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RESEARCH ARTICLE

Phylogeography and conservation of the endemic Hispaniolan Palm-Tanagers (Aves: *Phaenicophilus*)

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Abstract The Gray-crowned Palm-Tanager (*Phaenicophilus poliocephalus*), sometimes considered conspecific with its more widespread congener *P. palmarum*, is restricted to Haiti's Tiburon Peninsula, a biodiversity hotspot threatened by extensive habitat loss. We used a multilocus phylogeographic approach to identify evolutionarily distinct populations of *Phaenicophilus*. Mitochondrial haplotypes formed two reciprocally monophyletic groups separated by 5% uncorrected divergence. Genealogical patterns of differentiation at nuclear intron alleles were congruent with those of mtDNA, and the two species also differed in body size and shape. An ancient sea channel between the Tiburon Peninsula and mainland Haiti was likely a dispersal barrier that led to allopatric divergence, a hypothesis supported by our estimates of divergence times. Our results support the recognition of two Palm-Tanager species, confirming *P. poliocephalus* as Haiti's only endemic bird species and underscoring the need to protect the Tiburon Peninsula's single primary forest reserve.

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Introduction

Hispaniola, which supports more endemic bird species than any other Caribbean island, is a biodiversity "hotspot" where an exceptional concentration of endemic species is threatened by extreme habitat loss (Latta 2005; Myers et al. 2000; Rimmer et al. 2005; Sergile and Woods 2001). Habitat loss is particularly severe in Haiti, where subsistence farming and logging have reduced native forest cover to less than 2% of its original extent (Keith et al. 2003; Paryski et al. 1989). The sole remaining tracts of primary forest in Haiti are found in two poorly protected reserves, the Macaya Biosphere in the Massif de la Hotte on the Tiburon Peninsula and La Visite National Park in the Massif de la Selle in southern Haiti. Both reserves support unique species and populations of numerous taxa, including reptiles (Glor et al. 2003), amphibians (Hedges 1996), orchids (Dod 1984), and snails (Thompson 1986). Without further protections and vigorous enforcement, these remnant tracts may soon be lost (Sergile and Woods 2001, Woods and Ottenwalder 1992). Avian conservation in these regions has been confounded by a lack of basic information about endemic species (Latta 2005), although recent work has included surveys of bird communities (Davalos and Brooks 2001; Rimmer et al. 2005) and descriptions of basic natural history of threatened species (Rimmer et al. 2008). Molecular phylogeographic studies contribute to conservation efforts by identifying evolutionarily significant units (ESUs) that merit separate management, using reciprocal monophyly at mitochondrial loci as one criterion for focused action units of conservation

(Moritz 1994). These inferences can be strengthened by the use of multiple unlinked nuclear loci that provide independent assessments of evolutionary history (Rubinoff and Holland 2005), especially when the markers show congruent patterns of divergence. When such molecular studies are combined with studies of phenotypic variation across populations, identification of ESUs can facilitate the conservation of adaptive phenotypic variation and its underlying genetic variation (Crandall et al. 2000).

Phylogeographic approaches can also be used to assess the patterns and processes of diversification on Hispaniola as a whole. Hispaniola is unusual among islands in that it possesses multiple closely related endemic avian taxa (Diamond 1977; Coyne and Price 2000; Keith et al. 2003). A previous multilocus study on the phylogeography of one of the endemic avian species pairs, the *Calyptophilus* Chat-Tanagers, suggested that Hispaniola's paleogeographic history as separate North/South island blocks (Graham 2003) allowed allopatric divergence within the genus, leading to two distinctive species (Townsend et al. 2007). It is not known if the other avian endemic species pairs on Hispaniola share this North/South phylogenetic distinctiveness, but their current geographic distributions differ from *Calyptophilus* (Keith et al. 2003), suggesting that other geographic barriers or isolating mechanisms may have contributed to past diversification.

The Black-crowned Palm-Tanager (*Phaenicophilus palmarum*) and Gray-crowned Palm-Tanager (*P. poliocephalus*), another species pair endemic to Hispaniola, occupy parapatric ranges on Hispaniola: *poliocephalus* is restricted to the Tiburon Peninsula whereas *palmarum* occupies the remainder of the island (Keith et al. 2003; Fig. 1, this paper). These forms have sometimes been considered conspecific (Bond 1986; Hellmayr 1936) but they differ in adult crown and throat plumage color. McDonald and Smith (1994) described a hybrid zone in the contact region near the western edge of the Massif de la Selle, potentially indicating a lack of reproductive isolation between the taxa. However, their analysis of morphological variance (McDonald and Smith 1994) and allozymes (McDonald and Smith 1990) indicates low gene flow and selection against hybrids. *Phaenicophilus poliocephalus* has been identified as Near Threatened by the IUCN (Birdlife International 2000), persisting in a region of Haiti that is largely fragmented and deforested. Confirming the evolutionary distinctiveness of this taxon endemic to Haiti would further justify the protection of the last remaining forest tracts on the Tiburon Peninsula for conserving this and other species endemic to the region.

Our goals in this study were to use a combination of multilocus DNA sequence analyses and studies of morphological variation to: (1) identify phylogeographic population structure within the *Phaenicophilus* complex, (2) assess the validity of the current taxonomy of the genus, and (3) identify whether divergence in *Phaenicophilus* populations is coincident with potential current or past geographic barriers.

Methods

Sampling

Genetic samples were obtained as part of island-wide surveys of avian diversity (2002–2006). The individuals sampled for this study were not collected and vouchered as

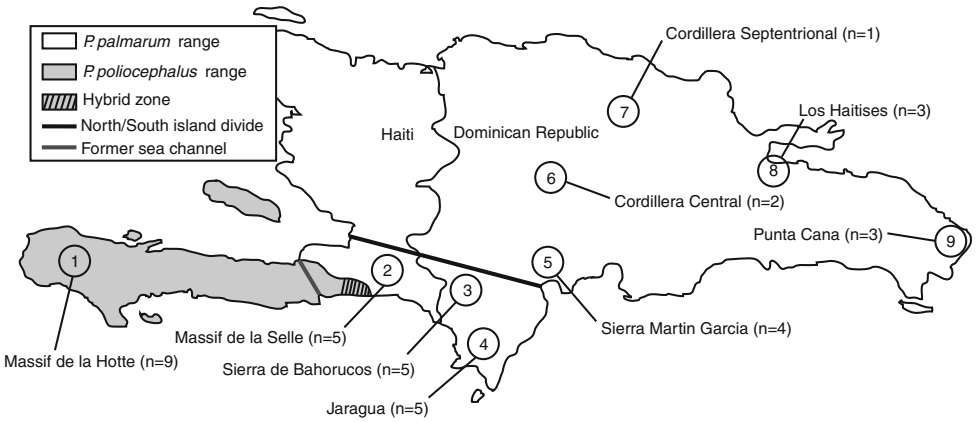

Fig. 1 Distribution of Palm-Tanagers and sampling sites in Hispaniola. *P. poliocephalus* occupies the Tiburon Peninsula (*gray*); *P. palmarum* occupies the rest of the island; both species occupy many habitats across a broad range of elevations. The hybrid zone is approximated by the hatched area. The *heavy black line* corresponds to the divide between the two paleoisland blocks that merged in the Miocene. The heavy *dark gray line* represents the sea channel that separated the Tiburon Peninsula from the southern island block as recently as the Pleistocene. *Circles* indicate the general sampling localities. *Numbers within circles* correspond to the locations in the haplotype networks (Fig. 2); n = sample size at each location

museum specimens because of concerns for their conservation status in Haiti, and because our sampling was part of ongoing multi-species mark-recapture studies of demography and survivorship (Latta et al. 2003; Rimmer et al. 2003, 2005; Rimmer and McFarland 2001). Samples of *Phaenicophilus* were obtained from eight localities within the range of *palmarum* and one locality within the range of *poliocephalus* (Fig. 1). Sample sizes per locality varied (Fig. 1), and a maximum of five individuals per *palmarum* location and nine individuals for the single *poliocephalus* location were sampled for molecular analyses. Birds were captured in mist nets and marked with permanent, individually numbered leg bands. From 99 birds, which included all individuals used in molecular analyses as well as 62 birds that were not genetically sampled, we measured seven morphological characters: exposed culmen length (from top of bill to tip), bill length (from distal end of nares to tip), bill width and depth (measured at distal end of nostrils) to the nearest 0.1 mm, wing length (unflattened chord) and tail length to the nearest 0.5 mm, and weight to the nearest 0.1 g. Approximately 80 µl of blood was taken from each individual with heparinized capillary tubes via brachial venipuncture with sterile 27-gauge hypodermic needles. Blood samples were stored in 0.5 ml blood lysis buffer (100 mM Tris-HCl, pH 8; 100 mM Na2 EDTA; 10 mM NaCl; 0.5% SDS; White and Densmore 1992).

Molecular analyses

We extracted DNA from each sample using Perfect gDNA Blood Mini kits (Eppendorf) following the manufacturer's protocol. We amplified and sequenced one mitochondrial gene and three nuclear introns for 28 *P. palmarum* and 9 *P. poliocephalus* individuals. Three of these loci were used to resolve evolutionary relationships among populations of *Calyptophilus* (Townsend et al. 2007), facilitating comparison between these datasets. Using the protocols of Lovette and Rubenstein (2007), we amplified and sequenced the mitochondrially encoded ND2 gene (1024 bp) with primers METb and TRPc (Eberhard and Bermingham 2004), beta-fibrinogen intron 5 (hereafter Fib-5, 551 bp) with primers Fib-5 and Fib-6 (Lovette and Rubenstein 2007), the Z-linked aconitase-1 intron 9 (Aco-9, 973 bp) with primers AcoI10F and AcoI10R2 (Barker et al 2008), and rhodopsin intron 1 (Rho-1, 865 bp) using the primers RHOf and RHOr (Primmer et al. 2002). Sequences were aligned by eye in Sequencher™ (Gene Codes). All individuals used in the molecular analysis were sexed genetically using an intron of the chromo-helicase DNA binding protein with the primers 2550F and 2718R (Fridolfsson and Ellegren 1999). The Z and W chromosome copies of this locus differ in length, allowing results to be read with gel electrophoresis. Sexing data were used to distinguish between females and homozygous males at the Z-linked Aco-9 intron.

Heterozygous intron sequences were resolved as individual alleles using PHASE 2.1 (Stephens et al. 2001; Stephens and Scheet 2005). For each individual, both alleles from Fib-5 and Rho-1 were included in analysis. For the Z-linked Aco-9 intron, two alleles were included for males and the single allele was included for females in analyses. We constructed statistical parsimony haplotype networks using TCS 1.21 (Clement et al. 2000) to estimate relationships among haplotypes and alleles. Haplotype networks divergent enough to remain unconnected at the 95% parsimony limit were arbitrarily connected between two haplotypes with the minimum pairwise divergence (i.e. ND2, Fig. 2). A 30 base-pair region in Rho-1 that contained a deletion including binucleotide repeats of variable length was excluded from analysis, because the number of mutation events could not be determined and this variability cannot be coded for in TCS.

The optimal model of sequence evolution for ND2 was chosen using the model selection analysis in TOPALi v2 (Milne et al. 2009) and applied in PAUP* (Swofford 2000) to analyze corrected nucleotide variation. We generated an estimate of divergence time using the model-corrected divergence between *palmarum* and *poliocephalus* calculated in PAUP* (Swofford 2000) for ND2 using a molecular rate of 2.1% (±0.1%) divergence per million years, the average rate of divergence for the mitochondrial Cytochrome b gene in birds (Weir and Schluter 2008).

We used Isolation with Migration-analytic (IMa) (Hey and Nielsen 2004, 2007; Nielsen and Wakely 2001) for multilocus coalescent analysis of divergence time and migration rates between the two recognized species of Palm-Tanager. IMa requires selectively neutral, recombination-free independent loci, assumes no genetic structure within each species, and assumes no gene flow from unsampled species. We sampled both species of *Phaenicophilus*, but do not test genetic structuring within either species. Simulation data suggest the Isolation with Migration model is robust to assumption violations of current population structure for all parameters (Strasburg and Rieseberg 2009) and structure in the ancestral population for the time parameter but not population size and migration parameters (Becquet and Przeworski 2009). We assumed the mitochondrial data to be recombination-free, and tested for recombination in all nuclear loci using the four-gamete test (Hudson and Kaplan 1985) in DnaSP 4.0 (Rozas et al. 2003). We selected the largest recombination-free segment with segregating sites for analysis. Neutrality was tested in these segments with DnaSP 4.0 (Rozas et al. 2003) using the Hudson-Kreitman-Aguade test (HKA) (Hudson et al. 1987). We used the Hasegawa-Kishino-Yano (HKY) model to fit our recombination-free segments

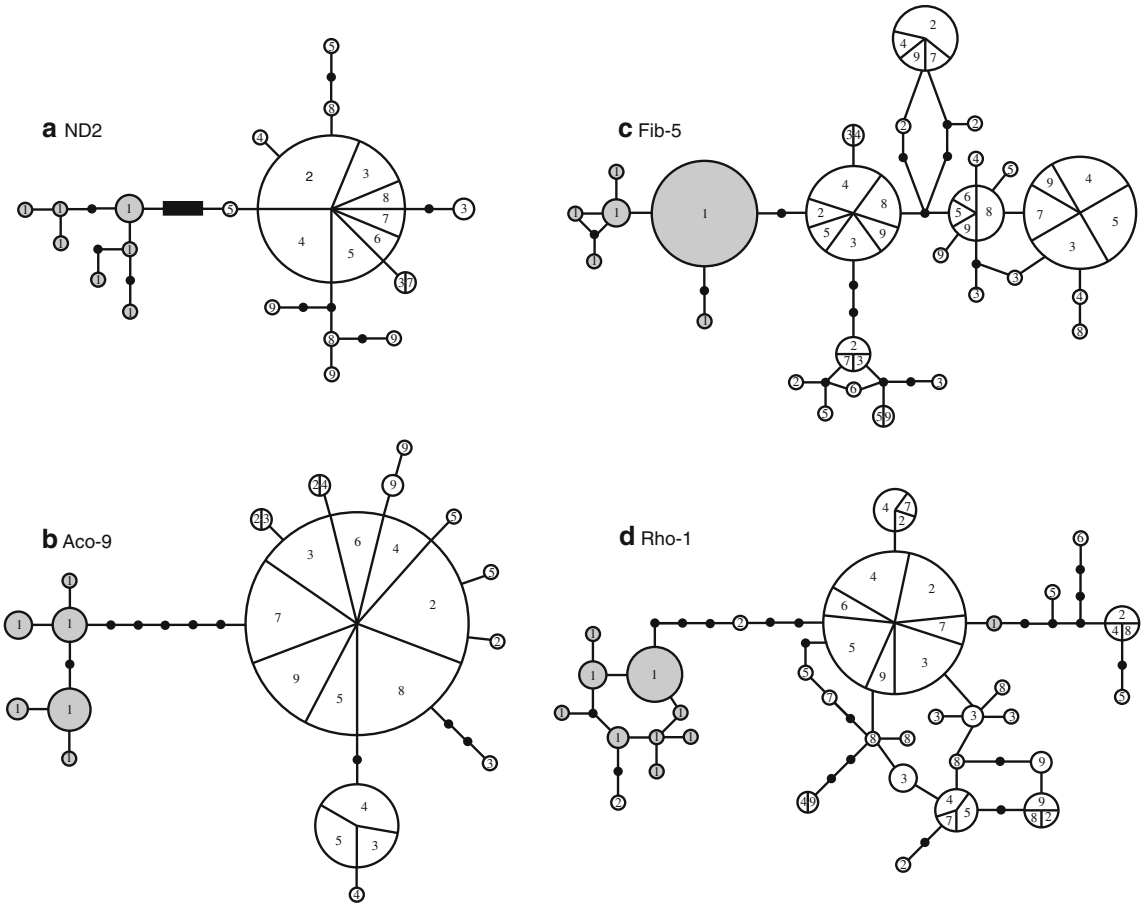

Fig. 2 Statistical parsimony haplotype networks. *Black dots* indicate inferred unsampled haplotypes separated by single substitutions. The *black bar* in ND2 represents 54 substitutions. The *gray shading* represents *P. poliocephalus* samples and the *white* represents *P. palmarum* samples. Numbers correspond to the sampling localities in Fig. 1

to the IMa model because of violation of the Infinite Sites model in our data: several sites were polymorphic with more than two bases. We ran multiple preliminary metropolis coupled Monte Carlo Markov chain simulations of variable length to optimize parameter bounds and mixing. We ran three final simulations with a burn-in of 1×10^6 steps, a geometric heat mode with a first heating parameter of 0.95 and a second heating parameter of 0.80, 30 chains, different random seeds for each simulation, and a total of 15 million steps with parameter effective sample sizes reached at least 100.

IM parameter outputs and posterior probability density distributions are scaled to the neutral mutation rate μ, the geometric mean of the neutral mutation rates of all loci, measured in substitutions/site/locus (s/s/l). To compare the multilocus coalescent estimate of divergence time with the mitochondrial clock estimate, we specified a molecular rate of 1.35×10^{-9} substitutions/site/year (s/s/y) for nuclear loci (Ellegren 2007) and a rate of 2.1×10^{-8} s/s/y for the mitochondrial locus, and calculated the geometric mean per-locus mutation rate for the dataset (1.03×10^{-6} substitutions/locus/year). We used this value to convert the peak estimate and 90% highest posterior density interval of the time parameter (scaled to the neutral mutation rate μ) to demographic units (years since divergence).

Morphological analyses

A scatterplot matrix indicated positive correlations among all of the seven morphometric variables. Using principal components analysis (PCA) on covariances, we reduced these variables to two meaningful components, using the criteria of a scree test (Cattell 1966). We examined size differences among sites with a one-way ANOVA on PC1 and PC2 scores with site as a between-group factor. When effects were significant, we used Tukey's HSD multiple comparison test to determine which levels were different. Sexes were not available from all birds in the sample and we did not include them as a factor in the model. All morphological analyses were performed using JMP 5.1 for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Phaenicophilus shows a similar pattern of historical divergence at all four loci that corresponds with its current taxonomic boundaries. Mitochondrial ND2 variation in *Phaenicophilus* is highly structured, with haplotypes from *P. palmarum* and *P. poliocephalus* forming two substantially divergent, reciprocally monophyletic groups (Fig. 2). The groups are separated by 54–61 nucleotide substitutions, representing 5.3–5.9% uncorrected divergence. ND2 variation among sampled *palmarum* populations is minimal: all but one sample locality for *palmarum* contained the most common *palmarum* haplotype. The three samples from locality 9 fall outside the common central haplotype. Variation within the single sampling location of *poliocephalus* is high, with seven haplotypes from nine individuals.

All nuclear intron loci form similar patterns: *poliocephalus* alleles formed a distinct group with varying levels of divergence from *palmarum* alleles (Fig. 2). The two groups are reciprocally monophyletic at both the Aco-9 and Fib-5 loci. Alleles at the Rho-1 locus show a pattern of divergence between *palmarum* and *poliocephalus*, but are not reciprocally monophyletic: one rare allele from each taxon is located within the other's haplotype group. At all nuclear loci, the divergence between clusters was much lower than in the mtDNA (Fig. 2), with a minimum of 0.4% uncorrected divergence at Fib-5 and 0.6% uncorrected divergence at Aco-9. All unique mitochondrial and nuclear sequences have been archived in GenBank (accession numbers FJ159166–FJ159208).

Model selection analysis in TOPALi found a Hasegawa-Kishino-Yano model (HKY + I + G) with invariant sites (pINV = 0.197) and rate heterogeneity ($\propto = 0.508$) as the optimal model for the ND2 dataset at all criteria (hierarchical likelihood ratio tests, Akaike information criterion, Bayesian information criterion). This model produced a mean corrected divergence of 7.0% (range: 6.6–7.7%) between *palmarum* and *poliocephalus*. Using the 2.1% (±0.1%) rate for mitochondrial data, this produces a mean divergence time estimate of 3.3 million years (±200,000 years).

The four-gamete test found evidence for recombination in Fib-5 and Rho-1. Selecting the longest recombination-free segment reduced these loci from 551 to 195 bp and 863 to 113 bp, respectively. No recombination was detected in Aco-9, so all 973 bp were used. The HKA tests were not significant at any locus ($P > 0.05$), indicating these segments are selectively neutral. Running the IM simulation with different random starting seeds in the final runs produced convergence for parameters' marginal posterior density distributions. Data from the longest run are presented. Posterior probability density distributions, scaled to the neutral mutation rate μ, for the six demographic parameters of the IMa model are shown in Fig. 3. Peak probabilities and 90% highest probability density (HPD) distributions for the demographic parameters are shown in Table 1. Converting the time parameter to years produced a mean divergence time estimate of 2.2 million years (HPD: 1.1 million years—undefined). The time parameter (t) did not produce a complete distribution, instead

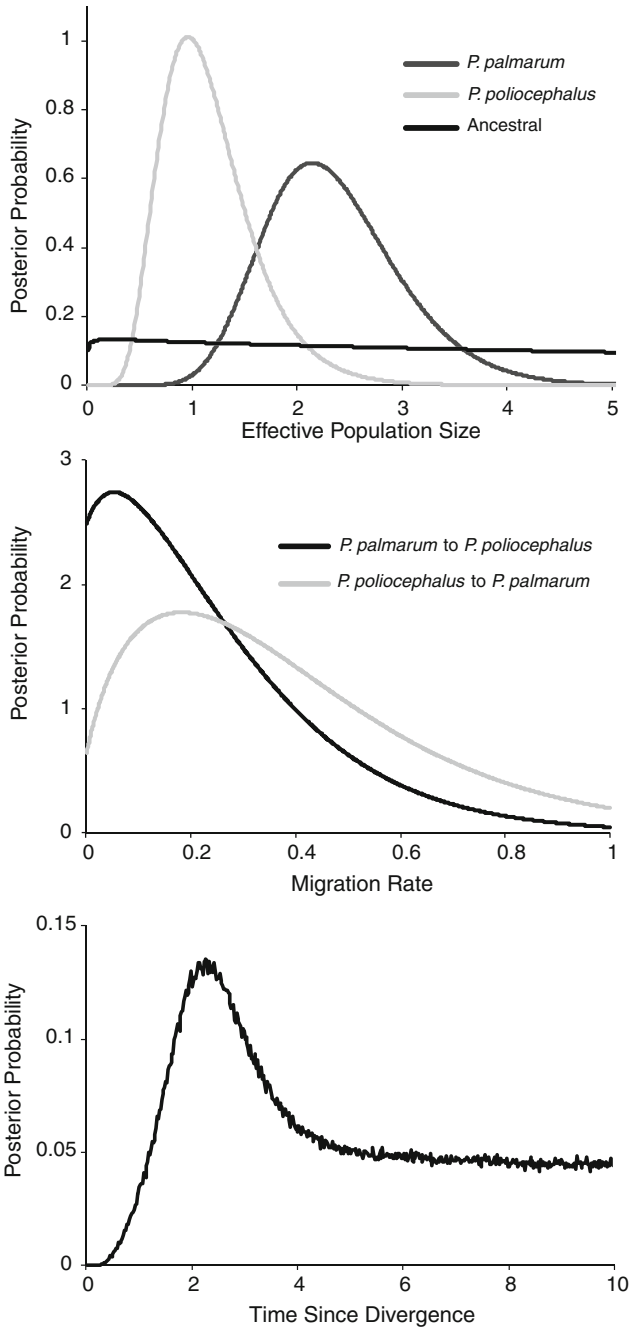

Fig. 3 Marginal posterior density distributions of the demographic parameters estimated in the IMa analysis. All *x*-axes are scaled to the neutral mutation rate

Table 1 Posterior probability peaks and 90% highest probability densities (HPD) for the six demographic parameters estimated in IMa

Parameter	Peak	HPD
palmarum effective population size	2.1400	1.3000–3.3720
poliocephalus effective population size	0.6457	0.4760–1.8360
Ancestral effective population size	0.9560	0.0050–8.7650
Introgression from *palmarum* to *poliocephalus*	1.0113	0.0005–0.5235
Introgression from *poliocephalus* to *palmarum*	0.2050	0.0005–0.7105
Time since divergence	2.2700	1.1300–undefined

reaching a distinct peak then plateauing at a lower probability for the rest of the parameter bounds (Maximum prior for t set to 20; distribution up to 10 shown in Fig. 3). Expanding the parameter bounds in preliminary runs did not change this pattern. The long asymmetric right tail of the HPD range (and thus the IMa divergence time estimate) is likely influenced by this plateau in the probability distribution and the prior bounds so it is left as undefined.

A PCA of the seven morphological measurements generated eigenvalues for the first two components of 23.9 and 4.3, accounting for 61.7 and 16.1% of the total variance, respectively. We retained PC1 as composite 'size' variable in subsequent analyses because it was positively correlated to each of the morphological traits. Factor loadings for the second component were negative for wing and culmen width but positive for all other measurements. We retained PC2 as a factor explaining 'shape' in relationship to size. A one-way ANOVA on PC1 and PC2 scores with site as a between-group factor revealed significant differences in size and shape among *Phaenicophilus* populations ($F(3,95) = 16.91$, $P < 0.0001$ for size and $F(3,95) = 7.15$, $P = 0.0002$ for shape; Fig. 4). Tukey's HSD test showed that birds from all measured populations of *palmarum* were significantly larger than the birds from the *poliocephalus* population ($P < 0.05$), and that *poliocephalus* individuals also differed in shape (PC2) from *palmarum* individuals from the Sierra de Bahoruco.

Discussion

Although *Phaenicophilus palmarum* and *P. poliocephalus* have sometimes been considered conspecific (Bond 1986; Hellmayr 1936), our molecular and morphological markers examined indicate that there are two lineages of Palm-Tanager congruent with the currently described species. Substantial mitochondrial divergence (5.3–5.9% uncorrected) separates these two reciprocally monophyletic lineages, a level of divergence that greatly exceeds the average mitochondrial divergence between sister species of bird in North America (1.9%, Johnson and Cicero 2004). Two of three nuclear loci were also reciprocally monophyletic and showed the same phylogeographic pattern, further indicating substantial divergence between these two lineages. Likewise, we found significant differences in body size and shape between them, corroborating earlier analyses of morphology and plumage color (McDonald and Smith 1994). Considered in concert, these genetic and

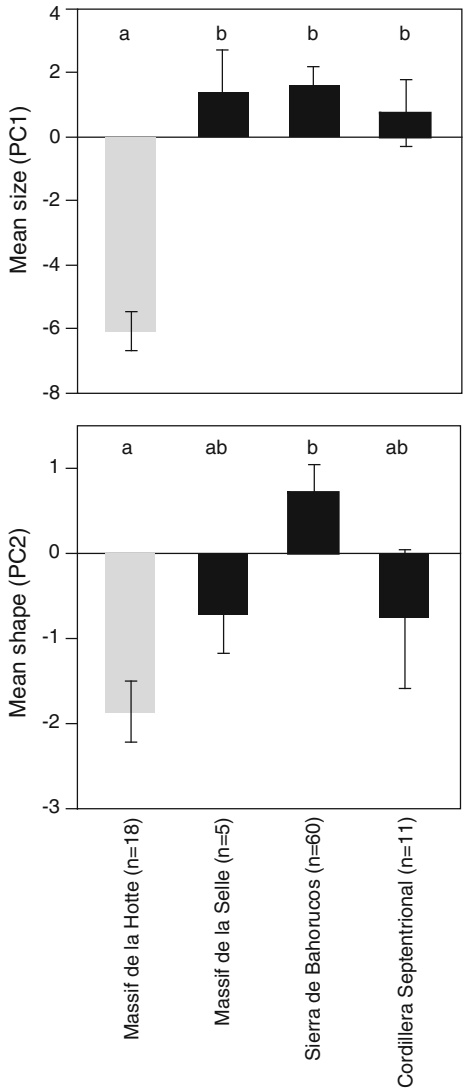

Fig. 4 Mean size (PC1) and shape (PC2) of birds from each sampling locality. Levels not connected by the same letter are significantly different. *Black bars* indicate *P. palmarum* populations, while the *grey bar* indicates the *P. poliocephalus* population. *N* = sample sizes

morphological data strongly suggest that the two Palm-Tanager lineages have undergone substantial historical divergence.

The two *Phaenicophilus* lineages are known to hybridize (McDonald and Smith 1994), indicating a lack of complete reproductive isolation. However, McDonald and Smith (1990, 1994) concluded that gene flow was restricted, and they suggested that the hybrid zone was an area of limited secondary contact with selection against hybrids. Although our sampling was not designed to directly examine migration across or near the hybrid zone, we did find a lack of reciprocal monophyly at the Rho-1 locus. This could represent introgression, as single alleles from each species (in *palmarum*, an allele from the sampling point closest to the hybrid zone) fall within the other species' haplotype group. However, incomplete lineage sorting is another potential explanation for the lack of reciprocal monophyly at this locus, given the longer coalescent time for nuclear loci relative to mitochondrial genes (Hoelzer 1997) and the very shallow divergences (2–6 fixed nucleotide substitutions, Fig. 2) at the other nuclear loci in this study. The reciprocal monophyly at most molecular markers, along with the morphological distinctions between lineages, suggests that gene flow between the two lineages, if currently continuing, is not pervasive.

Our two estimates of divergence time indicate the Palm-Tanager lineages likely split in the late Pliocene or early Pleistocene, 2–3 million years ago, a substantially greater estimate than previous analyses (50,000–260,000 years ago based on allozyme data, McDonald and Smith 1990). The mitochondrial clock estimate is greater than the peak time estimate generated by the multilocus dataset, a pattern found in other recent studies (Carling et al. 2010; Brumfield et al. 2008; Lee and Edwards 2008), although the 90% HPD of the multilocus time parameter completely overlaps the mitochondrial estimate. The use of mutation rates as molecular clocks in divergence estimates is subject to a number of important caveats. Molecular clocks are subject to large and difficult-to-calculate error, including rate heterogeneity within and between lineages, rate variation between loci, and errors in the fossil or geographic calibrations necessary to 'set' the clock (Arbogast et al 2002; Garcia-Moreno 2004; Lovette 2004). Despite these sources of error, the 'standard' avian mitochondrial clock rate of 2.1% divergence per million years has recently been validated by a large number of new calibrations (Weir and Schluter 2008) although rate variation among avian orders exists. Given these caveats, we avoided relying on a single molecular rate and instead used two different methods to generate a divergence time estimate that should be taken as a general approximation, not at hard point estimate.

The complex geological history of Hispaniola as multiple separate island blocks has presented multiple opportunities for the divergence of Palm-Tanagers and other taxa in allopatry. McDonald and Smith (1990) suggested that flooding of the low plain separating the two paleo-island blocks during high sea levels in Pleistocene interglacials allowed a population of *Phaenicophilus* to colonize the south island and speciate in allopatry. Their estimate of a young divergence time of 50,000–260,000 years supports this hypothesis. This scenario requires *P. palmarum* to have recently reinvaded the south island block, pushing out *poliocephalus* until they reach the present day distribution, with *poliocephalus* restricted to the Tiburon Peninsula. The current distributions, combined with our molecular estimates, suggest a more parsimonious alternative—these taxa diverged when the Tiburon Peninsula was separated from the southern paleo-island block by an ancient sea channel. This channel, previously recognized as the "Jacmel-Fauché depression" or "Bond's Line," lies in the Trouin Valley, following the Riviere Gauche between Jacmel and Carrefour Fauché (Keith et al. 2003). The peninsula, the core of *P. poliocephalus*' present range, merged with the rest of Hispaniola in the late Pleistocene approximately 100,000 years ago (Maurrasse and Rigaud 1982), as a result of uprising in the sea channel that separated it from the mainland. The current boundary between the taxa corresponds closely to this former water barrier and our divergence time estimates substantially predate the merging of the Tiburon Peninsula with the rest of Hispaniola. This suggests the ancient sea channel formed a barrier to gene flow, resulting in isolation and allopatric speciation.

A previous study characterized molecular and morphological variation in another Hispaniolan endemic species pair, the *Calyptophilus* Chat-Tanagers (Townsend et al. 2007). *Calyptophilus* has a phylogeographic pattern superficially similar to *Phaenicophilus*: two well-differentiated genetic groups with no other substantial differentiation. However, different geographic barriers appear to have promoted the diversification in these two genera. In *Calyptophilus*, the older north-south paleo-island division appears to have been the most important barrier to gene flow, as indicated by the ranges of the genetic groups and the much older divergence between them (peak estimate of 9.7 million years). In *Phaenicophilus,* gene flow was likely restricted by the more recent Tiburon Peninsula sea channel. Considered together, the likelihood of allopatric divergence in the histories of both *Calyptophilus* and *Phaenicophilus* argues against a scenario of speciation within one island mass. Instead, the complex geographic history involving multiple island blocks and changing sea levels has likely driven Hispaniola's endemic speciation events through allopatric divergence and insular speciation. Further analysis of other closely related endemic bird species on Hispaniola will provide insight into the relative frequencies of these modes of speciation for island birds.

The distinctiveness of *Phaenicophilus poliocephalus* at all genetic loci we examined, its high degree of divergence from *P. palmarum* in mtDNA, and its differences from *P. palmarum* in size, shape, and plumage support the continued recognition of *poliocephalus* as Haiti's only known endemic bird species, meriting separate management considerations both to conserve the historical legacy and functional diversity within *Phaenicophilus* (Crandall et al. 2000; Moritz 1994). *P. poliocephalus* is listed by the IUCN as Near Threatened due to severe habitat loss throughout its range (Birdlife International 2000), although it remains locally common in the Macaya Biosphere Reserve (Rimmer et al. 2005). Although additional populations of *poliocephalus* may persist elsewhere in the Tiburon Peninsula (McDonald and Smith 1990, 1994), no recent information is available concerning the status of this species outside of the Macaya Biosphere Preserve. Any other occupied sites would almost certainly lack legal protection. Without further protections and vigorous enforcement, this remnant tract and its endemic wildlife might soon be lost (Sergile and Woods 2001; Woods and Ottenwalder 1992). Strengthening conservation management of the Macaya Biosphere Reserve will help protect not only the Gray-crowned Palm-Tanager, a potential conservation flagship species, but also many less conspicuous flora and fauna endemic to the Tiburon Peninsula.

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