticalis	B 1694	LSUMNS	Peru: Pasco, Santa Cruz; ca. 9 km SSE Oxapampa
verticalis	B 1696	LSUMNS	Peru: Pasco, Santa Cruz; ca. 9 km SSE Oxapampa
verticalis	B 22788	LSUMNS	Bolivia: La Paz, Province B. Saavedra, 83 km by road E. Charazani
verticalis	B 22797	LSUMNS	Bolivia: La Paz, Province B. Saavedra, 83 km by road E. Charazani
Myioborus pictus	FMNH 2210	FMNH	Mexico: Sinaloa, El Batel
Wilsonia pusilla	47919	UWBM	USA: Washington; Wahkiakum, Cathlamet (JMB696)
W. canadensis	PA-WCA55	STRI	Panama: Colon Province, 2 km W Gatun locks
Ergaticus ruber	BMM-187	FMNH	Mexico: Michoacan; Pico de Tancitaro, 3 km N Zirimondiro
Cardellina rubrifrons	B 10178	LSUMNS	USA: Arizona: Santa Cruz Co.: GRNR CNYN

[a]Abbreviations: FMNH = Field Museum of Natural History; LSUMNS = Louisiana State University Museum of Natural Science; ZMUC = Zoological Museum of the University of Copenhagen; STRI = Smithsonian Tropical Research Institute; UWBM = University of Washington Burke Museum; KU = Kansas University Natural History Museum; MBM = University of Nevada Las Vegas, Barrick Museum of Natural History; and UNAM = Universidad Nacional Autónoma de Mexico.