The Condor 105:228–238

MITOCHONDRIAL DNA PHYLOGEOGRAPHY OF THE BAY WREN (TROGLODYTIDAE: *THRYOTHORUS NIGRICAPILLUS*) COMPLEX

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Abstract. The Bay Wren (*Thryothorus nigricapillus*) is distributed from Costa Rica to Ecuador and includes seven described subspecies, five of which occur in the Caribbean lowlands of Panama. The subspecies vary in plumage characters, with particularly striking differences between Bay Wrens from western Panama (to the north), and eastern Panama (to the south). We surveyed mitochondrial DNA (mtDNA) sequence variation from a geographically broad sample of Bay Wrens and compared the phylogeographic structure of mtDNA diversity with previously described patterns of morphological variation. The mtDNA-based phylogeographic reconstructions revealed a basal split separating populations in far eastern Panama and South America from those in central Panama through Costa Rica. These two clades are concordant with previous morphology-based groupings of *T. nigricapillus* subspecies into the ''*castaneus* group'' (*costaricensis*, *odicus*, *castaneus*, and *reditus*) and the ''*nigricapillus* group'' (*schottii*, *connectens*, and *nigricapillus*). Morphological intergradation between the two groups takes place in central Panama, but all intergrades possess the mtDNA haplotype of the *castaneus* group, suggesting that mitochondrial gene flow is introgressing from west to east. In spite of the marked body size and plumage variation present among subspecies of the *castaneus* group, mtDNA variation within this group was low. At a deeper phylogenetic level, the mtDNA data support recognition of the Riverside Wren, *T. semibadius*, as a full species. This taxon has sometimes been considered conspecific with *T. nigricapillus*, but the high mtDNA divergence between these species is consistent with previous suggestions that the morphological similarity results from convergence in plumage traits.

Key words: Bay Wren, Panama isthmus, phylogeny, phylogeography, plumage, speciation, Thryothorus nigricapillus.

Filogeografía del ADN Mitocondrial del Complejo de *Thryothorus nigricapillus*

Resumen. *Thryothorus nigricapillus* se distribuye desde Costa Rica hasta Ecuador e incluye siete subespecies, de las cuales cinco se encuentran en las tierras bajas caribeñas de Panamá. Las subespecies varían en plumaje, con diferencias particularmente notables entre *Thryothorus nigricapillus* del occidente de Panamá (hacia el norte), y aquellas del oriente de Panamá (hacia el sur). Examinamos la variación entre secuencias de ADN mitocondrial (mtADN) de una muestra geográficamente amplia de *Thryothorus nigricapillus* y comparamos la estructura filogeográfica de la diversidad de mtADN con patrones previamente descritos de variación morfológica. Las reconstrucciones filogeográficas basadas en las secuencias de mtADN revelaron una división basal entre las poblaciones del este de Panamá y Sudamérica, y las poblaciones que se encuentran desde el centro de Panamá hasta Costa Rica. Estos dos clados corresponden a las agrupaciones previamente definidas con base en caracteres morfológicos, dividiendo las subespecies de *T. nigricapillus* en dos grupos: el ''grupo *castaneus*'' (*costaricensis*, *odicus*, *castaneus* y *reditus*) y el ''grupo *nigricapillus*'' (*schottii*, *connectens* y *nigricapillus*). Entre los dos grupos ocurre intergradación morfológica en Panamá central, pero las formas intermedias tienen haplotipos de mtADN característicos del grupos *castaneus*, sugiriendo que el flujo genético mitocondrial es introgresivo de oeste

Manuscript received 29 July 2002; accepted 2 January 2003.
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a este. A pesar de la notable variación en tamaño corporal y plumaje entre las subespecies del grupo *castaneus*, la variación de mtADN dentro de este grupo fue baja. A un nivel filogenético más profundo, los datos de mtADN apoyan el reconocimiento de *T. semibadius* como especie. Este taxón ocasionalmente ha sido considerado coespecífico con *T. nigricapillus*, pero la marcada divergencia a nivel de mtADN entre estas especies es consistente con previas sugerencias de que la semejanza morfológica es resultado de convergencia en caracteres del plumaje.

INTRODUCTION

The location and dynamic geological history of the Isthmus of Panama renders it a region of particular interest for the study of genetic variation in Neotropical birds. The best-known effect of the Pliocene formation of the Panamanian landbridge was the mixing of distinct North and South American faunas in a process termed the Great American Biotic Interchange (Simpson 1980, Marshall 1988, Coates and Obando 1996, Webb 1997). In addition, the complex geographical development of the isthmian region has fostered intraspecific phenotypic and genetic variation among indigenous taxa (Peterson et al. 1992, Seutin et al. 1993, Bermingham and Martin 1998). One such case is the Bay Wren (*Thryothorus nigricapillus*), with five of seven described subspecies occurring in the Caribbean lowlands of Panama (Wetmore 1959, Paynter 1960, Wetmore et al. 1984).

The Bay Wren complex is of particular biogeographic interest because of the striking plumage variation among subspecies, their uncertain distributional boundaries, and the presence of a possible zone of secondary contact in east-central Panama. Subspecies at the northern (*T. n. costaricensis*) and southern (*T. n. nigricapillus*) extremes of the range are very different in appearance from one another, and the seven subspecies have sometimes been divided into two groups (AOU 1998).

Here we investigate the magnitude and geographic structure of mitochondrial DNA (mtDNA) variation among Panamanian and Ecuadorian subspecies of the Bay Wren. Genetic comparisons permit an independent assessment of population divergence, one that allows strong inference regarding the phylogenetic basis of phenotypic variation. If the magnitude of plumage differentiation is roughly proportional to the time populations have been isolated, the phenotypic differences that distinguish geographic populations of Bay Wren would indicate substantial periods of separation and predict commensurate levels of DNA nucleotide substitution and phylogenetic separation. Alternatively, the absence of significant phylogenetic divergence would suggest that plumage differences among subspecies of Bay Wren are more probably due to strong selective pressures or a small number of alleles with large phenotypic effect (e.g., Omland and Lanyon 2000). In either case, our mtDNA-based analysis of Bay Wrens provides a phylogeographic context for future comparative studies of intraspecific variation in isthmian birds.

METHODS

STUDY TAXA

The Bay Wren has a broad distribution in Central America and northern South America, with a range that extends from the Caribbean slope of Nicaragua south through Costa Rica and Panama into western Colombia and western Ecuador (Fig. 1). Throughout its range, the Bay Wren is a generally common resident of forests and second-growth borders, where it is typically found along streams and roadsides in the tropical and lower subtropical habitat zones (Wetmore et al. 1984, Stiles and Skutch 1989).

Differences in overall plumage types have led to the somewhat oversimplified recognition of two groups of subspecies: a northern ''*castaneus* group'' (*T. n. costaricensis*, *T. n. odicus*, *T. n. castaneus*, and *T. n. reditus*) and a southern ''*nigricapillus* group'' (*T. n. schottii*, *T. n. connectens*, and *T. n. nigricapillus*; AOU 1998). *Thryothorus n. costaricensis* and *T. n. castaneus* together were given the specific rank of *T. castaneus* early in the last century (Ridgway 1904) but have subsequently been considered conspecific with *T. nigricapillus*. Plumage and size differences are subtler among the races within each of these two main groups; Wetmore (1959) and Wetmore et al. (1984) treated this morphological variation in detail. The *T. nigricapillus* subspecies distributions presented in Figure 1 are based on the examination of 281 specimens listed in the Appendix.

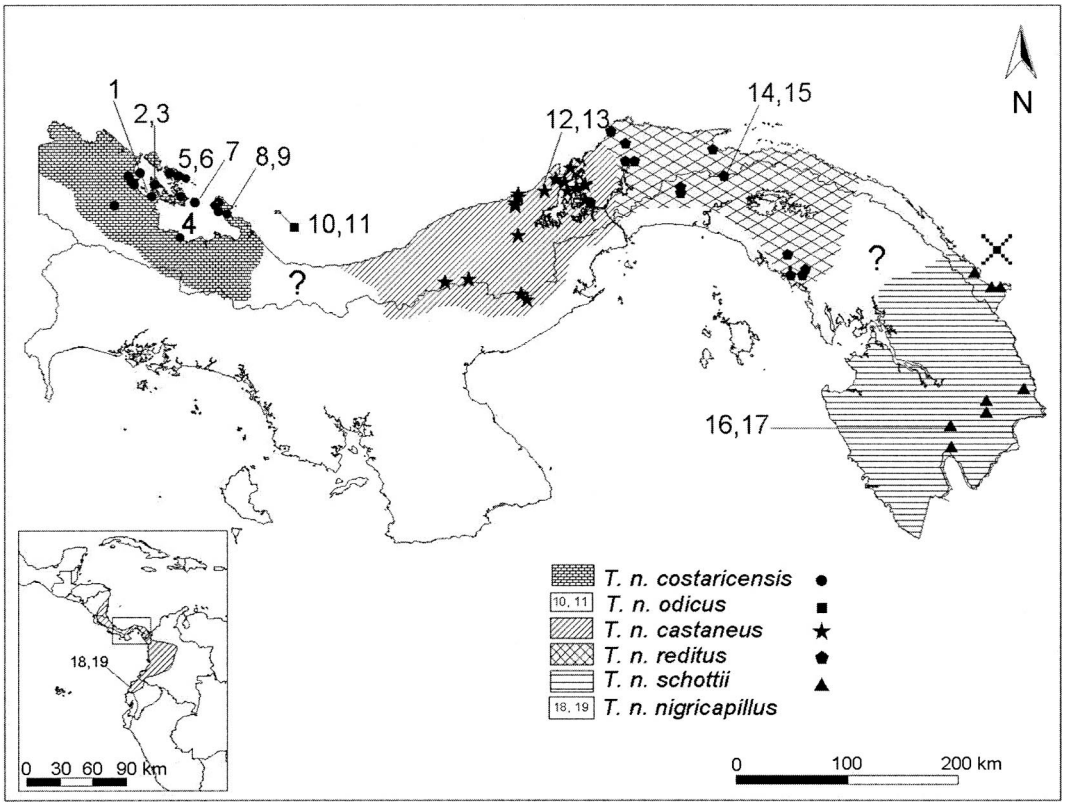

FIGURE 1. Geographic distribution of subspecies of *Thryothorus nigricapillus* in Panama. Dots indicate sampling locations; numbers indicate sequenced individuals listed in Table 1. Subspecies ranges are based on the specimens examined in the Appendix; ''?'' denotes regions where the boundaries between subspecies are not known. An X marks the type locality of *Thryothorus n. reditus*, drawing attention to the nomenclatural problem referred to in the Appendix. Inset depicts the entire Central and South American range of *T. nigricapillus*, including the two subspecies not found in Panama: *T. n. connectens* of southwest Colombia (not sampled) and *T. n. nigricapillus* of western Ecuador. The country of Panama is boxed on the inset map.

To avoid potential confusion over the use of ''*nigricapillus*'' to describe a subspecies, a subspecies complex, and a species, we hereafter use ''*T. n. nigricapillus*'' when referring to the Ecuadorian subspecies alone; ''*nigricapillus* group'' when referring to the subspecies complex composed of *T. n. nigricapillus*, *T. n. connectens*, and *T. n. schottii*; and ''Bay Wren'' or ''*T. nigricapillus*'' when referring collectively to all forms of the species.

In addition to the uncertain relationships among the seven subspecies of the Bay Wren, the Riverside Wren (*T. semibadius*) of southwestern Costa Rica has variously been considered conspecific with *T. nigricapillus* (Hellmayr 1934, Paynter 1960), placed in a superspecies complex with *T. nigricapillus* (Sibley and Monroe 1990), or given full species status (e.g., Wetmore 1959, Wetmore et al. 1984, AOU 1998). To test these hypotheses of relationship, we included a sample of *T. semibadius* in our reconstructions. Three specimens of *T. rutilus* (Rufous-breasted Wren) collected in central Panama provided an unambiguous outgroup for the phylogenetic analyses (Table 1).

MITOCHONDRIAL DNA ANALYSIS

Samples were obtained from the tissue collections of the U.S. National Museum of Natural History and the Museum of Natural Science at Louisiana State University, and the Academy of Natural Sciences, Philadelphia (Table 1). Total cellular DNA was extracted by phenol-chloroform extraction, followed by dialysis. We used the polymerase chain reaction (PCR) to amplify a 1040-base pair (bp) fragment of the mitochon-

TABLE 1. Geographic locations, museum specimen accession numbers, tissue identification numbers, and GenBank DNA sequence accession numbers for *Thryothorus nigricapillus* and outgroup *Thryothorus* species analyzed for a study of Bay Wren phylogeography. Museum abbreviations are as follows: ANSP, Academy of Natural Sciences, Philadelphia, Pennsylvania; LSU, Louisiana State University Museum of Natural Science, Baton Rouge, Louisiana; STRI, Smithsonian Tropical Research Institute, Balboa, Panama; USNM, U.S. National Museum of Natural History, New York. Localities are in Panama except where another country is listed.

Sample	Taxon	Museum	Specimen accession no.	Tissue catalog no.	GenBank accession no.	Collection location (Province; locality)
1	*T. n. costaricensis*	USNM	612420	B00494	AY103289	Bocas del Toro; Tierra Oscura, mainland S of Isla San Cristóbal
2	*T. n. costaricensis*	USNM	607004	B00302	AY103287	Bocas del Toro; Isla San Cristóbal, Bocatorito
3	*T. n. costaricensis*	USNM	607005	B00305	AY103288	Bocas del Toro; Isla San Cristobal, Bocatorito
4	*T. n. costaricensis*	USNM	614082	B01986	AY103284	Bocas del Toro; Chiriquí Grande
5	*T. n. costaricensis*	USNM	605408	B01745	AY103282	Bocas del Toro; Isla Bastimentos, Old Point
6	*T. n. costaricensis*	USNM	605409	B01762	AY103283	Bocas del Toro; Isla Bastimentos, Old Point
7	*T. n. costaricensis*	USNM	607871	B01103	AY103279	Bocas del Toro; Cayo Agua
8	*T. n. costaricensis*	USNM	607879	B01249	AY103280	Bocas del Toro; Península Valiente
9	*T. n. costaricensis*	USNM	607876	B01250	AY103281	Bocas del Toro; Península Valiente
10	*T. n. odicus*	USNM	613500	B01028	AY103277	Bocas del Toro; Isla Escudo de Veraguas
11	*T. n. odicus*	USNM	613508	B01029	AY103278	Bocas del Toro; Isla Escudo de Veraguas
12	*T. n. castaneus*	LSU	164291	B28552	AY103285	Colón; Achiote Road at Rio Providencia
13	*T. n. castaneus*	LSU	164292	B28559	AY103286	Colón; Achiote Road at Rio Providencia
14	*T. n. reditus*	LSU	163696	B26392	AY103290	Panamá; Serranía de San Blas; Chepo
15	*T. n. reditus*	LSU	163697	B26393	AY103291	Panamá; Serranía de San Blas; Chepo
16	*T. n. schottii*	LSU	108537	B2269	AY103292	Darién; Cana on E slope Cerro Pirre
17	*T. n. schottii*	LSU	108536	B2272	AY103293	Darién; Cana on E slope Cerro Pirre
18	*T. n. nigricapillus*	ANSP	180437	LSU B12047	AY103294	Ecuador, Pinchicha; Mindo, 1300 m
19	*T. n. nigricapillus*	ANSP	180436	LSU B12053	AY103295	Ecuador, Pinchicha; Mindo, 1300 m
20	*T. semibadius*	LSU	138767	B16101	AY103273	Costa Rica, Puntarenas; Rio Copey, 4 km E Jacó
21	*T. rutilus*	LSU	163699	B26902	AY103274	Panamá; Old Gamboa Road
22	*T. rutilus*	LSU	163700	B26903	AY103275	Panamá; Old Gamboa Road
23	*T. rutilus*	STRI	—	PA-THU66	AY103276	Panamá; Old Gamboa Road

drial genome from all individuals. The primer pair CO2GQL and CO3HMH (Bermingham 2003) was used to amplify a region spanning the full $tRNA^{Lys}$, ATPase 8, and ATPase 6 genes. PCR reaction components and conditions are detailed in Hunt et al. (2001). Amplification products were cleaned and checked via electrophoresis on low-melting-point agarose gels. We then conducted Dyedeoxy terminator cycle sequencing reactions (Applied Biosystems Division of Perkin Elmer, Inc., Fullerton, California). We sequenced partially overlapping portions of the light strand of the ATPase region with the primers CO2GQL, A8PWL, and a segment of the heavy strand with primer CO3HMH (Bermingham 2003). These products were then electrophoresed in an Applied Biosystems model 377 automated DNA sequencer.

Genetic data analyses. Sequenced fragments obtained using the three primers were aligned and proofread using Sequencher 3.1 (Gene Codes Corporation 1998). All products used in the analyses presented here had very clean chromatograms with a high signal-to-noise ratio, no conspicuous double peaks, and no confounding sequencing artifacts. Phylogenetic analyses were based on the 842-bp coding sequence of the complete ATPase 6 and ATPase 8 genes. These genes have a frame-shift overlap of 10 bp; this short region of overlap did not vary among our taxa and was excluded from analyses parameterized by codon position. Genetic distances among haplotypes were estimated using PAUP*4.0b10 (Swofford 2002); distances reported here are uncorrected percent divergence. Phylogenetic reconstructions among all haplotypes were generated via a maximum likelihood (ML) approach using the Markov chain Monte Carlo method (Steel 1994) implemented in MrBayes (Huelsenbeck and Ronquist 2001). This analysis employed the general time-reversible model (nst = 6), with site-specific rate variation partitioned by codon. Four chains were run for 500 000 generations and sampled every 1000 generations. Inspection of the resulting ML scores suggested that parameter and likelihood stationarity was reached by 10 000 generations; we discarded the topologies sampled from the first 20 000 generations. A majority-rule consensus of the remaining 480 sampled trees was generated in PAUP* to provide a phylogenetic hypothesis with associated posterior probability values for internal branches.

For comparison, distance- and parsimony-based reconstructions were also conducted using PAUP* and a variety of distance metrics and character weighting methods. As all highly supported (100% posterior probability) nodes in the ML tree were also invariably present in the neighbor-joining and maximum parsimony reconstructions, only the results of the ML analysis are presented in detail here.

Phylogenetic reconstructions of Bay Wrens identified a clade of very closely related haplotypes representing the *castaneus* group, which included all individuals sampled from central and western Panama. To examine the relationships of these *castaneus* group haplotypes in more detail, we generated a gene genealogy using the program TCS (Clement et al. 2000). This network-based approach enhances the interpretation of the relationships between closely allied haplotypes because it permits extant haplotypes to be derived from other extant haplotypes.

RESULTS

We obtained the complete sequence (842 nucleotides) of the ATPase 8 and ATPase 6 coding regions from a total of 23 samples of *Thryothorus*, including 17 *T. nigricapillus* sampled along a geographic transect spanning western Bocas del Toro province through the eastern Darién lowlands of Panama and two *T. nigricapillus* from Ecuador (Fig. 1). We also included in our analyses four samples representing two other species of *Thryothorus* (Table 1). All sequences have been accessioned in GenBank (see Table 1 for accession numbers). A total of 194 nucleotide sites varied among all 23 samples, and a total of 74 sites varied among the 17 unique haplotypes of *T. nigricapillus*.

The ATPase haplotypes of *T. nigricapillus* differed from those of *Thryothorus semibadius* and *T. rutilus* by 90–120 substitutions (10.7–14.3%). MtDNA distances between *T. nigricapillus* and *T. semibadius* (mean = 11.4%), two taxa sometimes considered conspecific (Hellmayr 1934, Paynter 1960), were nearly equivalent to the mean distance (13.5%) between *T. nigricapillus* and the morphologically more distinct *T. rutilus*. Divergence among mtDNA haplotypes within the Bay Wren complex was modest, with a maximum difference of 5.6%.

A deep basal bifurcation separated the 19 Bay Wren samples into two clades whose geographic ranges appear to meet in east-central Panama

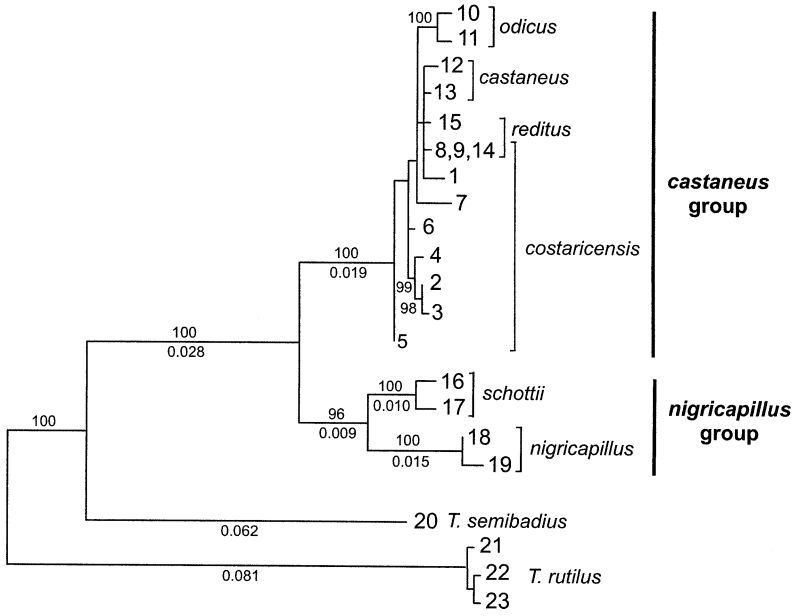

FIGURE 2. Phylogenetic relationships among individuals of *Thryothorus* based on the mitochondrial ATPase 6 and 8 genes. Individuals are numbered as in Table 1 and Figure 1. The consensus tree pictured represents the maximum likelihood analysis of mtDNA haplotypes. Numbers above branches indicate all posterior probability values >90%, and numbers below branches indicate uncorrected genetic distances based on mtDNA sequence divergence.

(Fig. 2). These clades correspond to the *castaneus* and *nigricapillus* subspecies groups (AOU 1998), and haplotypes from the two groups differed by 34–47 substitutions (4.0–5.6%). In the phylogenetic analysis, support for the reciprocal monophyly of these two haplotype groups was high (Fig. 2).

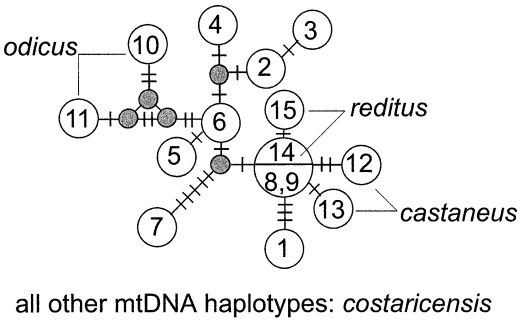

FIGURE 3. A genealogical network for the mtDNA haplotypes observed in the *castaneus* group, which includes the four *Thryothorus nigricapillus* subspecies from western and central Panama (*castaneus*, *costaricensis*, *odicus*, and *reditus*). Shaded circles indicate inferred ancestral haplotypes at network nodes; bars indicate the number of nucleotide substitutions between mtDNA haplotypes (e.g., individuals 2 and 3 differ at one nucleotide site, individuals 12 and 14 at two sites).

The *castaneus* group was represented by 13 unique haplotypes recovered from the 15 representatives of the subspecies *costaricensis*, *odicus*, *castaneus*, and *reditus*. The maximum divergence among the mtDNA haplotypes of the *castaneus* group was 10 nucleotide substitutions (1.2%). The haplotype network depicting the relationship of these very closely related haplotypes is shown in Figure 3. Although individuals sampled at particular locations tended to have similar haplotypes, there was little evidence of strong geographic structuring within this clade. For example, an identical mtDNA haplotype was recovered from individuals representing the subspecies *costaricensis* and *reditus* sampled at sites approximately 330 km apart (samples 8, 9, and 14 in Table 1 and Fig. 1). *Thryothorus n. odicus*, the best-differentiated subspecies in our sample of the *castaneus* complex, had a mean genetic distance of only 0.9% from the remainder of the subspecies in this group.

The *nigricapillus* group was represented by two samples of *T. n. schottii* from eastern Darién

province and two *T. n. nigricapillus* from Ecuador. Haplotypes from these two widely separated localities differed by 24–27 substitutions (2.9–3.2%), suggesting substantial geographically structured diversity within the *nigricapillus* group. Additional samples, particularly from Colombian populations of *T. n. connectens*, would be required to fully evaluate the pattern and magnitude of phylogeographic diversity within the South American representatives of this complex.

DISCUSSION

PHYLOGEOGRAPHIC VARIATION IN MAINLAND POPULATIONS OF THE BAY WREN

The most striking of our results was the large (4.0–5.6%) mtDNA divergence in pairwise comparisons between haplotypes representing Bay Wrens from far eastern Panama and South America (*nigricapillus* group) and those from central Panama through Costa Rica (*castaneus* group). This is concordant with the previous recognition of two distinct subspecies groups based on morphological criteria (AOU 1998). The degree of mtDNA sequence divergence within the Bay Wren equals or surpasses that observed between many avian sister species (e.g., Bermingham et al. 1992, Klicka and Zink 1997, Lovette et al. 1998). It is also similar in magnitude to the genetic differentiation among conspecific populations of freshwater fish distributed across the same region (Bermingham and Martin 1998, Perdices et al. 2002), suggesting that the same historical factors were responsible for the divergences within the Bay Wren and within isthmian freshwater fish. Bermingham and Martin (1998) posited that changes in sea level from the end of the Miocene through the early Pliocene might have caused several episodes of regional isolation across the rising isthmus, thus setting the stage for allopatric differentiation. The magnitude of mtDNA divergence between the *castaneus* and *nigricapillus* groups is consistent with the expansion of the common ancestor of these groups across the newly formed Panamanian isthmus approximately three million years ago (Coates and Obando 1996).

Although there is concordance between morphology and mtDNA at the level of the two major subspecies groups, within each group there is discordance. Plumage differences between *schottii* and *nigricapillus* are not striking (see Appendix), yet these two subspecies differ by roughly 3% mtDNA sequence divergence. Within the *castaneus* group, however, we found a reverse pattern in which subspecies showing marked differences in body size or plumage pattern were often indistinguishable on the basis of their mtDNA haplotypes. There is extensive variation in plumage traits from west to east in the subspecies *costaricensis*, *castaneus*, and *reditus*, with breast color changing from dark chestnut to white and ventral barring changing from absent or indistinct to extensive (Wetmore et al. 1984). Yet these three subspecies show only slight differences in mtDNA sequence (Fig. 2, 3).

Phenotypically, the two subspecies from central Panama, *T. n. castaneus* and *T. n. reditus*, are intergrades between *T. n. costaricensis* and *T. n. schottii*. The range occupied by *castaneus* and *reditus* could thus be considered a phenotypic hybrid zone in which no pure parental types occur, much as is seen, although over a much more limited area, in the northern and southern forms of the Variable Seedeater (*Sporophila americana*; Olson 1981). Although it is most like *costaricensis*, *castaneus* shows the influence of *nigricapillus*/*schottii* in the paler chestnut of the underparts, more extensive white throat, and greater frequency and extent of black ventral barring. The populations that Wetmore treated under the name *T. n. reditus* (Appendix) are extremely variable, but have much more white in the underparts and are always more heavily barred below than in *castaneus*. All retain considerable amounts of chestnut coloration in the underparts, which is not expressed in *schottii*. The demarcation between these two populations of intergrades is quite sharp, with *castaneus* being known from Coclé to the vicinity of the Panama Canal, and *reditus* occurring in the headwaters of the Río Chagres and eastward. One specimen from the former Canal Zone at Río Frijoles is referable to *reditus*. The point of contact between *castaneus* and *reditus* is extremely similar to that between the distinctive northern and southern forms of the Buff-rumped Warbler (*Basileuterus fulvicauda*; Wetmore et al. 1984).

The very abrupt transition from *castaneus* to *reditus* in the narrowest part of the Panamanian isthmus suggests that the two isolates might have come into contact at this point. In this scenario, mtDNA and presumably nuclear genes have flowed eastward toward South America to

produce the highly variable intergrades now called *reditus*. Morphological characters indicate that some *nigricapillus* nuclear genes have apparently introgressed in the opposite direction, giving rise to what is now recognized as *castaneus*. Nevertheless, the mtDNA evidence suggests that the zone of contact between the *castaneus* group and the *nigricapillus* group occurs not in the canal region but farther to the east on the Caribbean slope.

Differential introgression of plumage and mtDNA could result, for example, from female preference for males with dark breast barring on a white background. Analogous patterns of plumage introgression possibly driven by female choice have been well documented in a *Manacus* hybrid zone in western Panama (Parsons et al. 1993, Brumfield et al. 2001).

The partial discordance between patterns of genetic and morphological variation in the Bay Wren is intriguing given the absence of major geographic disjunctions in this species' continuous continental distribution. The vocal behavior of the subspecies *castaneus* has been studied in central Panama, and vocal duetting has been shown to be important in the contexts of sexual selection and social competition (Levin 1996a, 1996b), both of which could be linked with the origin and maintenance of the subspecific morphological diversity. Further sampling and behavioral studies, particularly in the zone where *reditus* and *schottii* meet, are key to understanding the mechanisms underlying the patterns described here.

DIFFERENTIATION IN THE ISLANDS OF BOCAS DEL TORO

The most pronounced genetic difference within the *castaneus* subspecies group, supported by a single mtDNA synapomorphy, was observed between the insular *odicus* and related mainland forms. The subspecies *T. n. odicus*, confined to Isla Escudo de Veraguas, is characterized by its much larger size (9–16%) and paler chestnut of the underparts (Wetmore 1959). Two other endemic subspecies of birds on Escudo de Veraguas are also much larger than their counterparts on the adjacent mainland: the Rufous-tailed Hummingbird (*Amazilia tzacatl handleyi*) and the Golden-collared Manakin (*Manacus vitellinus amitinus*). In addition, a larger, heavier bill distinguishes the Blue-gray Tanager (*Thraupis episcopus caesitia*) on the island from its mainland form. Escudo de Veraguas has been isolated from the mainland longer than any of the other islands of Bocas del Toro, about 8900 years, although this separation may simply be the last in a series of isolating events caused by fluctuating Pleistocene sea levels (Summers et al. 1997). An endemic species of sloth (Anderson and Handley 2001) provides an additional measure of the relative isolation of Isla Escudo de Veraguas, which appears to be reflected in the mtDNA sequence divergence (0.06–1.2%) between *odicus* and the remainder of the *castaneus* group. Nonetheless, the genetic divergence of *odicus* only slightly exceeds nucleotide diversity in the *castaneus* group, and the overall similarity of mtDNA haplotypes is revealed in the genealogical network presented in Figure 3. The maximum mtDNA divergence between *odicus* and related subspecies in the *castaneus* group is almost three times lower than observed between subspecies in the *nigricapillus* group.

We detected no morphological or genetic differences among individuals of *costaricensis* from three islands in Chiriquí Lagoon or between the islands and the adjacent mainland of Bocas del Toro. These islands were separated from the mainland in the mid- to late Holocene from 4700 to 1000 years ago (Anderson and Handley 2001), so either insufficient time has passed to accumulate measurable phenotypic and mtDNA differences among populations, or dispersal has been sufficient to prevent differentiation. It is interesting to note that with the exception of House Wrens (*Troglodytes aedon*) around the artificial pasturelands of Isla San Cristobal, the Bay Wren is the only one of a diverse fauna of eight species of wren in lowland Bocas del Toro that occurs on the islands.

RELATIONSHIPS OF *T. SEMIBADIUS* AND *T. NIGRICAPILLUS*

The Riverside Wren (*Thryothorus semibadius*) of the Pacific lowlands of southwestern Costa Rica and extreme western Panama was treated as a subspecies of *T. nigricapillus* by Hellmayr (1934) and Paynter (1960). However, Wetmore (1959) and Slud (1964) argued for specific separation and Wetmore et al. (1984:94) noted that *T. semibadius* differed ''definitely and completely'' from all of the forms of *T. nigricapillus*. It is smaller, has a crown the same color as the back, and its barring results from feathers with three black bars, whereas the ventral feathers of

nigricapillus have two black bars, suggesting that these taxa may have evolved black barring independently. Nevertheless, the two continue to be considered a superspecies (AOU 1998).

The only apparent reason for regarding *T. semibadius* and *T. nigricapillus* as close relatives is their allopatric distribution combined with barred underparts, although the barred forms of *T. nigricapillus* are those farthest removed geographically from *T. semibadius*. If geography and barring are the criteria, then *T. semibadius* shares an allopatric distribution with other congeners with barred underparts such as the Banded Wren (*T. pleurostictus*), which occurs from Mexico to northwestern Costa Rica, also in Pacific lowlands. It would perhaps fit even better into the disjunct range of the Spot-breasted Wren (*T. maculipectus*), with subspecies ranging from Mexico to Costa Rica and with supposed outliers *T. m. paucimaculatus* and *T. m. sclateri* (the most similar to *T. semibadius*) in Ecuador and Peru. These last have also been treated as subspecies of *T. rutilus* (e.g., Hellmayr 1934).

Our mtDNA-based analyses identified 11.4% sequence divergence between *semibadius* and members of the Bay Wren clade, and therefore strongly support the recognition (Wetmore et al. 1984, AOU 1998) of *semibadius* as a species-level taxon distinct from *T. nigricapillus*. Owing to the fact that we have only sampled three of the 27 species of *Thryothorus* (AOU 1998), we can only speculate based on geographic and phenotypic considerations and level of mtDNA divergence that *T. semibadius* and *T. nigricapillus* probably do not have a sister-group or superspecies-level relationship. Clearly there is much yet to be learned about relationships within this complex genus of wrens.

ACKNOWLEDGMENTS

This project was initially inspired by conversations in the field with Donna Dittmann and Steve Cardiff after collecting several *T. n. schottii*, and we thank them for their suggestions and insights. We thank the U.S. National Museum of Natural History, the Louisiana State University Museum of Natural Science Collection of Genetic Resources, and the Academy of Natural Sciences, Philadelphia, for granting us loans of tissue samples from their holdings. We are very grateful to the countries of Panama, Costa Rica, and Ecuador for approving and supporting the collection of these samples. We thank J. Hunt for his expert technical assistance, Deborah Siegel and Grethel Grajales for mapping the geographical localities of Bay Wrens in Panama, and Douglas Causey for lending specimens from the Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts. The Smithsonian Institution Molecular Systematics Program and a Smithsonian Tropical Research Institute short-term fellowship to MAG funded this research.

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APPENDIX

Bay Wren specimens examined for this study and a nomenclatural note regarding the name *Thryothorus n. reditus*. All specimens are from the National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM), except as marked from the Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts (MCZ). Place names are standardized according to the following sources: Costa Rica (Slud 1964), Panama (USBGN 1990), Colombia (Paynter 1997), Ecuador (Paynter 1993).

Thryothorus nigricapillus costaricensis ($n = 69$)

Nicaragua: Río Escondido (2); Greytown (2); Los Sabalos (1). **Costa Rica:** Alajuela: Río Frio (3); Cartago: Bonilla (1); Pacuare (2, including Paqua [*sic*]); Reventazón (1); Volcán Turrialba (1); Limon: Jiménez (6); Siquirres, 16 km south (1); San José: Guayabo = Guayabal? (1); San José (2). **Panama:** Bocas del Toro: Río Changuena, 727 m (1); Boca del Drago, Changuinola Canal (1); Almirante, Milla 2 (1); Río Oeste (7); Valle de Agua (3); Tierra Oscura (2); Isla Bastimentos (9); Cayo Nancy (5); Isla San Cristóbal (4); Isla Popa (4); Cayo Agua (4); Cayo Patterson (1); Playa Verde, Península Valiente (1); Punta Alegre, Península Valiente (4).

Thryothorus nigricapillus odicus ($n = 16$)

Panama: Bocas del Toro: Isla Escudo de Veraguas (16, including holotype).

Thryothorus nigricapillus castaneus ($n = 51$)

Panama: Coclé: Boca de Uracillo (6); Cascajal (2); Cerro La India Dormida (2); Tigre, head of Río Guabal (3); El Valle, head of Río Mata Ahogada (2). Colón: El Chilar, Río Indio (5); Río Membrillar, Río Indio (3); Chilar, Quebrada Sereña (1); Chilar, Quebrada Torno Rompido (3); Colón (former Panama Canal Zone): Gatún (9); Río Indio, near Gatún (7); Río Indio, near mouth (2); Frijoles (1); Lion Hill (2); Marajal (1); Monte Lirio, Río Gatún (1); Pipeline Road (1).

Thryothorus nigricapillus reditus (*n* = 42)

Panama: Colón (former Panama Canal Zone): Gamboa, Río Frijoles (1). Colón: Cerro Bruja (1); Portobelo (3). Panamá: Estación Hidro El Candelaria (10); Estación Hidro El Peluca (4); San Miguel, Cerro Azul (2); Bajo Grande, Cerro Azul (1); Quebrada Jorón, Chiman (1); Río Corotú, Chiman (3); Río Majé, Charco del Toro (4); Quebrada Cauchero, Cerro Chucantí (9); San Blas: Mandinga (3).

There is a nomenclatural problem associated with the name *reditus*. Griscom (1932) based this subspecies on a series from easternmost San Blas, Panama, at Permé (the type locality, Fig. 1) and Puerto Obaldía. The subspecies was supposed to differ from *schottii* in having the throat white, as in nominate *nigricapillus*, with no mention of the characters Wetmore (1959) later attributed to *reditus* elsewhere in Panama. The white throat, as mentioned previously, appears sporadically elsewhere in *schottii*. Wetmore (1959:21) considered birds from Puerto Obaldía and Armila, only a few kilometers to the east of Permé, to be referable to *schottii* and that Permé was ''barely within the range'' of *reditus*. We examined nine paratypes of *reditus* from Permé and Puerto Obaldía and found the amount of white in the throat to be variable. There was a hint of a more chestnut wash in the underparts of one specimen from Permé, so that it remains possible that some genetic influence from the *reditus* form may extend as far east as Permé, although this would have to be demonstrated through DNA analysis. But otherwise *reditus* would have to be synonymized with *schottii* and a new name given to the birds listed above that Wetmore recognized under *reditus*.

Thryothorus nigricapillus schottii (*n* = 91)

Panama: San Blas: Permé (5 MCZ), Armila (1), Puerto Obaldia (1 USNM, 4 MCZ). Darién: Tacarcuna Village (2); Púcuro (3); Río Paya mouth (9); Cana (7). **Colombia:** Cordoba: Río Salvajín, Río Esmeralda (5); Socorré, Río Sinú (4). Bolivar: Volador (1); Regeneración (3). Antioquia: Alto Bonito (4); La Bodega (1); El Pescado (6); El Real (3); Villa Arteaga (8); Hacienda Belén (5). Chocó: Acandí (3); Nuquí (7); Río Jurubidá (3); Río Truandó (2, syntypes). Valle del Cauca: Buenaventura (1); San José (1); Punto Muchimbo, Río San Juan (2).

Thryothorus nigricapillus connectens (*n* = 5)

Colombia: Cauca: Guapí (2); Narino: Guayacana (2); Barbacoas (1).

Thryothorus nigricapillus nigricapillus (*n* = 7)

Ecuador: No locality (1). Guayas: Guayaquil (2); Huerta Negra (4).

In *T. n. nigricapillus* of Ecuador, the underparts are white, becoming brownish or buff posteriorly, and finely barred with black except on the throat and upper breast. In *T. n. schottii* of Colombia and eastern Panama, the barring tends to be heavier and more extensive on the throat. There is considerable variation, however, and birds with white, unbarred throats may appear almost anywhere in the range. A female from El Real, Antioquia, Colombia, is pale, with a white throat and fine ventral barring, and would be difficult to separate from a series of *T. n. nigricapillus*. A male from the same locality is dark and very heavily barred nearly to the chin, and a second male is intermediate. The subspecies *T. n. connectens* of southern Colombia, as implied by its name, was supposed to bridge the differences between *nigricapillus* and *schottii* but was synonymized with *schottii* by Wetmore (1959).