The Condor 103:248–258

PHYLOGENETIC RELATIONSHIPS IN BEARDED MANAKINS (PIPRIDAE: *MANACUS*) INDICATE THAT MALE PLUMAGE COLOR IS A MISLEADING TAXONOMIC MARKER[1]

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Abstract. The piprid genus *Manacus* is composed of four allospecies that are readily distinguishable by differences in male plumage color. Electrophoretic data for two populations of each of the four forms plus seven outgroup piprid taxa were collected from 32 isozyme loci and used to infer phylogenetic relationships. Each *Manacus* form was monophyletic, with the exception of *M. manacus*, in which the trans-Andean (west of the Andes) population was sister to *M. vitellinus*, rather than to its conspecific cis-Andean (east of the Andes) population. This controversial relationship, supported by the synapomorphic allele PGM-2[b] as well as allele frequencies at ADA, GOT-1 and LGG, is consistent with general biogeographic patterns in the region, but indicates that male plumage color is an unreliable taxonomic marker. Reconstruction of male plumage color on the tree confirms that gold plumage is a derived state in *M. vitellinus*, a finding consistent with the possibility that gold plumage is an evolutionary novelty in *vitellinus* which has spread recently under positive selection. Among piprids, there was strong support for a group composed of *Antilophia*, *Chiroxiphia*, and *Corapipo*, and for a group composed of *Pipra mentalis*, *P. fasciicauda*, and *Dixiphia pipra*. *Manacus* is more closely related to the *P. mentalis* + *P. fasciicauda* + *D. pipra* group. The isozymes supported *Lepidothrix* as the basal taxon of those examined.

Key words: Andes, biogeography, hybrid zone, Manacus, *manakins, protein electrophoresis, systematics.*

Relaciones Filogenéticas en Saltarínes Barbados (Pipridae: *Manacus*) Indican que el Color del Plumaje del Macho es un Marcador Taxonómico Poco Confiable

Resumen. El género *Manacus* se compone de cuatro aloespecies que son fácilmente distinguibles por diferencias en el color del plumaje del macho. Datos electroforéticos para dos poblaciones de cada una de las cuatro formas, más siete taxa de grupos externos de pípridos (outgroups) fueron colectados de 32 loci de isoenzimas y fueron utilizados para inferir relaciones filogenéticas. Cada forma de *Manacus* fue monofilética, con excepción de *M. manacus*, en el cual la población trans-Andina (oeste de los Andes) fué cercana a *M. vitellinus*, y no a la población conespecífica cis-Andina (oriente de los Andes). Esta relación controversial, soportada por el alelo sinapomórfico PGM-2[b] al igual que por las frecuencias alélicas en ADA, GOT-1 y LGG, es consistente con los patrones biogeográficos generales en la región, pero indica que el caracter color del plumaje del macho no es un marcador taxonómico confiable. La reconstrucción del color del plumaje del macho en el árbol confirma que el plumaje dorado es un estado derivado en *M. vitellinus*, un hallazgo consistente con la posibilidad de que el plumaje dorado sea una novedad evolutiva en *vitellinus* que se ha dispersado recientemente bajo selección positiva. Entre los pípridos, se observó evidencia fuerte para un grupo compuesto por *Antilophia*, *Chiroxiphia* y *Corapipo*, y también para un grupo compuesto de *Pipra mentalis*, *P. fasciicauda* y *Dixiphia pipra*. *Manacus* está más estrechamente relacionado con el grupo de *P. mentalis*, *P. fasciicauda* y *D. pipra*. Las isoenzimas mostraron a *Lepidothrix* como el taxón basal entre todos los examinados.

INTRODUCTION

Manakins (Pipridae) are small, frugivorous inhabitants of forests throughout the Neotropics, remarkable for their lek mating behavior and striking secondary sexual plumage traits. *Ma-*

[1] Received 27 April 2000. Accepted 20 November 2000.

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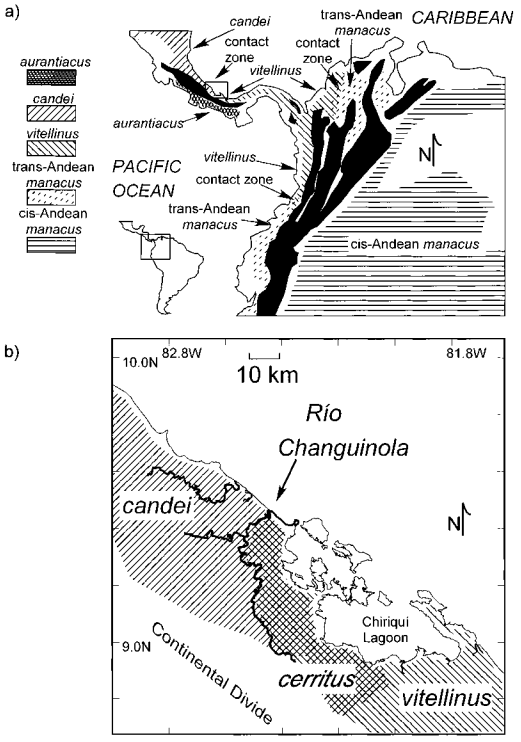

FIGURE 1. a) Schematic of *Manacus* distribution in southern Central America and northwestern South America. Mountains above 1000 m elevation are in black. b) Enlargement of *candei-vitellinus* contact zone in western Panama where hybrid ''*cerritus*'' is found.

nacus comprises four allospecies (Fig. 1a), each readily diagnosable by differences in the color of the definitive male throat and belly plumage (collar color is the same as throat color in all taxa): (1) white throat with yellow belly, *M. candei* (White-collared Manakin); (2) gold throat with green belly, *M. vitellinus* (Golden-collared Manakin); (3) orange throat with green belly, *M. aurantiacus* (Orange-collared Manakin); and (4) white throat with gray belly, *M. manacus* (White-bearded Manakin). Members of the genus are highly sexually dichromatic, with females of the four forms differing subtly in the shade of their drab green plumage. First-year males are virtually indistinguishable from females in plumage.

The four forms of *Manacus* have been treated most commonly as distinct, but closely related, species (Sibley and Monroe 1993, AOU 1998), although some authors have considered them members of a single polytypic biological species, *M. manacus* (Snow 1975, Traylor 1979). There are some named taxa within the four main groups (Traylor 1979), but geographic variation within the four is limited to clinal changes in the shade of the throat or belly plumage; the primary hues by which the four groups are defined do not change (Brumfield, unpubl. data). For example, the throat of *M. v. vitellinus* from central Panama is a golden yellow shade whereas *M. v. viridiventris* of extreme eastern Panama has a brighter, more lemon-yellow throat (Griscom 1929).

Previous studies of *Manacus* biogeography, reproductive ecology, and hybrid zones suggest a complex interaction of allopatric differentiation, hybridization, and sexual selection in the genotypic and phenotypic evolution of the four forms (Chapman 1926, 1935, Snow 1962, Haffer 1967, Lill 1974, Parsons et al. 1993). In Colombia, there are two contact zones west of the Andes between *vitellinus* and *manacus*, one in northern Colombia and one in Pacific coastal forest near the Ecuadorean border (Fig. 1a). In western Panama, a narrow zone of introgression unites *candei* and *vitellinus* (Fig. 1b). Introgressed forms in this region have been treated as a distinct species, *M. cerritus* (Hellmayr 1929), but subsequent genetic studies confirmed that *cerritus* is a hybrid (Parsons et al. 1993, Brumfield 1999). The hybrid zone between *candei* and *vitellinus* in western Panama is unusual in that several male plumage traits appear to have introgressed across it (Parsons et al. 1993, Brumfield 1999). Steep and coincident clines in a series of genetic and morphological characters define the main transition between the two forms along the western shore of the Chiriquí Lagoon, Bocas del Toro, Panama (Fig. 1b). However, the transition in male throat and belly color occurs at the Río Changuinola, 50 km to the west.

Because of the skewed male reproductive success associated with the lek mating system of manakins (Lill 1974), male secondary sexual traits are likely to be under strong selection and could cross the hybrid zone rapidly once recombined into the alternate genome (Barton 1979). Parsons et al. (1993) suggested that such a process was responsible for the differential introgression of *vitellinus* traits. This possibility can be tested in several ways. Behavioral experiments (McDonald, unpubl.) have been used to directly examine the role of gold male plumage in male-male competitive interactions. An indirect phylogenetic test is also possible. The idea

that gold plumage traits of *vitellinus* are under positive selection and are currently spreading to replace white traits suggests that they may have arisen recently. This implies that white will be the inferred ancestral condition for throat color when plumage traits are mapped onto a phylogeny of the genus.

Such a scenario would explain the leapfrog pattern of variation (Remsen 1984) in which the ranges of three white-throated populations (*candei, manacus* in Pacific Colombia, and *manacus* in northern Caribbean Colombia) straddle the range of the gold-throated form (*vitellinus*). Leapfrog variation occurs frequently in organisms with linear distributions (Remsen 1984), presumably because new traits arising in the middle of an elongated range result in a pattern wherein morphologically similar forms at the ends of the distribution are partitioned on either side of the novel form. If the gold throat trait is spreading from a point of origin in Panama or northern Colombia, it might well produce a split in the range of the white-throated forms west of the Andes.

A common distribution pattern for birds of humid lowland forest in northwestern South America is for a strictly trans-Andean form or forms to occur west of the Andes, while a strictly cis-Andean form or forms occurs east of the Andes (Hilty and Brown 1986). We follow Haffer's (1967) usage of trans-Andean for the tropical lowlands west of the Eastern Andean Cordillera and cis-Andean for those east of the Eastern Andean Cordillera. Morphological (Cracraft and Prum 1988, Prum 1988) and genetic (Brumfield and Capparella 1996) studies have typically shown that all trans-Andean forms within a species group are monophyletic. This pattern presumably arises because the high Andes, coupled with the drier vegetation of the Caribbean coast, present a formidable barrier to dispersal of lowland humid forest birds.

The distribution of *Manacus* deviates from this general pattern by the presence of two disjunct populations of *M. manacus* (a predominately cis-Andean form), west of the Andes in the Caribbean lowlands of northern Colombia and in the Pacific lowlands of northwestern Peru, western Ecuador, and southwestern Colombia (Fig. 1a). This anomalous distribution prompted Chapman (1926) and Haffer (1967) to propose that the trans-Andean *M. manacus* populations were the result of recent dispersal of cis-Andean populations across or around the Andes (Dispersal Hypothesis). Their hypothesis leads to the testable prediction that all *M. manacus* populations form a clade. An alternative hypothesis derived from the biogeographic pattern described above is that trans-Andean *M. manacus* populations represent the ancestral plumage condition, from which other trans-Andean forms (i.e., *vitellinus, candei*) have arisen in situ (Vicariance Hypothesis). This hypothesis predicts that all trans-Andean forms are monophyletic. The idea that yellow-throated plumage traits have arisen recently and are spreading under positive selection provides a plausible mechanism to produce the current distribution of plumage color types under the Vicariance Hypothesis. A well-resolved phylogeny of the group will allow discrimination between the Dispersal and Vicariance Hypotheses.

METHODS

Three to five individuals from each of two populations were sampled for each of the four *Manacus* plumage groups (Table 1). Samples from the trans-Andean population of *manacus* in northern Colombia were unavailable. At least four individuals from each of seven other piprid species were used as outgroups. The outgroup taxa represent several nested sister clades to *Manacus* as determined from behavioral and syringeal characters (Prum 1990, 1992). To maximize representation of the diverse and probably polyphyletic genus *Pipra* (Prum, pers. comm.), we sampled one species from each of Ridgely and Tudor's (1994) groupings.

Protein electrophoresis was performed on Titan III cellulose acetate plates (Helena Laboratories Inc., Beaumont, Texas) according to methods described by Richardson et al. (1986). Tissue homogenates were prepared by grinding approximately 50 mg of heart, liver, and pectoral muscle in 300 μl of distilled water or homogenization buffer (2 mM magnesium chloride, 0.2 mM dithiothreitol, and 0.25 M sucrose). The mixture was spun in a Brinkman 5415C Eppendorf centrifuge at 13 000 rpm for 10 min. The resulting supernatant was divided into 25-μl aliquots and frozen (−80°C) for subsequent electrophoretic experiments. Running conditions for all loci appear in Brumfield (1999). Electromorphs were coded alphabetically in order of relative mobility from the origin with the most anodally migrating allele as ''a.'' Among-gel

TABLE 1. Localities and specimen numbers for 4 *Manacus* and 7 other piprid taxa used in this study. Specimen numbers refer to the institutional frozen tissue catalogs of the United States National Museum of Natural History (USNM), the Louisiana State University Museum of Zoology (Baton Rouge; LSUMZ), the Field Museum of Natural History (Chicago; FMNH), and the Academy of Natural Sciences (Philadelphia; ANSP).

Population	Locality	Specimen number
Manacus vitellinus (central Panama)	PANAMA: prov. Colón; Soberania National Park	USNM 1858–1862
M. vitellinus (western Panama)	PANAMA: prov. Bocas del Toro; Valiente Peninsula	USNM 1224, 1251, 1299, 1359, 1380
M. candei (western Panama)	PANAMA: prov. Bocas del Toro; N bank Río Teribe	USNM 1920–1924
M. candei (Costa Rica)	COSTA RICA: prov. Limón; 11 km by road W of Guapiles	LSUMZ 16157, 16282, 16287, 16297, 16298
M. aurantiacus (western Panama)	PANAMA: prov. Chiriqui; 12.5 km by road N of Puerto Armuelles	USNM 2313, 2315, 2316, 2322, 2323
M. aurantiacus (Costa Rica)	COSTA RICA: prov. Puntarenas; Río Copey	LSUMZ 16096, 16097, 16105
M. manacus (cis-Andean)	PERU: depto. Loreto; 157 km by river NNE of Iquitos	LSUMZ 2583, 2678
	PERU: depto. Loreto; S Río Amazonas	LSUMZ 4801, 4910, 4912
M. manacus (trans-Andean)	ECUADOR: prov. Esmeraldas; Alto Tambo	ANSP 2394, 2407
	ECUADOR: prov. Manabi; Machalilla	ANSP 2954
	ECUADOR: prov. Esmeraldas; El Placer	LSUMZ 12013, 12029
Antilophia galeata	BOLIVIA: depto. Santa Cruz	LSUMZ 13815, 13835, 13866, 13881, 13882
Chiroxiphia pareola	PERU: depto. Loreto; 5 km N of Amazonas	LSUMZ 6864, 7031, 7056, 7118, 7181
Corapipo leucorrhoa	PANAMA: prov. Darién; about 6 km NW of Cana	LSUMZ 2085, 2087, 2089, 2091, 2092
Pipra mentalis	PANAMA: prov. Bocas del Toro; Cayo Agua	USNM 1084, 1089, 1095, 1096, 1143
P. fasciicauda	PERU: depto. Madre de Dios	FMNH 4316, 4349, 4355
	BRAZIL: Para; Altamira	USNM 6828
Dixiphia pipra	GUYANA: Essequibo; Waruma River	USNM 5013, 5016, 5076, 5094, 5123
Lepidothrix suavissima	GUYANA: Essequibo; Waruma River	USNM 5065, 5156, 5217, 5224, 5262

comparisons of all alleles were made by rerunning selected individuals on cross-correlation gels.

BIOSYS-1 (Swofford and Selander 1981) was used to compute the genetic distances D_C and D_N (Cavalli-Sforza and Edwards 1967, Nei 1978). GENEPOP (Raymond and Rousset 1995) was used to perform exact tests for genic differentiation between or among populations. Exact tests estimate the probability that allele counts in the population samples under comparison were drawn from the same distribution (based on pooled counts). As a preliminary exploration of phylogenetic structure in the data, a suite of distance analyses was performed. UPGMA (Sneath and Sokal 1973), neighbor-joining (Saitou and Nei 1987), and minimum-evolution (ME, Kidd and Sgaramella-Zonta 1971) analyses with Cavalli-Sforza and Edwards (1967) chord distances were performed. The strongly supported nodes in these analyses were used as constraints in the parsimony analysis.

Frequency parsimony analysis (FREQPARS, available electronically via ftp at lms.si.edu, Swofford and Berlocher 1987) was used as the principal cladistic method of analysis for the allele frequency data. This method treats individual loci as characters and allele frequency arrays as character states, a method that is less sensitive to sampling error than coarser ''presence-absence'' coding strategies (Swofford and Berlocher 1987). Optimal topologies have the minimum amount of allele frequency change across the tree, with change measured as pairwise Manhattan distances summed across all loci. Under the MANAD criterion (Swofford and Berlocher 1987) implemented in the FREQPARS program, allele frequency arrays at internal nodes are un-

restricted. Additional frequency parsimony analyses were performed using PAUP*, thus facilitating searches of tree space (Berlocher and Swofford 1997, Swofford 1998). A limitation of the PAUP* method is that it implements the MANOB criterion (Swofford and Berlocher 1987), for which allele frequency arrays at internal nodes are restricted to arrays observed in at least one of the terminal taxa. Using PAUP*, 1000 unconstrained bootstrap replicates of loci were performed using ten random addition tree searches per replicate and TBR branch swapping. The maximum number of saved trees was set to 500.

RESULTS

GENETIC VARIATION

Levels and patterns of variation at 32 presumed genetic loci from 25 enzyme systems were resolved for all ingroup and outgroup taxa (data available upon request). Exact tests identified ADA, GOT-1, GSR, LGG, PGM-2, PP-1, and VL as significantly heterogeneous ($P \leq 0.05$) within *Manacus*. PGM-2 exhibited a striking geographic pattern in which a derived allele (PGM-2^b) was shared at high frequency by the two populations of *vitellinus* and the trans-Andean population of *manacus*. PGM-2^b is considered derived because it is unique to these three populations and because another allele (PGM-2^d) occurs at high frequency in all other *Manacus* populations and in all outgroup taxa. A pairwise exact test of genic differentiation at PGM-2 between the trans-Andean and cis-Andean *manacus* populations was highly significant ($P = 0.003$). In contrast, there was only weak differentiation when comparing the trans-Andean *manacus* population with *vitellinus* populations from central Panama ($P = 0.05$) and from western Panama ($P = 0.09$).

Exact tests for genic differentiation at ADA, GOT-1, and LGG provided additional support for a closer genetic relationship between *vitellinus* and trans-Andean *manacus* than between the two *manacus* populations. At all three loci, there was significant (GOT-1, $P = 0.03$; LGG, $P = 0.01$) or nearly significant (ADA; $P = 0.06$) differentiation between the two *manacus* populations, and no significant differentiation ($P > 0.05$) between the *vitellinus* and trans-Andean *manacus* populations.

Genetic distances were consistently lower between conspecific populations (D_N range 0.000 to 0.038) than between heterospecifics (range 0.011 to 0.089, Table 2). The one exception was in the comparison between the two populations of *manacus,* where the trans-Andean population of *manacus* had lower genetic distances to the two populations of *vitellinus* ($D_N = 0.011$ and 0.027) than to the cis-Andean *manacus* population ($D_N = 0.038$). Even without PGM-2 data, distances from the trans-Andean *manacus* population to either *vitellinus* population are equal to or lower than the distance to its cis-Andean conspecific population ($D_N = 0.012$ to central Panama *vitellinus,* 0.024 to western Panama *vitellinus,* 0.024 to cis-Andean *manacus*).

DISTANCE ANALYSES

The shortest minimum-evolution topology (2.323 steps, Fig. 2) was the same as that found using the neighbor-joining algorithm. In this tree, the trans-Andean *manacus* population is sister to a monophyletic *vitellinus,* with cis-Andean *manacus* basal to this clade. The UPGMA tree was similar to the ME tree, except that the cis-Andean *manacus* population is basal to the *candei* + *aurantiacus* clade. Changes in the position of cis-Andean *manacus* in the two distance analyses reflect the short internal branch between cis-Andean *manacus* and the basal *Manacus* node (Fig. 2).

Locus-exclusion analyses were performed to assess the strength of phylogenetic relationships. The terminal *vitellinus* + trans-Andean *manacus* clade was still present after excluding PGM-2, indicating additional allele frequency support for the controversial relationship. This support derives largely from GOT-1 and LGG, which are significantly differentiated between the two *manacus* populations. In the PGM-2 excluded tree, cis-Andean *manacus* moved to a position sister to a clade comprising *vitellinus,* trans-Andean *manacus,* and *candei.*

FREQUENCY PARSIMONY ANALYSIS

The number of tree topologies for 15 taxa was larger than we could practically evaluate with the ''usertree'' method (Swofford and Berlocher 1987) under the MANAD criterion implemented in FREQPARS. Therefore, we evaluated a subset of trees ($n = 42\,525$) in which the following groups were constrained to cluster together based on their stability in distance analyses: 1) *Antilophia*, *Chiroxiphia*, and *Corapipo*; 2) *Pipra*

TABLE 2. Genetic distance matrix based on 32 loci resolved for all taxa. Upper matrix, unbiased genetic distance (D_N); lower matrix, chord distance (D_C).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1) *vitellinus* (C Panama)	****	0.000	0.038	0.034	0.071	0.078	0.046	0.011	0.392	0.406	0.333	0.224	0.215	0.199	0.506
2) *vitellinus* (W Panama)	0.117	****	0.052	0.043	0.081	0.088	0.061	0.027	0.378	0.394	0.316	0.219	0.227	0.206	0.501
3) *candei* (W Panama)	0.212	0.252	****	0.000	0.028	0.035	0.024	0.046	0.362	0.385	0.290	0.215	0.205	0.192	0.458
4) *candei* (Costa Rica)	0.175	0.203	0.127	****	0.026	0.032	0.026	0.045	0.345	0.373	0.281	0.196	0.197	0.184	0.450
5) *aurantiacus* (W Panama)	0.244	0.269	0.182	0.154	****	0.000	0.060	0.082	0.349	0.379	0.325	0.236	0.246	0.229	0.504
6) *aurantiacus* (Costa Rica)	0.260	0.284	0.203	0.178	0.036	****	0.067	0.089	0.352	0.382	0.332	0.244	0.253	0.236	0.513
7) *manacus* (cis-Andean)	0.232	0.258	0.201	0.189	0.246	0.262	****	0.038	0.361	0.381	0.267	0.231	0.219	0.199	0.424
8) *manacus* (trans-Andean)	0.145	0.185	0.215	0.183	0.245	0.261	0.210	****	0.401	0.411	0.338	0.247	0.226	0.204	0.525
9) *Antilophia galeata*	0.532	0.536	0.516	0.506	0.503	0.503	0.525	0.531	****	0.136	0.176	0.379	0.399	0.366	0.604
10) *Chiroxiphia pareola*	0.551	0.551	0.536	0.527	0.528	0.528	0.542	0.550	0.368	****	0.237	0.418	0.395	0.332	0.586
11) *Corapipo leucorrhoa*	0.502	0.497	0.477	0.466	0.487	0.495	0.462	0.500	0.397	0.455	****	0.384	0.386	0.366	0.544
12) *Pipra mentalis*	0.430	0.437	0.416	0.400	0.418	0.427	0.437	0.434	0.518	0.542	0.526	****	0.172	0.116	0.472
13) *Dixiphia pipra*	0.430	0.444	0.414	0.405	0.425	0.434	0.437	0.428	0.518	0.540	0.529	0.360	****	0.109	0.412
14) *Pipra fasciicauda*	0.422	0.437	0.404	0.400	0.419	0.429	0.418	0.412	0.517	0.511	0.525	0.312	0.329	****	0.512
15) *Lepidothrix suavissima*	0.581	0.585	0.562	0.557	0.571	0.578	0.554	0.583	0.621	0.617	0.600	0.564	0.538	0.586	****

mentalis and *P. fasciicauda*; 3) all *Manacus*; 4) all *vitellinus*; and 5) all *aurantiacus.* This analysis of all 32 loci included 9 constant characters, 10 variable but parsimony-uninformative characters, and 13 parsimony-informative characters. The analysis resulted in five most-parsimonious trees (102.55 steps), the strict consensus of which is illustrated (Fig. 3b). The most striking feature of the most-parsimonious trees is that *manacus* is paraphyletic in all five. Because of the low genetic distance (D_N = 0.000) between the two *candei* populations, we consider *candei* to be monophyletic in spite of the lack of strong cladistic support. Figure 3a illustrates the one tree in which *candei* is monophyletic.

A PAUP* *unconstrained* branch and bound search under the MANOB criterion found four most-parsimonious trees (103.15 steps, CI = 0.87), with topologies equivalent to four of the five most-parsimonious trees (102.55 steps) from the FREQPARS search (increased length due to MANOB restriction). One of the most-parsimonious trees identified by FREQPARS was not recovered because its length under MANOB increased to 103.35, a length that includes ten other MANOB trees. Bootstrap support was highest for a group composed of *Antilophia, Corapipo,* and *Chiroxiphia* (94%), the *Manacus* clade (89%), and a group composed of *Pipra mentalis* and *P. fasciicauda* (82%). Reasonable support was also present for the monophyly of *vitellinus* (75%) and of *aurantiacus* (75%).

Strict consensus trees from the FREQPARS search and the PAUP* search were identical, suggesting that the MANOB criterion is a good approximation of the MANAD criterion for this data set. PAUP* was therefore used for all further parsimony analyses. Loci identified as significantly heterogeneous among *Manacus* populations (ADA, GOT-1, GSR, LGG, PGM-2, PP-1, VL; see Genetic Variation section above) were excluded sequentially, a branch and bound search performed, and the strict consensus tree examined. This procedure demonstrated that the paraphyly of *manacus* is due solely to the synapomorphic allele PGM-2^b, which unites *vitellinus* and the trans-Andean population of *manacus* in a terminal clade. Without this locus, *manacus* and *vitellinus* become terminal sister clades with both forms monophyletic. The removal of GSR and PP-1 results in collapse of the branches supporting the monophyly of *vitellinus* and *aurantiacus,* respectively. Removal of ADA or VL re-

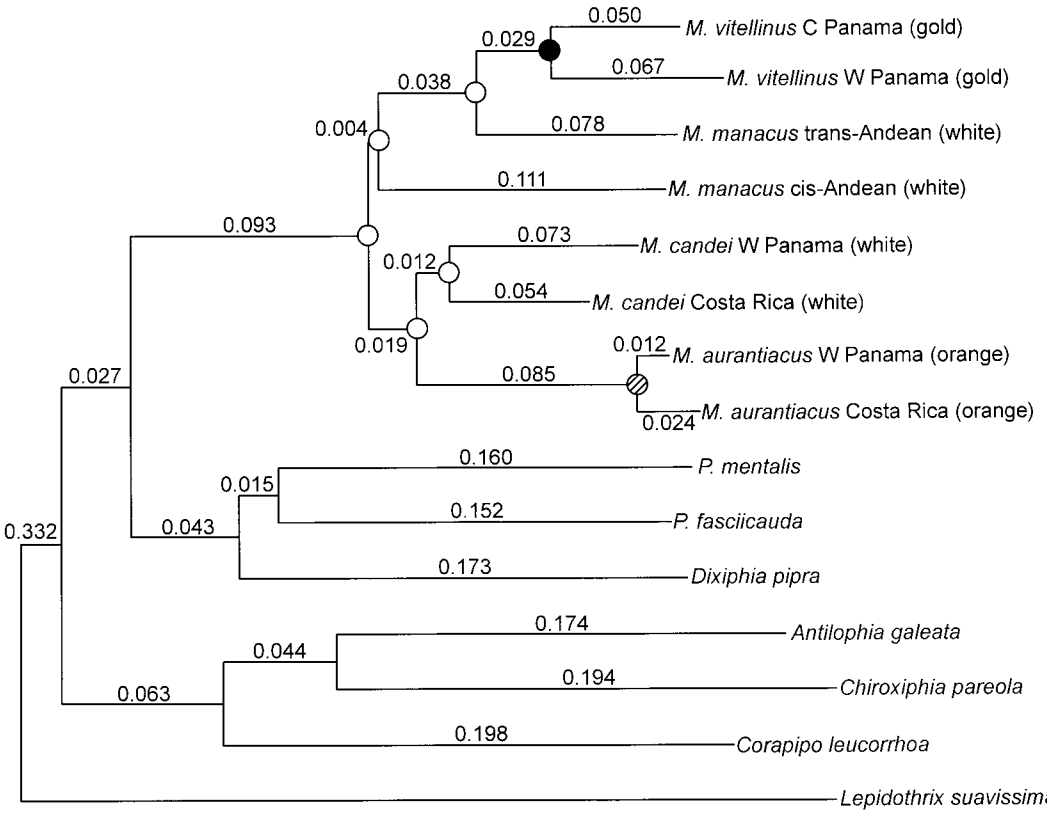

FIGURE 2. Minimum evolution tree of *Manacus* relationships based on D_C matrix. Tree is drawn with midpoint rooting. Male throat colors are given for each *Manacus* population. Reconstructed ancestral throat color states are denoted by circles: filled circle is gold male throat plumage, unfilled circle is white, and diagonally shaded circle is orange.

sults in the cis-Andean population of *manacus* moving from its position in Figure 3a to a position sister to the western Panama population of *candei.* Excluding LGG or GOT-1 did not change the results from the full analysis. Other analyses included creating a single operational taxonomic unit for each form by combining allele frequency data from conspecific populations, and excluding populations near the contact zone in western Panama. Results of these analyses were consistent with the conclusions of the full analysis.

DISCUSSION

EVOLUTIONARY RELATIONSHIPS

Other than the evolutionary relationships implied by taxonomic classifications, no hypotheses concerning phylogenetic relationships within *Manacus* have been previously published. In the present study, monophyly of the genus, of *aurantiacus,* and of *vitellinus* is supported by all phylogenetic analyses, and monophyly of *candei* is supported by the very low genetic distance between the two populations examined. The most remarkable phylogenetic result is the node uniting the *vitellinus* clade with the trans-Andean population of *manacus* (bootstrap = 65%), a relationship seemingly at odds with male plumage colors. Such a relationship has not been suggested in any previous studies of *Manacus.* Although a single allele, PGM-2^b, provides the bulk of the parsimony support, support from additional loci is indicated by the persistence of the node in distance trees constructed from matrices that were calculated without PGM-2 data.

Monophyly of *candei* populations is clearly indicated in the genetic distances (Table 2) and distance trees (Fig. 2), despite a lack of strong cladistic support. There is moderate support (parsimony bootstrap = 59%) for a terminal clade

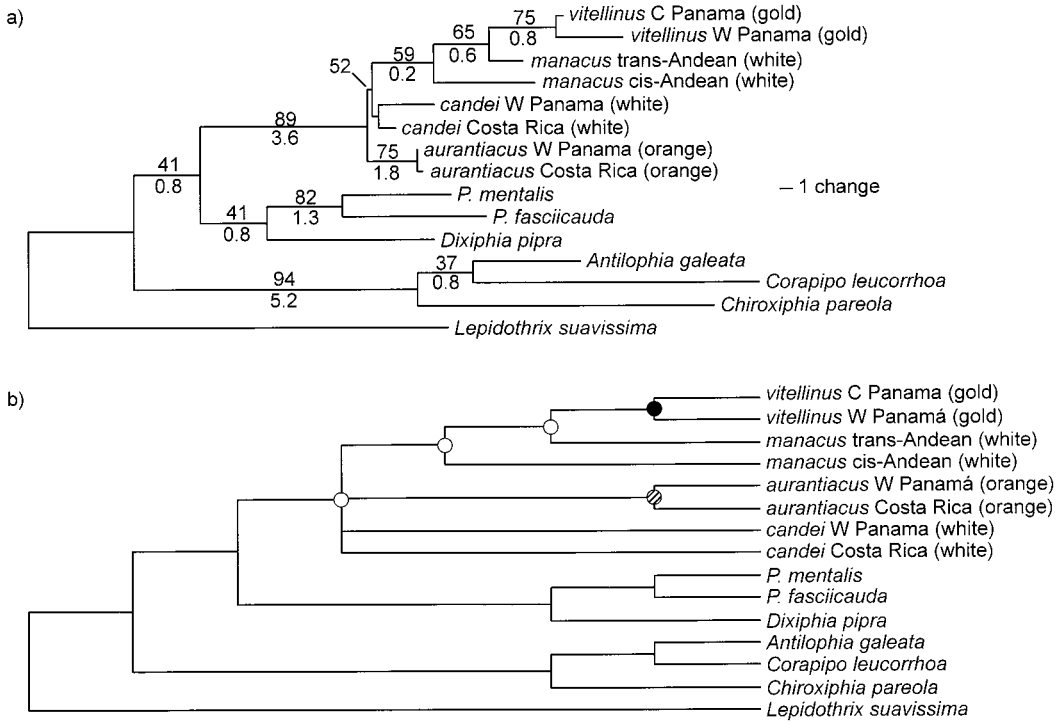

FIGURE 3. a) Phylogenetic reconstruction of *Manacus* relationships based on isozyme allele frequency data. Tree depicted is one of five most-parsimonious trees (102.55 steps) found using FREQPARS. Bootstrap support is given above branches, and decay index below branches. Two FREQPARS steps are equivalent to a single allelic substitution. Tree rooted to *Lepidothrix* based on its uniformly high genetic distances to other piprid taxa. b) Strict consensus of five most-parsimonious trees. Ancestral node throat color states are as in Figure 2.

composed of *vitellinus* and *manacus* (Fig. 3a). The phylogenetic relationships of *candei* and *aurantiacus* are more uncertain. In the frequency parsimony analysis, *candei* is sister to the *vitellinus* + *manacus* clade, with *aurantiacus* as the basal *Manacus* taxon. In distance analyses, *aurantiacus* and *candei* are both monophyletic, and form a sister clade to *vitellinus* + *manacus.* Resolving the relationships of *aurantiacus* and *candei* will require more data, but the isozyme trees do not corroborate the close relationship of *aurantiacus* and *vitellinus* implied by their treatment as members of a single group (Haffer 1967).

BIOGEOGRAPHY

The isozyme phylogeny contradicts the prediction of the Dispersal Hypothesis that *manacus* is a monophyletic form. Instead, the predictions of the Vicariance Hypothesis are partially borne out, because trans-Andean *manacus* is monophyletic with *vitellinus,* and *manacus* is paraphyletic (Fig. 3b). The isozyme phylogeny suggests a more complex vicariant history, however, because cis- and trans-Andean populations are not neatly partitioned into two clades. Rather, the base of the tree is composed of the trans-Andean forms *aurantiacus* and *candei.* However, the cis-Andean *manacus* population is close to the base of the *Manacus* clade in distance analyses, so a tree with cis-Andean *manacus* basal is probably not inconsistent with the isozyme data. Thus, the data are consistent with the Vicariance Hypothesis and show reasonably strong support for the close relationship of *vitellinus* and trans-Andean *manacus.* Samples from additional populations, particularly in northwestern South America, are needed to better characterize the historical pattern of diversification.

EVOLUTION OF MALE SECONDARY SEXUAL TRAITS

As an indirect test of the hypothesis that the gold plumage of *vitellinus* is a recently derived trait that is spreading into neighboring white-plum-

aged forms, male plumage color was mapped onto the isozyme phylogeny (Fig. 2, 3b); *vitellinus* was coded as having gold throat plumage, *aurantiacus* as having orange plumage, and *candei* and *manacus* as having white throat plumage. All outgroups were coded with a fourth ''not gold, orange, or white'' character state, because none have gold, orange, or white throats.

The inferred state of the common ancestor of *manacus* and *vitellinus* is white-throated, based on most-parsimonious reconstructions on both the parsimony (Fig. 3b) and distance tree (Fig. 2). This implies that gold throat plumage is an autapomorphic state in *vitellinus* that has evolved from a white-plumaged ancestor. This provides a resolution for the seeming conflict between male throat color and the close relationship between trans-Andean *manacus* and *vitellinus.* If white throat plumage is a symplesiomorphy for trans-Andean and cis-Andean *manacus* populations, it provides no cladistic information and is not in conflict with the isozyme phylogeny.

The inferred state at the base of the *Manacus* tree is also white (Fig. 2, 3b), although our confidence in that conclusion is tempered by equivocal phylogenetic resolution at the base of the tree. Also, a case could be made that the gold plumage of *vitellinus* and orange plumage of *aurantiacus* represent a single ''pigmented throat'' character state. We therefore reconstructed plumage colors on the phylogeny with *vitellinus* and *aurantiacus* coded in that manner. Under this coding, the common ancestor of *manacus* + *vitellinus* was still white, but the inferred state at the base of the parsimony tree changed from white to ambiguous. However, on the distance tree, the basal node as well as the common ancestor of *candei* and *aurantiacus* changed from white to ambiguous. All other reconstructed states remained as in the previous analysis.

PIPRID RELATIONSHIPS

We compared the isozyme tree with previous hypotheses of piprid relationships proposed by Lanyon (1985) on the basis of isozymes and by Prum (1990, 1992) on the basis of behavior and syringeal anatomy. As in the present study, Lanyon (1985) lacked many representative piprid species. Of the taxa Lanyon included, the following are common to both of our studies: *Manacus manacus, Dixiphia pipra, Chiroxiphia pareola,* and *Corapipo* sp. Considering these taxa, Lanyon's (1985) phylogenetic trees are entirely consistent with ours.

Prum's (1990) analysis of piprid relationships was comprehensive, including representatives of all major piprid groups. Notably, the isozyme phylogeny corroborates Prum's (1990) conclusion that *Manacus* is monophyletic. Regarding the closest relative of *Manacus,* Prum (1992) proposed that the genus was sister to a clade composed of *Antilophia* and *Chiroxiphia.* In the isozyme analysis, *Antilophia* and *Chiroxiphia* do cluster together in the distance tree (Fig. 2), but the sister taxon to that clade is *Corapipo,* not *Manacus.* There is no possible rooting of either the distance or parsimony tree that would corroborate the sister relationship of *Manacus* with the *Antilophia* + *Chiroxiphia* clade. Constraining *Antilophia, Chiroxiphia,* and *Manacus* to cluster together requires eight additional frequency parsimony steps, equivalent to four amino acid substitutions. The isozyme data suggest instead that *Manacus* is sister to a clade composed of *Pipra mentalis, P. fasciicauda,* and *Dixiphia pipra,* assuming the tree is correctly rooted along the branch leading to *Lepidothrix,* the longest branch.

According to Prum's (1992) tree, *Corapipo* should represent the most basal piprid of the taxa analyzed in this study. Genetic distances between *Lepidothrix* and all other taxa are uniformly high, however, suggesting that it represents the best *ad hoc* candidate for the basal piprid if rates of molecular evolution are roughly equivalent among taxa (Table 2). Rather than having a basal position in the isozyme tree, *Corapipo* clusters with *Antilophia* and *Chiroxiphia* due to the shared, derived alleles LDH-1^a, LDH-2^c, PGD^a, and PGM-1^d.

There is some parsimony support for a clade of *Antilophia* and *Corapipo* based on the shared allele GPI^c that is fixed in each form and found in no other. In the distance tree, on the other hand, *Antilophia* is sister to *Chiroxiphia,* a relationship consistent with Prum's (1992) tree. However, this structure is largely due to autapomorphic differentiation of *Corapipo* at GOT-1, GSR, ICD-1, and VL.

The grouping of *P. mentalis, P. fasciicauda,* and *D. pipra* in the isozyme trees (Fig. 2, 3) is supported by the synapomorphic allele LDH-2^a. The placement of *D. pipra* basal to *P. mentalis* + *P. fasciicauda* is consistent with Prum's (1990) phylogeny, but the similar genetic dis-

tances among the three (D_N range = 0.109–0.172, Table 2) provide some evidence for retaining *D. pipra* in the genus *Pipra.*

ACKNOWLEDGMENTS

D. Dobkin, S. J. Hackett, J. Garcia-Moreno M., C. Huddleston, W. Koenig, R. Prum, and two anonymous reviewers provided constructive criticisms of the manuscript. We are grateful to the collectors and curatorial staffs of the Louisiana State University Museum of Zoology (Baton Rouge), the Field Museum of Natural History (Chicago), and the Academy of Natural Sciences (Philadelphia) for lending specimens to us, and to Marcella Santaella for providing the Spanish abstract. We thank INRENARE for granting collecting permits in Panama. M. Leone, G. Maggiori, E. Bermingham, and others at the Smithsonian Tropical Research Institute greatly facilitated our work in Panama. This work was funded in part by a Smithsonian Predoctoral Fellowship to RTB.

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