

## PHYLOGEOGRAPHY OF THE RUFOUS-TAILED HUMMINGBIRD (*AMAZILIA TZACATL*)

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**Abstract.** The Rufous-tailed Hummingbird (*Amazilia tzacatl*) is a common resident of the neotropical lowlands of Middle America and northern South America. The Escudo Hummingbird (*A. t. handleyi*), the most distinctive of the five subspecies of *A. tzacatl*, is endemic to Isla Escudo in Caribbean western Panama. Mitochondrial DNA (mtDNA) sequence variation from across most of the species' range showed five well-defined but shallow clades (maximum uncorrected distance 2.4%) that only partially agreed with subspecific taxonomy. A widespread Middle American clade ranges from southeastern Mexico to central Caribbean Panama and includes birds collected on the Bocas del Toro archipelago. The Escudo Hummingbird fell within this clade and was slightly differentiated (two unique substitutions; uncorrected distance ~0.2–0.5%). Two additional clades occur in the Pacific regions of southern Middle America. A fourth clade is endemic to eastern Panama (eastern Panamá and Darién provinces); a fifth is found in northwestern South America and in Darién. Secondary contact between clades occurs at three sites: between clades I and III in northwestern Costa Rica, between clades I and II in western Panama, and between clades IV and V near the Panama–Colombia border. The last case is likely due to recent expansion into the region from two directions. Thus the history of *Amazilia tzacatl* demonstrates a tendency for the formation of monophyletic mtDNA clades, likely as the result of geographic isolation, but also a propensity for secondary contact of these clades, a phenomenon recovered in many other phylogeographic studies of neotropical birds.

**Key words:** *Amazilia tzacatl*, Middle America, mitochondrial DNA, phylogeography, Rufous-tailed Hummingbird, secondary contact.

### Filogeografía de *Amazilia tzacatl*

**Resumen.** El colibrí *Amazilia tzacatl* es un residente común de las tierras bajas neotropicales de América Central y del norte de América del Sur. La subespecie *A. t. handleyi* es la que más se diferencia de *A. tzacatl* de las cinco subespecies, y es endémica de la Isla Escudo en el Caribe occidental de Panamá. La variación en la secuencia de ADN mitocondrial (ADNmt) a lo largo de la mayor parte del área de distribución de la especie mostró cinco clados bien definidos pero poco profundos (distancia máxima no corregida de 2.4%), que sólo concordaron parcialmente con la taxonomía subespecífica. Un clado de América Central ampliamente distribuido se distribuye desde el sudeste de México hasta el centro caribeño de Panamá e incluye aves colectadas en el archipiélago de Bocas del Toro. Los individuos de la subespecie *A. t. handleyi* cayeron dentro de este clado y se diferenciaron muy poco (dos sustituciones únicas, distancia no corregida de ~0.2–0.5%). Dos clados adicionales se presentan en las regiones del Pacífico del sur de América Central. Un cuarto clado es endémico del este de Panamá (este de Panamá y las provincias de Darién); un quinto clado se encuentra en el noroeste de América del Sur y en el Darién. El contacto secundario entre clados ocurre en tres sitios: entre los clados I y III en el noroeste de Costa Rica, entre los clados I y II en el oeste de Panamá, y entre los clados IV y V cerca de la frontera entre Panamá y Colombia. El último caso se debe probablemente a una expansión reciente en la región desde dos direcciones. Así, la historia de *A. tzacatl* demuestra una tendencia a la formación de clados monofiléticos de ADN mitocondrial, probablemente como resultado del aislamiento geográfico, pero también una propensión al contacto secundario de estos clados, un fenómeno comúnmente descrito en otros estudios filogeográficos de aves neotropicales.

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## INTRODUCTION

Phylogeographic studies give us the ability to “ground truth” existing hypotheses about factors that shape contemporary avian diversity. Nearly one in three bird species is found in the Neotropical Region, making this the most species-rich region for birds on Earth. Although the greatest number of bird species is found in the vast lowlands of this region, only a handful of these species have undergone detailed phylogeographic analysis (Marks et al. 2002, González et al. 2003, Lovette 2004a, Cheviron et al. 2005, Aleixo 2006, Dingle et al. 2006, Cabanne et al. 2007, 2008, Nyári 2007, Miller et al. 2008). The phylogeographic complexity of many of these taxa is greater in Middle America than in South America (e.g., Marks et al. 2002, González et al. 2003, Dingle et al. 2006, Nyári 2007, Miller et al. 2008; see also Bermingham and Martin 1998). This complexity may stem from multiple factors, including the presence of two narrow land bridges (the Isthmus of Tehuantepec and the Isthmus of Panama; González et al. 2003, Dingle et al. 2006, Barber and Klicka 2010) that may have restricted gene flow and multiple colonizations of Middle America by lineages originating in South America (Marks et al. 2002, Dingle et al. 2006, Nyári 2007, Miller et al. 2008).

We chose to study the phylogeography of the Rufous-tailed Hummingbird (*Amazilia tzacatl*) for several reasons. First, this species geographically overlaps several Middle American taxa for which phylogeographic studies have been completed (e.g., González et al. 2003, Miller et al. 2008, Miller et al. 2010), enabling interspecific comparisons across this region.

Second, *Amazilia tzacatl* is one of only a few landbirds found on both Caribbean and Pacific islands off southern Middle America, providing an excellent opportunity for studies of biogeography, ecology, and evolution. The population on Isla Escudo, which lies off of the Caribbean coast of Veraguas, Panama, is of particular interest because these birds are 25% larger than and differ notably in plumage from the mainland forms, whereas birds on the nearby Bocas del Toro archipelago are essentially the same size and color as mainland birds. Finally, two recent expeditions to eastern Darién Province, Panama, obtained the first specimens of this species from this region, enabling us to investigate phylogeographic patterns in a neotropical bird naturally expanding its range (see Discussion).

## STUDY TAXON

The Rufous-tailed Hummingbird is a common resident of the tropical and subtropical zones in the neotropics, with a range that spans 28° of latitude from southeastern Mexico to southwestern Ecuador and western Venezuela (Skutch 1931, Schuchmann 1999). It inhabits clearings, gardens, brushy coastal habitats, gallery and mangrove forests, and humid forest edges but can also occasionally be found inside dense

forest or above 1200 m (Skutch 1931, Wetmore 1968, Schuchmann 1999). Common throughout its range, this species is often the most abundant hummingbird in the communities in which it is found (Skutch 1931; pers. obs.).

Currently, *A. tzacatl* is considered to have five subspecies, with the nominate form occurring over most of the species' range (southwestern Mexico to eastern Panama). The island endemic *A. t. handleyi* (Wetmore, 1963, the Escudo Hummingbird), found only on Isla Escudo de Veraguas, Panama, was initially described as a distinct species on the basis of its considerably larger size and darker plumage (Wetmore 1959, 1963, 1968; Fig. 1), but subsequently it has been classified as a subspecies of *A. tzacatl* (e.g., Ridgely 1976, American Ornithologists' Union 1998). Otherwise, Rufous-tailed Hummingbirds are rather uniform in appearance throughout their range, with only subtle differences in size and plumage distinguishing the remaining mainland subspecies (Wetmore 1968, Weller and Schuchmann 1999). These other subspecies include *fuscicaudata* (northern Colombia and western Venezuela), *jucunda* (western Colombia and northwestern Ecuador), and *brehmi* (Weller and Schuchmann 1999; restricted range in Nariño, southern Colombia). South American birds are smaller than their counterparts in Middle America. Among the South American forms, differences appear to be due largely to clinal variation in bill and wing measurements and in the coloration of the margins of rectrices (Weller and Schuchmann 1999) rather than discrete phenotypic groups. Both Chapman (1917) and Cory (1918) questioned whether *jucunda* is distinguishable from *fuscicaudata*. Finally, multiple ornithological surveys during the latter part of the 20<sup>th</sup> century showed a distributional gap between Middle and South American populations in the eastern Darién Province of Panama until at least the mid-1980s and perhaps into the early 2000s (Wetmore 1968, Robbins et al. 1985).

## METHODS

We sampled 99 individuals from 32 different localities within the range of *A. tzacatl*. From the availability of specimens, we chose localities to maximize geographic coverage within the species (Fig. 2A, Appendix). Few specimens were available from South America, but in Middle America we sampled up to 10 individuals per location when possible. With the exception of one individual from Isla Escudo de Veraguas, all the samples represent vouchered museum specimens (full specimen information is listed in the Appendix). We also obtained from GenBank sequences of 20 closely related hummingbird species from seven genera for use as potential outgroups (McGuire et al. 2007).

From all individuals, we sequenced the entire mitochondrial gene for NADH dehydrogenase subunit II. We extracted, amplified, and sequenced DNA by following standard procedures (e.g., Miller et al. 2008); at times we employed an internal sequencing primer (ND2-HUM525:

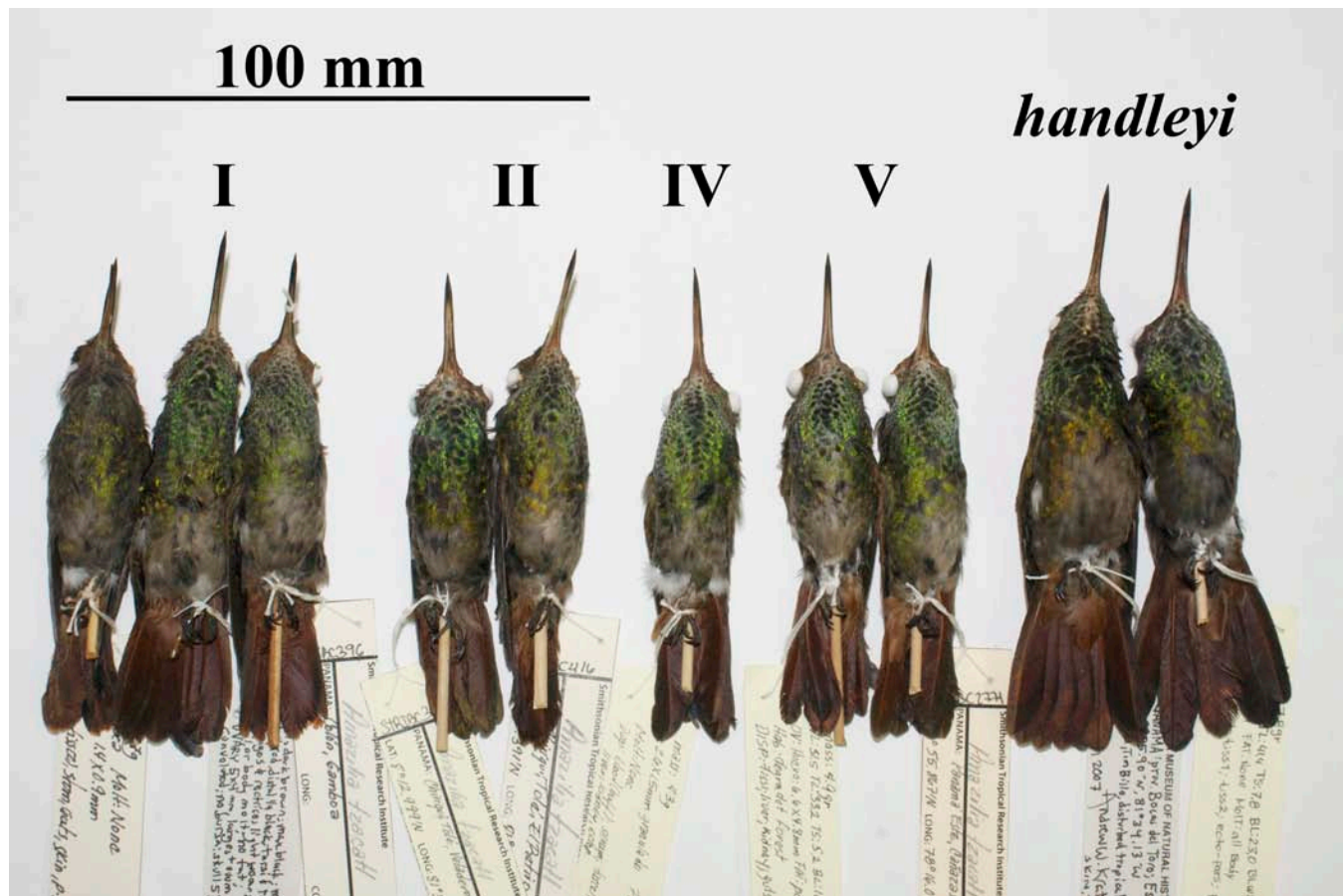


FIGURE 1. Specimens of *Amazilia tzacatl* representing the phenotypic variation found across mtDNA clades. Numbers refer to clade designation in text, *handleyi* refers to birds from Isla Escudo.

5'-CCGAAAATCCTAGCCTTCT-3'). We aligned sequence data by eye with Sequencher 4.6 (Gene Codes Corporation, Ann Arbor, MI). We generated a median-joining haplotype network (Bandelt et al. 1999) with Network 4.516 (fluxus-engineering.com). Using MrBayes 3.1 (Ronquist and Huelsenbeck 2003), we also generated a Bayesian phylogeny with *A. yucatanensis* and *A. rutila* as our outgroup taxa after a preliminary survey of 20 hummingbird species. The AIC criterion in MrModelTest (Nylander 2004) identified the GTR + G model as the best fit to our molecular data. We ran two runs, each with ten Markov chains for 12 000 000 generations, sampling one tree every 1000 generations. We used AWTY software (Nylander et al. 2008) to explore stationarity and convergence in the Markov chain. Stationarity was reached quickly (after approximately 30 000 generations), so we discarded the first 100 trees from each run as burn-in (100 000 generations) and used the remaining 23 802 trees to construct a consensus tree.

We inferred population-demographic parameters from estimates of Tajima's  $D$  (Tajima 1989), Fu's  $F_s$  (Fu 1997), and Romis-Onsins and Rozas' (2002)  $R_2$ , calculated with DnaSP version 5 (Librado and Rozas 2009). We used both Fu's  $F_s$  and

$R_2$  because of the superior behavior of  $R_2$  with small sample sizes and  $F_s$ 's better performance with large sample sizes. We applied these statistical analyses to the five observed mitochondrial clades (see Results) and to island and nearby mainland populations of interest. We assessed the statistical significance of observed values of  $F_s$  and  $R_2$  by comparisons to expected values obtained from 50 000 coalescence simulations using a model of constant population size (Librado and Rozas 2009).

Finally, we evaluated the effect of geographic distance on genetic differences between sampled birds (i.e., isolation by distance) via a Mantel test implemented in the program Alleles in Space (Miller 2005). This program assesses the correlation between genetic and geographic distances among individuals rather than populations in a study set, which may be appropriate for datasets such as ours that have many sampling sites with only a few sampled individuals. Uncorrected pairwise sequence divergence was correlated with pairwise geographic distance for all possible individual pairs of *Amazilia tzacatl* in our study set (except for FN01199, which was only a partial sequence). We determined the significance of the correlation by comparing the observed correlation to random correlations based on 10 000 permutations.



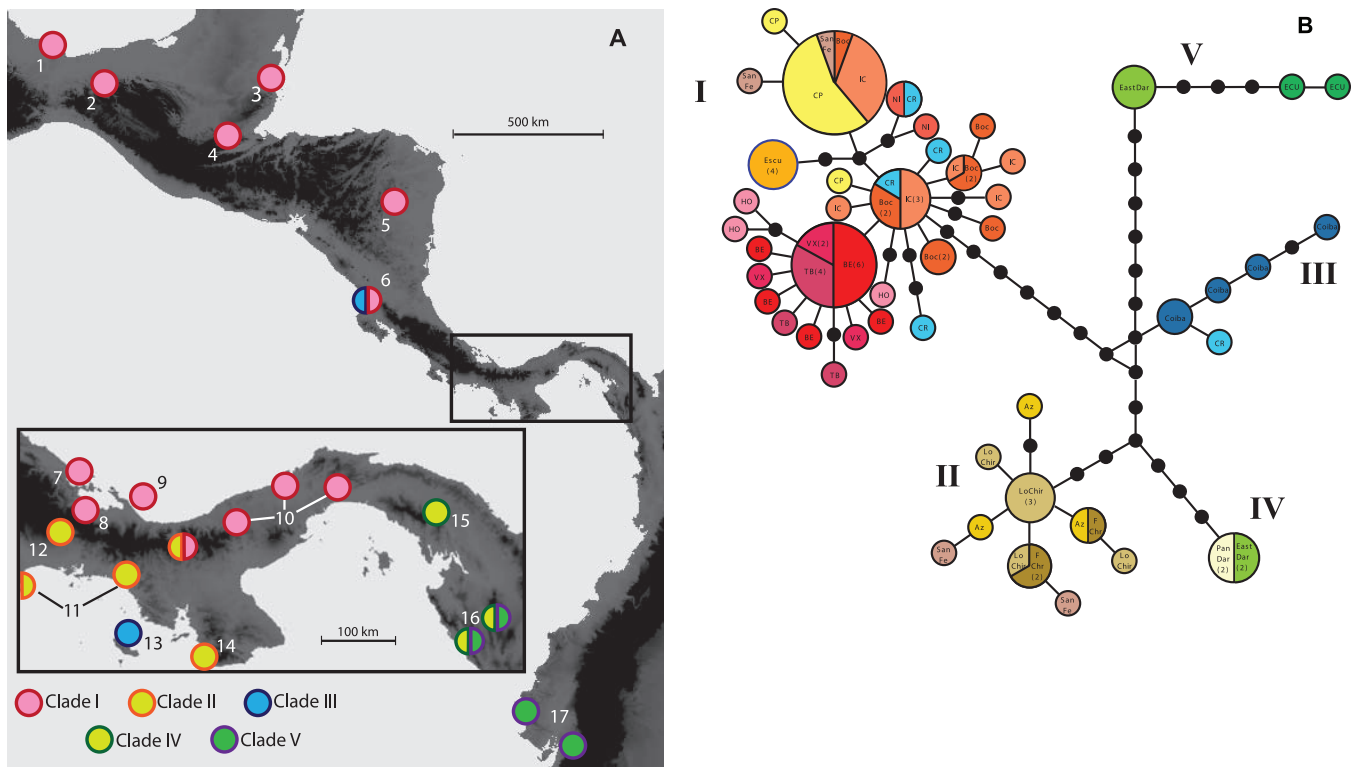


FIGURE 2. (A) Distribution of specimens of *Amazilia tzacatl* collected for this study. Colors represent mtDNA clades recovered from that location; broken circles represent sites with two clades recovered; see Fig. 3. 1, Veracruz, Mexico; 2, Tabasco, Mexico; 3, Toledo, Belize; 4, Atlantida, Honduras; 5, La Luz, Nicaragua; 6, Guanacaste, Costa Rica; 7, Isla Colón, Panama; 8, Bocas del Toro mainland, Panama (multiple sites); 9, Isla Escudo de Veraguas, Panama; 10, central Panama; 11, Chiriquí lowlands, Panama (multiple sites); 12, Chiriquí foothills, Panama; 13, Santa Fé, Panama; 14, Azuero, Panama; 15, Isla Coiba, Panama; 16, Panamá–Darién border, Panama; 17, eastern Darién, Panama; 18, Pacific Ecuador; see Appendix for site details. (B) Minimum-spanning tree of ND2 sequences from *Amazilia tzacatl*. The population from Isla Escudo, originally described as a species, is circled in blue. Black dots indicate haplotypes unrecovered in our tree. This topology is congruent with the results of a Bayesian phylogenetic analysis (not shown). See Appendix for population codes.

The isolation of Isla Escudo from the mainland, and presumably the divergence between the Escudo Hummingbird and mainland *Amazilia tzacatl*, has been estimated at 9000 years before present (Summers et al. 1997, Anderson and Handley 2001). Similarly, the isolation of Isla Coiba, and presumably its fauna, from the mainland, has been dated to sea-level rise at the beginning of the Holocene (Castroviejo 1997). To test these biogeographic hypotheses, we ran coalescence simulations, using the software IM (Hey and Nielsen 2004). Each simulation paired an island to an adjacent mainland population: (1) Isla Escudo vs. Bocas del Toro mainland, (2) Isla Coiba vs. Bocas del Toro mainland, and (3) Isla Colón (part of the Bocas del Toro archipelago) vs. Bocas del Toro mainland. In the first two cases we assumed no gene flow since divergence, because with no shared haplotypes we have no evidence for gene flow, and the results of the simulations are likely to be poor without such an assumption being made (J. Hey, pers. comm.). Likewise, in all analyses we assumed a generation time of one year, which is reasonable for hummingbirds (Bleiweiss 1998). In the case of Isla Colón vs. the mainland, we ran simulations with and without gene flow in

the models (in the latter case, gene flow—migrations scaled to the effective population size—was allowed to vary up to a maximum of 1). In all four cases, we ran simulations  $3.5 \times 10^7$  generations, sampling every 10 generations after discarding the first  $4 \times 10^6$  generations as burn-in. IM generates a likelihood distribution of divergence time,  $T$ , measured in coalescence units. To convert  $T$  into years ( $t$ ), we divide  $T$  by the per-locus mutation rate. ND2 divergence in birds has been estimated to be between 2% and 5% (Lovette 2004b; DaCosta and Klicka 2008), translating into a lineage-specific mutation rate of between 1% and 2.5% per million years. Alternatively, Ho et al. (2007) argued that mutation rates estimated from phylogenetic studies are biased downward relative to intra-specific (“instantaneous”) mutation rates because in the short term most mutations are represented by segregating sites that will be fixed over time, in effect hiding their occurrence; accordingly, they estimated an instantaneous mutation rate for avian coding mtDNA of 7.5% per million years. Thus, for each simulation, we used these three mutation-rate estimates (1%, 2.5%, and 7.5% per million years) to generate three likelihood-density curves of Escudo vs. mainland divergence

times. The approach of Ho et al. (2007) is controversial (e.g., Bandelt 2007, Emerson 2007), and we apply this unconventional mutation rate to achieve an extreme lower limit for our estimates of divergence times. All simulations were run three times to ensure convergence.

## RESULTS

We obtained 1041 bp of ND2 data from 99 individuals of *A. tzacatl* ranging from southern Mexico to south-central Ecuador (Fig. 2A, Appendix). Eighty of 1041 sites (7.8%) were variable, with 37 singleton variable sites and 43 parsimony-informative sites; only one site was represented by three nucleotide states. In total, we found 48 unique haplotypes. The haplotype network and Bayesian phylogenetic tree recovered a similar geographic relationship of the Rufous-tailed Hummingbird's mtDNA haplotypes. Both showed five main clades, shallow divergences between clades, minor substructure within clades, and secondary contact between clades. We present the haplotype network because of its superior visual clarity (Fig. 2B).

The most heavily sampled clade ranges from northern Mexico south along both the Caribbean and Pacific slopes to Costa Rica and then along the Caribbean slope to central Panama (clade I, subspecies *tzacatl* and *handleyi*). A second clade included individuals from the Pacific lowlands and foothills of southwestern Panama (clade II, subspecies *tzacatl*), and a third clade contained all five birds sampled from Isla Coiba off the Pacific coast of western Panama plus one bird from Guanacaste, Costa Rica (clade III, subspecies *tzacatl*). A fourth clade included birds sampled from eastern Panama: the border between Panamá and Darién provinces and eastern Darién Province (clade IV; the subspecies of birds from this region has not been established), and a fifth clade included the two birds from northwestern Ecuador and the remaining birds from eastern Darién (clade V, *jucundalfuscicaudata*).

At the population level, secondary contact of mtDNA clades occurred at three locations: (1) between clades I and II at Santa Fe, Veraguas, Panama; (2) between clades I and III in Guanacaste in northwestern Costa Rica; and (3) between clades IV and V in eastern Darién, Panama, near the border with Colombia. At the Costa Rican contact zone the average uncorrected pairwise divergence ( $D_{xy}$ ; Nei 1987) between clades I and III was 1.2%, at the Santa Fe contact zone between clades I and II it was 1.6%, and at the Darién contact zone between clades IV and V it was 1.7%. The largest  $D_{xy}$  distance between haplotypes in our dataset was 2.2%, between clades I and V; the average pairwise distance between these two clades was 1.8%.

The four birds sampled from Isla Escudo had a unique haplotype otherwise unrepresented in clade I, although the average pairwise divergence between Escudo birds and those from the adjacent Bocas del Toro mainland was quite low (0.30%). In contrast, birds from Isla Colón, an island within the nearby Bocas del Toro archipelago, shared all haplotypes with birds from the mainland. Thus, although the raw pairwise divergence between Isla Colón and the mainland was similar to that between Escudo and the mainland (0.25% vs. 0.30%), the net nucleotide difference ( $D_A$ ; Nei 1987) was markedly lower on Escudo (0.03% vs. 0.17%). Likewise, although we found no variation in haplotypes on Isla Escudo, Isla Colón and mainland Bocas del Toro had similar nucleotide diversities (Table 1).

We found no evidence for distance per se as a correlate of genetic differences in the full dataset (Pearson's  $r = 0.027$ ,  $P = 0.28$ ), rejecting the hypothesis that isolation by distance explains the phylogeographic patterns we observed in *Amazilia tzacatl*. We did find a significant effect of isolation by distance within clade I ( $r = 0.336$ ,  $P < 0.0001$ ) but did not find such an effect in clade II ( $r = 0.085$ ,  $P = 0.75$ ). We did not test for isolation by distance in clades III–V because of limited sample size.

TABLE 1. Summary statistics of populations of *Amazilia tzacatl*.  $N$  = sample size,  $\pi$  = nucleotide diversity,  $\theta_w$  = Watterson's theta,  $D_T$  = Tajima's  $D$ ,  $F_s$  = Fu's  $F$ ,  $R_2$  = Romis-Onsins and Rozas'  $R_2$ . Significance of  $F_s$  and  $R_2$  was determined by coalescence simulations.<sup>a</sup>

Population or clade	$n$	$\pi$	$\theta_w$	$D_T$	$F_s$	$R_2$
Clade I	65	0.00307	0.00790	-2.01*	-19.16****	3.19***
Isla Escudo	4	0.00000	0.00000	—	—	—
Isla Colón	13	0.00241	0.00248	-0.10	-0.45	0.14
Bocas del Toro mainland	9	0.00213	0.00283	-1.11	-1.90	0.14
Clade II	15	0.00174	0.00266	-1.30	-4.95***	0.88**
Azuerio Peninsula	3	0.00256	0.00256	—	-0.34	0.12*
Clade III	6	0.00205	0.00210	-0.14	-1.97*	0.19
Isla Coiba	5	0.00192	0.00184	0.27	-1.01	0.25
Clade IV	4	0.00000	0.00000	—	—	—
Clade V	4	0.00304	0.00262	1.54	0.81	0.28

<sup>a</sup>Levels of significance: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ .

Our IM estimates of the time since the Escudo and mainland populations split peaked at 132 000 years before present (ybp), 52 400 ybp, or 17 200 ybp, depending on the mutation rate employed (see Methods). Likewise, only 0.1%, 0.5%, or 8.5% of the density distribution of posterior likelihood was at or below 9000 ybp (Fig. 3), the estimated date for the isolation of Isla Escudo (Summers et al. 1997, Anderson and Handley 2001), suggesting that gene flow between the Escudo Hummingbird and mainland populations ceased well before the beginning of the Holocene. Simulations for the split between Isla Colón, in the nearby Bocas del Toro archipelago, and the Bocas del Toro mainland provided qualitatively different results. Without migration (gene flow) in the model, divergence time estimates peaked at 11 200 ybp, 4300 ybp, or 1600 ybp, depending on the mutation rate assumed, with 17%, 48% or >96% of the estimate below 5200 ybp (Fig. 3), the estimated date of the separation of Isla Colón from the mainland (Anderson and Handley 2001). In the simulations in which gene flow was allowed, likelihood curves were essentially flat; although the highest point was at  $t = 0$ , these results are consistent with data insufficient for proper estimation of divergence time.

The simulation results for the divergence time between Isla Coiba and the Chiriquí lowlands were even more strongly inconsistent with a Holocene separation. In this case, average pairwise divergence was 0.94% (net divergence: 0.71%), and nucleotide diversity was only slightly lower on Isla Coiba than on the adjacent mainland (0.19% vs. 0.26%; Table 1). Estimated divergence time peaked at 419 400 ybp, 171 500 ybp, or 56 500 ybp with a corresponding 0.00%, 0.00%, and 0.16% of the posterior distribution at or below 10 000 ybp (Fig. 3), depending on the mutation rate employed.

Finally, clades I and II, representing populations from mainland Middle America north and west of the Panama Canal watershed, were the only groups that had strongly significant negative values of Fu's  $F_s$  and  $R_2$  ( $P < 0.01$  to  $P < 0.0001$ ), indicating population expansions. Observed  $F_s$ , but not  $R_2$ , was marginally significant for clade III (e.g.,  $0.5 > P > 0.01$ ), but it became insignificant after a Bonferroni adjustment of  $\alpha$  for multiple tests. The remaining clades did not show signs of demographic expansion (Table 1).

## DISCUSSION

Our phylogeographic analyses recovered five clades within *Amazilia tzacatl*, consistent with other studies that have shown high degrees of phylogeographic structure in widespread neotropical birds (Marks et al. 2002, González et al. 2003, Lovette 2004a, Cheviron et al. 2005, Aleixo 2006, Dingle et al. 2006, Cabanne et al. 2007, 2008, Nyári 2007, Miller et al. 2008). Another similarity to these other studies is that mtDNA clades are not congruent with recognized subspecies: clade V contains both *jucunda* and *fuscicaudata*, and clade I includes both the nominate subspecies and *handleyi*. In fact, the nominate subspecies is divided into three distinct clades, and thus

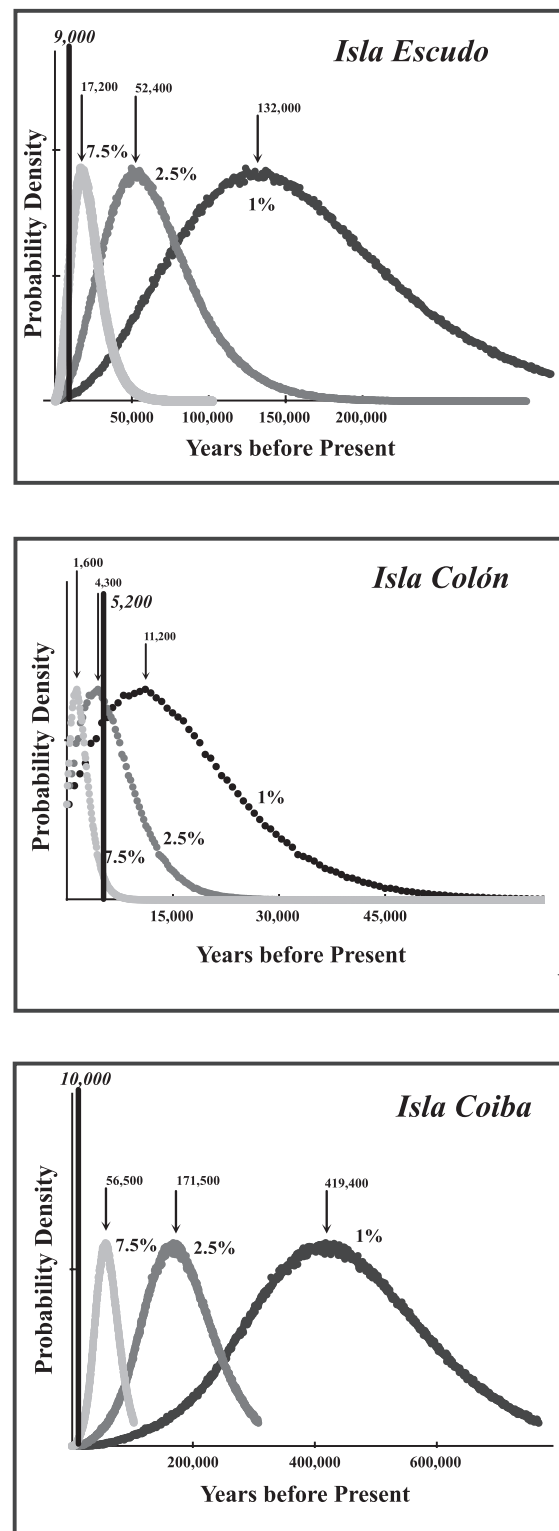


FIGURE 3. Estimated times of divergence from the mainland for the island populations of Isla Escudo, Isla Colón (both in the Bocas del Toro archipelago), and Isla Coiba based on coalescence simulations and three divergence times. In each graph, a thick vertical line indicates the estimated date of last isolation of the island as a consequence of Holocene sea-level rise.

pairwise distances between some members of this subspecies are almost as great as the largest intraspecific pairwise distance in our dataset (1.8% vs. 2.2%). This result reiterates the observation that in many cases the processes of mtDNA coalescence and gene flow are not integrally connected to phenotypic divergence (Winker 2009, 2010).

Despite these similarities with other studies, divergences between clades of *Amazilia tzacatl* were relatively modest (greatest divergence in our samples equaled 2.4%) and lower than those in many other lowland neotropical birds. For example, Marks et al. (2002) found up to 7.8% variation between Middle American and Amazonian clades of *Glyphorynchus spirurus* (Wedge-billed Woodcreeper). Similarly, three other studies found deeper divergences among more or less continuously distributed lowland birds in Panama: in *Thryothorus nigricapillus* (Bay Wren) González et al. (2003) found up to 5.6% divergence between Darién (eastern) and central Panama, in *Schiffornis turdina* (Thrush-like Schiffornis) Nyári (2007) found 8.4% divergence between western and eastern Panama, and in *Mionectes oleagineus* (Ochre-bellied Flycatcher) Miller et al. (2008) found 3.7% divergence between western and central Panama and 2.4% divergence between central and eastern Panama, although species limits have not been robustly tested in these cases.

In the full dataset, we found no evidence for isolation by distance, suggesting that past geographic barriers to gene flow, and not geographic distance per se, are responsible for the phylogeographic patterns we recovered. Within clades, we found no evidence for isolation by distance in clade II, but we did find a significant correlation between genetic and geographic distances between sampled birds when we restricted the analysis to clade I. Miller et al. (2010) suggested that the population genetics of clade I may have been partially shaped by range expansion at the beginning of the Holocene; interestingly, Klicka et al. (2011) recovered a similar pattern of significant isolation by distance in the northern clade of the Hairy Woodpecker (*Picoides villosus*), which they argued experienced a substantial range expansion after the last glacial maximum, whereas a southern clade of that species did not show effects of isolation by distance, so they posited that its range has not expanded since the last glacial maximum. Whether a relationship exists between isolation by distance and range expansions by birds since the last glacial maximum awaits further investigation.

Our study found three regions of secondary contact between lineages: northwestern Costa Rica, west-central Panama, and eastern Darién, Panama. In the first two cases, secondary contact between Caribbean and Pacific haplotypes occurs at the only two locations in Costa Rica and western Panama where the continental divide drops to below 1000 m above sea level (see figure 6 in Stiles 1983). This suggests that the genetic structure between the widespread clade I and more narrowly restricted clades II and III is largely a consequence of the geographic barrier of the Central American highlands rather than any intrinsic reproductive isolating mechanism.

The contact zone in eastern Darién is recent. In 2003, we collected two Rufous-tailed Hummingbirds at Piñas Bay in southeastern Darién, and in 2006 we collected three additional birds at Cana in the upper Tuira valley in eastern Darién. To our knowledge these represent the first specimens from eastern Darién Province and likely represent a range expansion during the last 20 or so years: during an extensive 2-month survey of southeastern Darién in 1945 and 1946, Wetmore (1946, 1959) did not find the Rufous-tailed Hummingbird. Similarly, during a month-long survey of the avifauna in and around Cana in 1982 Robbins et al. (1985) apparently did not observe or collect the species. It is common in gardens and similar habitats, and forages deliberately, mostly below 2 m. Thus it is unlikely that experienced ornithologists such as those on these previous expeditions overlooked such a conspicuous bird. Instead, it is likely that deforestation in eastern Panama and adjacent northwestern Colombia has allowed the range and population of *Amazilia tzacatl* to expand, bringing formerly allopatric populations into parapatry and even syntopy. At both of our collecting sites (Piñas Bay and Cana) in eastern Darién, we recovered both eastern Panama and South America haplotypes (clades IV and V), which differ by about 1.5%, showing that this former distributional gap has been colonized from both directions.

Our study represents the first genetic comparison of the Escudo Hummingbird with other populations of *Amazilia tzacatl*. Isla Escudo harbors a diversity of endemic vertebrates surprising for such a diminutive (4.3 km<sup>2</sup>) island. Of the 8–10 landbird species presumed to breed on Escudo, four are endemic subspecies (Wetmore 1959, 1963); likewise, two of the nine species of mammals that occur on Escudo are endemic (Kalko and Handley 1994). Summers et al. (1997) and Anderson and Handley (2001) provided similar paleogeographical reconstructions of Escudo based on an analysis of submarine topography and likely changes in sea level since the beginning of the Holocene; both concluded that Escudo was isolated from the mainland approximately 9000 ybp and that the islands of the Bocas del Toro archipelago became isolated between 5200 and 1000 ybp. We recovered no variation in the ND2 sequences of four Escudo Hummingbirds, consistent with what would be predicted for a population on an extremely small island that does not receive immigrants from the adjacent mainland. More surprisingly, these represented a private haplotype (two unique substitutions) not recovered in mainland birds, which is unexpected at the beginning of the lineage-sorting process (Omland et al. 2006).

Coalescence simulations of divergence times indicate that even with an extremely high mutation-rate estimate, the isolation of the Escudo Hummingbird likely occurred before 9000 ybp (posterior probability for  $t \leq 9000$  ybp = 0.15 vs. posterior probability for  $t > 9000$  ybp = 0.85; Bayes factor  $\approx 5$ : substantial support on the scale of Jeffreys 1961). The accuracy of this estimate depends on our assumptions of mutation rate and generation time. Additionally, it is possible that some mainland birds



are more closely related to Escudo birds but that we failed to sample such birds or that their haplotypes have gone extinct on the mainland. This may be unlikely, however, given the breadth of our mainland sampling and the signal of demographic expansion of clade I (see Fig. 2B and Table 1).

Ultimately, further sampling and additional loci will be required to provide a more precise estimate of the Escudo Hummingbird's divergence time, but our finding of a divergence from mainland relatives greater than expected is echoed by phylogeographic studies of two other Escudo vertebrates. González et al. (2003) found that the Escudo endemic form of *Thryothorus nigricapillus* (subspecies *odicus*) has two private mtDNA haplotypes that differ from those of adjacent mainland populations by 0.6–1.2%, and Wang and Shaffer (2008) found that an endemic color morph of the poison frog *Dendrobates pumilio* differs in mtDNA by at least 3.6% from other populations. As Summers et al. (1997) suggested, the sea level rise at 9000 ybp was probably the last of a series of sea-level changes that alternately isolated and unified the Escudo landmass to the mainland over a much longer time. Therefore, it is likely that these three vertebrates are relicts of much earlier isolation.

Likewise, four specimens from Isla Coiba, off the Pacific coast of western Panama, diverged in ND2 by 0.9% from populations in adjacent mainland southwestern Panama (clade II), and results from coalescence simulations are inconsistent with divergence at the beginning of the Holocene or more recently, even if an extremely high mutation rate is assumed (posterior probability for  $t \leq 10\,000$  ybp = 0.99 vs. posterior probability for  $t > 9000$  ybp = 0.01; Bayes factor  $\approx 59$ , very strong support; Jeffreys 1961). As in the case of the coalescence estimates for Escudo, these divergence estimates for Coiba are dependent on the estimates of mutation rate and generation time and assume that there is not substantial population structure in southwestern Panama that was incompletely sampled or that existed at the time of isolation and has subsequently gone extinct on the mainland. The presence of a clade II haplotype in northwestern Costa Rica that was not recovered in southwestern Panama is consistent with these scenarios. Nonetheless, despite our extensive sampling, the four Coiba birds, plus one of the five from northwestern Costa Rica, made up the entirety of clade II. Without additional sampling of birds and loci, it is unclear whether the finding of a clade II haplotype in northwestern Costa Rica (where all other samples belong to clade I; see Fig. 2B) represents long-distance dispersal or gene flow from Coiba to northwestern Panama or incomplete lineage sorting. Nevertheless, the absence of haplotypes shared by Coiba and the adjacent mainland provides another example of mtDNA divergence greater than expected from an island whose latest isolation from the mainland, like that of Escudo, is dated to around the beginning of the Holocene ( $\sim 10\,000$  ybp, Castroviejo 1997).

Like many other neotropical bird species, mainland *Amazilia tzacatl* comprises several distinctive mtDNA clades,

although divergences between these clades are shallower than in many other neotropical birds. Similarly, we recovered sites of secondary contact between these clades, and such secondary contact is also common in neotropical birds (e.g., Brumfield 2005, Miller et al. 2008, 2010, Vázquez-Miranda 2009). These results show a general pattern of geographic isolation followed by secondary contact without the generation of obvious biological species, a phenomenon that deserves greater attention. Likewise, the phylogeographic patterns recovered for both the Escudo and the Coiba populations of *Amazilia tzacatl* require further study. In both cases, these islands likely harbor relict populations representing biological diversity not found in mainland populations. Both Isla Escudo and Isla Coiba have been overlooked by systematists and warrant more comprehensive studies to ensure proper management of their endemic populations.

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## LITERATURE CITED

- ALEIXO, A. 2006. Historical diversification of floodplain forest specialist species in the Amazon: a case study with two species of the avian genus *Xiphorhynchus* (Aves: Dendrocolaptidae). *Biological Journal of the Linnean Society* 89:383–395.
- AMERICAN ORNITHOLOGISTS' UNION. 1998. Check-list of North American Birds, 7th ed. American Ornithologists' Union, Washington, D.C.
- ANDERSON, R. P., AND C. O. HANDLEY. 2001. A new species of three-toed sloth (Mammalia: Xenarthra) from Panama, with a review of the genus *Bradypus*. *Proceedings of the Biological Society of Washington* 114:1–33.
- BANDELT, H.-J., P. FORSTER, AND A. RÖHL. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16:37–48.
- BANDELT, H.-J. 2007. Clock debate: when times are a-changin': time dependency of molecular rate estimates: tempest in a teacup. *Heredity* 100:1–2.
- BARBER, B., AND J. KLICKA. 2010. Two pulses of diversification across the Isthmus of Tehuantepec in a montane Mexican bird fauna. *Proceedings of the Royal Society B* 277:2675–2681.
- BRUMFIELD, R. T. 2005. Mitochondrial variation in Bolivian populations of the Variable Antshrike (*Thamnophilus caerulescens*). *Auk* 122:414–432.



- BERMINGHAM, E., AND A. P. MARTIN. 1998. Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. *Molecular Ecology* 7:499–517.
- BLEIWEISS, R. 1998. Slow rate of molecular evolution in high-elevation hummingbirds. *Proceedings of the National Academy of Sciences USA* 95:612–616.
- CABANNE, G. S., F. R. SANTOS, AND C. Y. MIYAKI. 2007. Phylogeography of *Xiphorhynchus fuscus* (Passeriformes, Dendrocolaptidae): vicariance and recent demographic expansion in southern Atlantic forest. *Biological Journal of the Linnean Society* 91:73–84.
- CABANNE, G. S., F. M. D'HORTA, E. H. R. SARI, F. R. SANTOS, AND C. Y. MIYAKI. 2008. Nuclear and mitochondrial phylogeography of the Atlantic forest endemic *Xiphorhynchus fuscus* (Aves: Dendrocolaptidae): Biogeography and systematics implications. *Molecular Phylogenetics and Evolution* 49:760–773.
- CASTROVIEJO, S. 1997. Flora y fauna del Parque nacional de Coiba (Panamá): Inventario preliminar. Agencia Española de Cooperación Internacional, Madrid, Spain.
- CHAPMAN, F. W. 1917. The distribution of bird life in Colombia: a contribution to a biological survey of South America. *Bulletin of the American Museum of Natural History* 36:1–729.
- CHEVIRON, Z. A., S. J. HACKETT, AND A. P. CAPPARELLA. 2005. Complex evolutionary history of a neotropical lowland forest bird (*Lepidothrix coronata*) and its implications for historical hypotheses of the origin of neotropical avian diversity. *Molecular Phylogenetics and Evolution* 36:338–357.
- CORY, C. B. 1918. Catalogue of birds of the Americas. *Field Museum of Natural History Zoological Series* vol. 13, part 2, no 1.
- DACOSTA, J. M., AND J. KLIICKA. 2008. The Great American Interchange in birds: a phylogenetic perspective with the genus *Trogon*. *Molecular Ecology* 17:1328–1343.
- DINGLE, C., I. J. LOVETTE, C. CANADAY, AND T. B. SMITH. 2006. Elevational zonation and the phylogenetic relationships of the *Henicorhina* wood-wrens. *Auk* 123:119–134.
- EMERSON, B. C. 2007. Alarm bells for the molecular clock? No support for Ho et al.'s model of time-dependent molecular rate estimates. *Systematic Biology* 56:337–345.
- FU, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147:915–925.
- GONZÁLEZ, M. A., J. R. EBERHARD, I. J. LOVETTE, S. L. OLSON, AND E. BERMINGHAM. 2003. Mitochondrial DNA phylogeography of the Bay Wren complex. *Condor* 105:228–238.
- HEY, J., AND R. NIELSEN. 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics* 167:747–760.
- HO, S. Y. W., B. SHAPIRO, M. J. PHILLIPS, A. COOPER, AND A. J. DRUMMOND. 2007. Evidence for time dependency of molecular rate estimates. *Systematic Biology* 56:515–522.
- JEFFREYS, H. 1961. *Theory of probability*. Clarendon Press, Oxford, UK.
- KALKO, E. K. V., AND C. O. HANDLEY. 1994. Evolution, biogeography, and description of a new species of fruit-eating bat, genus *Artibeus* Leach (1821), from Panama. *Zeitschrift für Säugetierkunde* 59:257–273.
- KLIICKA, J., G. M. SPELLMAN, K. WINKER, V. CHUA, AND B. T. SMITH. 2011. A phylogeographic and population genetic analysis of a widespread, sedentary North American bird: the Hairy Woodpecker (*Picoides villosus*). *Auk* 128:346–362.
- LIBRADO, P., AND J. ROZAS. 2009. A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452.
- LOVETTE, I. J. 2004a. Molecular phylogeny and plumage signal evolution in a trans Andean and circum Amazonian avian species complex. *Molecular Phylogenetics and Evolution* 32:512–523.
- LOVETTE, I. J. 2004b. Mitochondrial dating and mixed support for the “2% rule” in birds. *Auk* 121:1–6.
- MARKS, B. D., A. P. CAPPARELLA, AND S. J. HACKETT. 2002. Historical relationships among neotropical lowland forest areas of endemism as determined by mitochondrial DNA sequence variation within the Wedge-billed Woodcreeper (Aves: Dendrocolaptidae: *Glyphorhynchus spirurus*). *Molecular Phylogenetics and Evolution* 24:153–167.
- MCGUIRE, J. M., C. C. WITT, D. L. ALTSHULER, AND J. V. REMSEN JR. 2007. Phylogenetic systematics and biogeography of hummingbirds: Bayesian and maximum likelihood analyses of partitioned data and selection of an appropriate partitioning strategy. *Systematic Biology* 56:837–856.
- MILLER, M. J., E. BERMINGHAM, J. KLIICKA, P. ESCALANTE, F. S. RAPOSO DO AMARAL, J. T. WEIR, AND K. WINKER. 2008. Out of Amazonia again and again: episodic crossing of the Andes promotes diversification in a lowland forest flycatcher. *Proceedings of the Royal Society B* 275:1133–1142.
- MILLER, M. J., E. BERMINGHAM, J. KLIICKA, P. ESCALANTE, AND K. WINKER. 2010. Neotropical birds show a humped distribution of within-population genetic diversity along a latitudinal transect. *Ecology Letters* 13:576–586.
- MILLER, M. P. 2005. Alleles in space: computer software for the joint analysis of interindividual spatial and genetic information. *Journal of Heredity* 96:722–724.
- NEI, M. 1987. *Molecular evolutionary genetics*. Columbia University Press, New York.
- NYÁRI, A. S. 2007. Phylogeographic patterns, molecular and vocal differentiation, and species limits in *Schiffornis turdina* (Aves). *Molecular Phylogenetics and Evolution* 44:1Nylander, J. A. A. 2004. MrModeltest2. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- NYLANDER, J. A. A., J. C. WILGENBUSCH, D. L. WARREN, AND D. L. SWOFFORD. 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* 24:581–583.
- OMLAND, K. E., J. M. BAKER, AND J. L. PETERS. 2006. Genetic signatures of intermediate divergence: population history of Old and New World holarctic ravens (*Corvus corax*). *Molecular Ecology* 15:795–808.
- RIDGELY, R. S. 1976. *A guide to the birds of Panama*. Princeton University Press, Princeton, NJ.
- ROBBINS, M. B., T. A. PARKER III, AND S. E. ALLEN. 1985. The avifauna of Cerro Pirre, Darién, eastern Panama. *Ornithological Monographs* 36:198–232.
- ROMIS-ONSINS, S. E., AND J. ROZAS. 2002. Statistical properties of neutrality tests against population growth. *Molecular Biology and Evolution* 19:2092–2100.
- RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
- SCHUCHMANN, K. L. 1999. Family Trochilidae (hummingbirds), p. 468–680. *In* J. del Hoyo, A. Elliot, J. Sargatal [EDS.], *Handbook of the birds of the world*, vol. 5. Barn-owls to hummingbirds. Lynx Edicions, Barcelona.
- SKUTCH, A. F. 1931. Life history of Rieffer's Hummingbird (*Amazilia tzacatl*) in Panama and Honduras. *Auk* 48:481–500.
- STILES, F. G. 1983. Systematics of the southern forms of *Selasphorus* (Trochilidae). *Auk* 100:311–325.
- SUMMERS, K., E. BERMINGHAM, L. WEIGT, S. MCCAFFERTY, AND L. DAHLSTROM. 1997. Phenotypic and genetic divergence in three

- species of dart-poison frogs with contrasting parental behavior. *Journal of Heredity* 88:8–13.
- TAJIMA, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585–595.
- VÁZQUEZ-MIRANDA, H., A. G. NAVARRO-SIGÜENZA, AND K. E. OMLAND. 2009. Phylogeography of the Rufous-naped Wren (*Campylorhynchus rufinucha*): speciation and hybridization in Mesoamerica. *Auk* 126:765–778.
- WANG, I. J., AND H. B. SHAFFER. 2008. Rapid color evolution in an aposematic species: a phylogenetic analysis of color variation in the strikingly polymorphic strawberry poison-dart frog. *Evolution* 62:2742–2759.
- WELLER, A. A., AND K. L. SCHUCHMANN. 1999. Geographical variation in the southern distributional range of the Rufous-tailed Hummingbird, *Amazilia tzacatl* De la Llave, 1832: a new subspecies from Nariño, southwestern Colombia. *Journal of Ornithology* 140:457–466.
- WETMORE, A. 1946. The birds of San José and Pedro González islands, Republic of Panama. *Smithsonian Miscellaneous Collections* 106:1–60.
- WETMORE, A. 1959. The birds of Isla Escudo de Veraguas, Panama. *Smithsonian Miscellaneous Collections* 139:1–27.
- WETMORE, A. 1963. Systematic notes concerned with the avifauna of Panama. *Smithsonian Miscellaneous Collections* 145:1–11.
- WETMORE, A. 1968. Birds of the Republic of Panama, part 2. Columbidae (pigeons) to Picidae (woodpeckers). *Smithsonian Institution Press*, Washington, DC.
- WINKER, K. 2009. Reuniting phenotype and genotype in biodiversity research. *BioScience* 59:657–665.
- WINKER, K. 2010. Subspecies represent geographically partitioned variation, a goldmine of evolutionary biology, and a challenge for conservation. *Ornithological Monographs* 67:6–23.

APPENDIX. Population, decimal latitude and longitude, GenBank number, museum and voucher, and mtDNA clade of specimens of *Amazilia tzacatl* analyzed in this study. Population names agree with abbreviations in Fig. 3. Museum abbreviations: ANSP, Academy of Natural Sciences of Philadelphia; LSUMZ, Louisiana State University Museum of Natural Science; MBM, Marjorie Barrick Museum of Natural History, University of Nevada, Las Vegas; STRI, Smithsonian Tropical Research Institute Bird Collection, UAM, University of Alaska Museum.

Veracruz (VX) 18.57, –95.08			
EU983301	UAM	TUX1120	I
EU983302	UAM	PEP22504	I
EU983303	UAM	PEP2505	I
EU983304	UAM	PEP2512	I
Tabasco (TB) 17.33, –93.61			
EU983305	UAM	CAM404	I
EU983306	UAM	CAM350	I
EU983307	UAM	CAM376	I
EU983308	UAM	CAM324	I
EU983309	UAM	CAM454	I
EU983310	UAM	CAM399	I
Belize (BE) 17.50, –88.57			
EU983311	UAM	KSW2148	I
EU983312	UAM	KSW3688	I
EU983313	UAM	KSW3698	I
EU983314	UAM	KSW3699	I
EU983315	UAM	KSW3740	I
EU983316	UAM	KSW3792	I
EU983317	UAM	KSW2074	I
EU983318	UAM	KSW2210	I
EU983319	UAM	KSW2754	I
EU983320	UAM	KSW2788	I
Honduras (HO) 15.75, –89.92			
EU983321	MBM	JK01122	I
EU983322	MBM	GAV2089	I
EU983323	MBM	JK01081	I
Nicaragua (NI) 13.73, –89.92			
EU983324	MBM	DAB1022	I
EU983325	MBM	DAB1027	I
Costa Rica (CR) 10.85, –85.6			
EU983381	MCZ	FN02425	I

(Continued)

APPENDIX. Continued.

EU983382	MCZ	FN02800	I
EU983383	MCZ	FN03040	I
EU983384	MCZ	FN01386	I
EU983385	MCZ	FN03040	III
Bocas1 (Boc) 9.22, –82.41			
EU983364	STRI	JTW248	I
EU983374	STRI	JTW231	I
Bocas2 (Boc) 8.79, –82.19			
EU983372	MBM	GMS1994	I
EU983375	MBM	JK06222	I
EU983376	MBM	JK06138	I
EU983377	MBM	JK06143	I
EU983378	MBM	JK06217	I
EU983379	MBM	JMD758	I
EU983380	MBM	JMD766	I
Isla Colón (IC) 9.35, –82.26			
EU983359	UAM	MJM1895	I
EU983355	UAM	MJM1899	I
EU983361	UAM	MJM1897	I
EU983358	UAM	MJM1893	I
EU983363	UAM	MJM1889	I
EU983358	UAM	MJM1896	I
EU983357	UAM	MJM1892	I
EU983356	UAM	MJM1891	I
JN036614	UAM	MJM1894	I
JN036615	UAM	MJM1898	I
JN036616	FMNH	AWK3218	I
JN036617	FMNH	AWK3199	I
JN036618	FMNH	AWK3221	I
Escudo (Escu) 9.1, –81.57			
EU983389	FMNH	AWK3260	I
EU983390	FMNH	AWK3265	I
EU983391	FMNH	AWK3269	I
EU983392	FMNH	AWK3278	I
CenPan1 (CP) 8.79, –80.54			
EU983328	UAM	MJM278	I
EU983332	UAM	MJM205	I

(Continued)

## APPENDIX. Continued.

CenPan2 (CP) 9.2, -79.49			
EU983329	UAM	MJM420	I
EU983326	UAM	MJM570	I
EU983327	UAM	MJM590	I
EU983330	UAM	MJM1034	I
EU983331	UAM	MJM1035	I
EU983334	UAM	KSW4380	I
EU983333	UAM	KSW4381	I
CenPan3 (CP) 9.22, -79.99			
EU983336	MBM	JK04274	I
CenPan4 (CP) 8.95, -79.7			
EU983335	UAM	MJM596	I
Coiba 7.53, -81.86			
EU983337	LSUMZ	B46732	III
EU983338	LSUMZ	B46653	III
EU983339	LSUMZ	B46696	III
EU983340	LSUMZ	B46680	III
EU983341	LSUMZ	B46646	III
Azuero (Az) 7.24, -80.97			
EU983342	MBM	JMD170	II
EU983343	MBM	JK04207	II
EU983344	MBM	JMD175	II
LoChir1 8.26, -81.85			
EU983345	UAM	MJM1874	II
EU983346	UAM	MJM1875	II
LoChir2 8.39, -82.73			
EU983347	MBM	GMS2201	II
LoChir3 8.21, -81.64			
JN036609	STRI	MJM5531	II

## APPENDIX. Continued.

JN036610	STRI	MJM5583	II
JN036611	STRI	MJM5584	II
LoChir4 8.2, -82.88			
EU983350	MBM	JMD896	II
F Chir 8.77, -82.42			
EU983349	UAM	KSW4509	II
EU983348	UAM	KSW4508	II
JN036612	STRI	MJM6245	II
Santa Fé (Sanfe) 8.53, -81.13			
EU983351	MBM	GMS1004	II
EU983352	MBM	GMS1005	II
EU983353	MBM	JK04176	I
EU983354	MBM	JMD125	II
PanDar1 8.91, -79.33			
EU983365	MBM	GMS1947	IV
EU983366	MBM	GMS1887	IV
PanDar2 8.93, -78.27			
JN036613	STRI	MJM7090	V
EastDar1 7.76, -77.68			
EU983367	UAM	JMM1088	IV
EU983368	UAM	KSW4849	V
EU983369	UAM	JMM1025	V
EastDar2 7.57, -78.19			
EU983370	STRI	JTW610	IV
EU983371	STRI	JTW721	V
Ecuador1 (ECU) -1.53, -80.73			
EU983387	ANSP	3333	V
Ecuador1 (ECU) -2.55, -79.35			
EU983386	ANSP	3638	V

(Continued)